PBA2023

33rd International Symposium on

Pharmaceutical and Biomedical Analysis

ABSTRACTS & PROCEEDINGS

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02-06 July 2023
Ankara University
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FOREWORD

Dear Friends and Colleagues,

On behalf of the Organizing Committee, we cordially invite you to join us for the 33rd International Symposium on Pharmaceutical and Biomedical Analysis (PBA 2023) to be held between July 2nd and 6th, 2023, in Ankara University Faculty of Pharmacy.

PBA 2023 will cover all aspects where pharmaceutical and biomedical analysis plays a role, including fundamental and applied sciences. It will offer plenary and keynote presentations on cutting-edge topics by internationally renowned leaders of the field, followed by contributed talks and poster presentations to stimulate interdisciplinary discussions as its long traditions of it. A young researcher's session will be organized to provide opportunities for and encourage Ph.D. students and postdocs to share their findings.

It has long been said that Türkiye is a bridge between Europe and Asia, and now we can expand that to all continents as we are happy to welcome premier analytical chemists and other chemistry-related scientists from all over the world to enrich our knowledge with their lectures and presentations.

In addition to the scientific program, exciting social events are being planned. Amazing sights, tranquil places off the beaten trail, world-renowned cuisine, and a very rich cultural tradition await you.

We look forward to your participation in PBA 2023 in July 2023 and to welcoming you to our capital city Ankara.

Prof. Dr. Sibel A. OZKAN
Chair of the Symposium

COMMITTEES

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Kelly Zhang*, USA

(permanent committee member)*

PROGRAM

July 2, 2023			
07.30-17.00	**Registration**		
	Lecture Hall 1 (A1)	**Lecture Hall 2 (A2)**	**Lecture Hall 3 (A3)**
08.30-15.00	**COURSE 1** **Analytical Challenges in Drug Development: Need in Orthogonal Chromatographic Methods** **Vladimir Ioffe**	**COURSE 2** **SPME: New Developments and Pharmaceutical Applications** **Janusz Pawliszyn, Wei Around Zhou, Barbara Bojko, Ezel Boyaci**	**COURSE 3** **Sensors and Applications** **Alberto Escarpa, Mamas Prodromidis, Gustavo Rivas**
10.45-11.00	Coffee Break	Coffee Break	Coffee Break
11.00-12.30	**COURSE 1 (Continues)**	**COURSE 2 (Continues)**	**COURSE 3 (Continues)**
12.30-13.30	Lunch	Lunch	Lunch
13.30-15.00	**COURSE 1 (Continues)**	**COURSE 2 (Continues)**	**COURSE 3 (Continues)**
	Lecture Halls		
17.00-17.45	**Opening Ceremony**		
17.45-18.30	**Session Chairs: Sibel A. Ozkan, Gustavo Rivas** **PL 1 - Bezhan Chankvetadze** Recent Challenges and Trends in Enantioselective Analysis of Chiral Drugs		
19.15-21.00	**Welcome Reception**		

PROGRAM

July 3, 2023		
	Hall A	
09.00-09.45	**Session Chairs: Bezhan Chankvetadze, Attila Felinger** **PL 2 - Frantisek Svec** (Hi)story of chromatography: Monoliths	
09.45-10.00	**Coffee Break**	
	Hall A	**Hall B**
	Session Chairs: Bezhan Chankvetadze, Attila Felinger	**Session Chairs: Zhengjin Jian, Jacques Crommen**
10.00-10.30	**KL 1 - Szabolcs Beni** Teaching the old dog new tricks: characterization of sugammadex and its various complexes	**KL 2 - Ann Van Schepdael** Impact of sterilization on the chemical composition of a selection of active pharmaceutical ingredients
10.30-11.00	**KL 3 - Emmanuelle Lipka** From analytical to preparative scale in chiral supercritical fluid chromatography: economy, challenge, opportunity	**KL 4 - Sandra Furlanetto** New trends in Quality Control: New compendial and ICH guidelines outlining Quality by Design (QbD) principles
11.00-11.20	**IL 1 - Paola Peluso** Integrating experimental and computational approaches to study enantioselective recognition in liquid-phase enantioseparations	**IL 2 - Vladimir Ioffe** Not Only Reverse Phase… A Need to Use Alternative HPLC Techniques for Pharmaceutical Analysis
11.20-11.35	**OP 1 - Tamar Khatiashvili** Photochemical immobilization of cellulose tris(3-chloro-5-methylphenylcarbamate) on silica and its use for separation of enantiomers in high-performance liquid chromatography	**OP 2 - Chee-Leong Kee** Analytical methodologies and challenges in the analysis of synthetic phosphodiesterase type 5 inhibitors (PDE-5i) found as adulterants in health supplements
11.35-11.50	**OP 3 - Baochun Shen** The Enantiseparation of Chiral Compounds by Chiral Stationary Phases	**OP 4 - Murat Ozdemir** Quantitative Analysis of Estradiol and Estradiol Metabolites by UPLC-MS/MS Method
11.50-13.30	**Lunch and Vendor Seminars (Metrohm)** **Pablo Fanjul Bolado** Recent advances in Raman and Surface Enhanced Raman Spectroelectrochemistry in analytical applications	
	Session Chairs: Paola Peluso, Emmanuelle Lipka	**Session Chairs: Chiara Fanali, Ann Van Schepdael**
13.30-14.00	**KL 5 - Gerhard Scriba** Analyte Complexation and Chiral Separations in Capillary Electrophoresis	**KL 6 - Attila Fellinger** Thermodynamics of Liquid Chromatography
14.00-14.20	**IL 3 - Antonio Salgado** NMR studies on the elucidation of the (unusual) structure of selector-selectand aggregates between daclatasvir and some cyclodextrins	**IL 4 - Aalessandra Gentili** Adavances in Solid Phase Extraction: the evolution of carbon-based sorbents
14.20-14.35	**OP 5 - Ann Gogolashvili** Mechanistic studies on enantioselective noncovalent selector-selectand interactions using capillary electrophoresis, nuclear magnetic resonance spectroscopy and isothermal titration microcalorimetry	**OP 6 - Lukasz Ciesla** Novel solutions in drug discovery from complex natural matrices for the prevention and treatment of neurodegenerative diseases

PROGRAM

14.35-14.50	**OP 7 - Pavel Jáč** Development of capillary electrophoresis-mass spectrometry method for the simultaneous analysis of boswellic acids and non-steroidal anti-inflammatory drugs	**OP 8 - Dönay Yuvali** Determination of Tamoxifen in Biological Fluids Using Liquid-Phase Microextraction and Liquid Chromatographic Analysis
14.50-15.05	**OP 9 - Nevin Oztekin** Separation and determination of enantiomers of 2-hydroxyglutaric acid in urine by capillary electrophoresis with contactless conductivity detection	**OP 10 - Sercan Yildirim** A deep eutectic solvent-based ferrofluid for vortex-assisted liquid-liquid microextraction of nonsteroidal anti-inflammatory drugs from environmental waters
15.05-16.15	**Coffee Break and Poster Session-1**	
	Session Chairs: Sandra Furlanetto, Szabolcs Beni	**Session Chairs: Alain Walcarius, Alberto Escarpa**
16.15-16.45	**KL 7 - Kenji Hamase** Three/four-Dimensional Chiral HPLC Analysis of Amino Acids in Mammals; Their Origins, Regulation Systems and Diagnostic Values	**KL 8 - Zbigniew Brzozka** Cell-on-a-chip microsystems – alternative in vitro models to study effectiveness of medical therapies
16.45-17.00	**OP 11 - Chiharu Ishii** Selective Determination of D-Amino Acids in Human Plasma Using a Two-Dimensional LC-MS/MS System Toward Early-Diagnosis of Chronic Kidney Disease	**OP 12 - Uzeyir Dogan** Development of a method for quantification of some metallic content of Humulus lupulus L. plant with ICP-OES
17.00-17.15	**OP 13 - Rusudan Kakava** Synthesis of chiral sulfoxides and study of structure-retention and structure-enantioselectivity relationships	**OP 14 - Leyla Karadurmus** A molecularly imprinted polymer-based electrochemical sensor for the determination of Emtricitabine
17.15-17.30	**OP 15 - Markus Ganzera** Mycosporine Like Amino Acids – Isolation and Analysis of Promising Marine Sunscreens	**OP 16 - Aytekin Uzunoglu** Inkjet-Printing of MXene/Holey CNT-based Inks for the Construction of Flexible Nicotine Sensors
17.30-17.45	**OP 17 - F. Bedia Erim** Biopolymers as antibacterial wound dressing and controlled drug release agents	**OP 18 - Inci Uludag** An Immunosensor Using A Specific Aptamer as A Bioreceptor for Quantification of Sarcosine
19.00-19.30	**Ministry of Culture & Tourism Ankara State Turkic World's Music & Folk Dance Ensemble**	

PROGRAM

July 4, 2023			
	Hall A		
09.00-09.45	**Session Chair: Zuhre Senturk** **PL 3 - Jean-Michel Kauffmann** Trends in Analytical Chemistry		
09.45-10.00	**Coffee Break**		
	Hall A	**Hall B**	**Hall C**
	Session Chairs: Zbigniew Brzozka, Susana Campuzano	**Session Chairs: Stig Pedersen-Bjergaad, Michal Markuszewski**	**Session Chairs: Jiri Homola, Mamas Prodromidis**
10.00-10.30	**KL 9 - Can Dincer** Disposable sensors for next-generation point-of-care diagnostics	**KL 10 - Andras Guttman** Size, charge, and N-glycosylation variant analysis of the anti-COVID-19 monoclonal antibody bamlanivimab by capillary gel electrophoresis	**KL 11 - Federico Marini** New Chemometric-Based Strategies for Pharmaceutical and Biomedical Analysis
10.30-11.00	**KL 12 - Alain Walcarius** Interest oriented mesoporous silica films for the electrochemical detection of biologically relevant species	**KL 13 - Serge Rudaz** From untargeted metabolomics to longitudinal targeted monitoring in clinics	**KL 14 - Ashraf Ghanem** Chirality post COVID: Challenges, Adaptations, and Future Prospects
11.00-11.20	**OP 19 - Mehmet Bulent Ozer** Acoustic Particle Manipulation in 3-D Printed Acoustophoretic Microfluidic Chips	**IL 6 - Chiara Fanali** Recent results in food sample preparation employing deep eutectic solvents	**IL 7 - Daniel Quesada-González** Lateral Flow Tests: Signal Enhancement Methods and Future Trends for Diagnostics
11.20-11.35	**OP 22 - Dilsat Ozkan-Ariksoysal** An Overview of Current Approaches in Quantum Dot-Based Electrochemical DNA Biosensors and Their Potential for Use in the Diagnostic Kit Field	**OP 20 - Mustafa Celebier** Unveiling the Metabolic Signature of Preeclampsia: A Promising Analytical Tool for Early Detection	**OP 21 - Ezgi Salmanli** A New Competitive Lateral Flow Immunoassay Test for Rapid Detection of Total Antibody in Vaccinated Puppies Serum Against Canine Parvovirus-2
11.35-11.50		**OP 23 - Jingwu Kang** Drug screening based on proteomics	**OP 24 - Aysun Dincel** Simultaneous spectrophotometric analysis of emtricitabine and tenofovir disoproxil fumarate in pharmaceutical formulation
11.50-12.05	**Symposium Photo**		
12.05-13.30	**Lunch and Vendor Seminars (CAS Life Sciences and SciFinder Discovery Platform)** **Görkem Mergen** CAS Life Sciences and SciFinder Discovery Platform		
	Session Chairs: Andras Guttman, Barbara Bojko	**Session Chairs: Federico Marini, Bekir Salih**	**Session Chairs: Kenji Hamase, Gabriella Masolini**
13.30-14.00	**KL 15 - Zuhre Senturk** The Story of the 100-Year Path of Voltammetry, Starting with Drops of Mercury and Reaching the Mysterious Shores of the Brain	**KL 16 - Martin Vogel** Electrochemistry/mass spectrometry – a versatile tool to mimic the metabolism of drug substances	**KL 17 - Zhengjin Jiang** Rapid purification and separation of antibody drugs based on epitope-mimetic peptide recognition technology

PROGRAM

14.00-14.20	**IL 8 - Francesco Busardo** Development of enantioselective high-performance liquid chromatography-tandem mass spectrometry method for quantitative determination of methylone and some of its metabolites in oral fluid	**IL 9 - Sezgin Bakirdere** Trends in analytical strategies for the determination of pharmaceuticals	**IL 10 - Marcello Locatelli** Emerging challenges for Analytical Chemistry in the Pharmaceutical and Biomedical Analysis
14.20-14.35	**OP 25 - Annagiulia Di Trana** The role of analytical pharmacotoxicology in adressing the main functions of the National Early Warning System on NPS	**OP 26 - Fahad S Aldawsari** An analytical debate regarding nitrite analysis in raw materials associated with nitrosamine occurrence in finished products; the importance of selecting a precise testing methodology	**OP 27 - Fezile Ozdemir** The Effect of Pharmacokinetic Genes Polymorphisms to the Plasma Clozapine and Norclozapine Levels in Patients with Schizophrenia
14.35-14.50	**OP 28 - Véronique Gilard** Benchtop-NMR for the detection of undeclared drugs in e-liquids and aphrodisiac honeys	**OP 29 - Onur Bender** Targeting FLT3/ITD mutation in acute myeloid leukemia: lessons from natural compounds	**OP 30 - Ummuye Nur Tuzun** Electrochemical development of poly(L-alanine)-gold nanoparticles-modified electrode for the determination and mechanism of antipsychotic drug olanzapine
14.50-15.05	**OP 31 - Demet Dincel** Therapeutic drug monitoring of the free and total valproic acid in human plasma by LC-MS/MS	**OP 32 - N.Neslihan Bozkurt** LC-MS/MS-Based Quantitative Analysis of A Tricyclic Antidepressant and Its Major Metabolite	**OP 33 - Abd Al Rahman Mohammd Faez Asfour** Investigation of New Thiazole Derivatives and their Biological Effects
15.05-16.15	**Coffee Break and Poster Session-2**		
	Session Chairs: Markus Ganzera, Martin Vogel	**Session Chairs: Daniel Quesada-González , Francesco Busardo**	**Session Chairs: Alessandra Gentili, Sezgin Bakirdere**
16.15-16.45	**KL 18 - Davy Guillarme** Innovative chromatographic strategies to improve the characterization of oligonucleotides	**KL 19 - Stig Pedersen-Bjergaad** Electromembrane extraction of peptides	**KL 20 - Bekir Salih** Current state-of-the-art Ion Mobility and Mass Spectrometry-based Analyses of Pharmaceuticals and Biomolecules
16.45-17.00	**OP 34 - Guo Jialiang** Screening of trypsin inhibitors in Cotinus coggygria Scop. extract using at-line nanofractionation coupled with semi-preparative reverse-phase liquid chromatography	**OP 35 - Jonathan Maurer** Peptide assays: an ounce of pre-analytics is worth a pound of cure	**OP 36 - Nadia Bounoua** An overview in the chiral separation of azole compounds using some analytical methods
17.00-17.15	**OP 37 - Khaldun Mohammad Al Azzam** Enhancement of Apixaban's Solubility and Dissolution Rate by Inclusion Complex (β-Cyclodextrin and Hydroxypropyl β-Cyclodextrin) and Computational Calculation of their Inclusion Complexes	**OP 38 - Joanna Bogusiewicz** The lipidomic landscape of different mutations in human brain tumors.	**OP 39 - Anil Yilmaz** Synthesis of Novel Oxime and Benzofuran Chemical Frameworks Possessing Potent Anticholinesterase Activity: A SAR Study Related to Alzheimer Disease

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17.15-17.30	**OP 40 - Zeynep Ozdemir** Quantification of Carbamazepine Profile in Human Plasma by GC-MS	**OP 41 - Mine Ensoy** Vulpinic Acid Induces Ferroptosis by Inceasing ROS Level and Changing Expression Profile of Ferroptosis Related Genes in Breast Cancer	**OP 42 - Nuran Gökdere** Green synthesis and characterization of silver nanoparticles using Pyracantha Coccinea M.J. Roem and their antibacterial, antibiofilm and anti quorum sensing activities
17.30-17.45	**OP 43 - Sara Tengattini** HILIC-MS-driven optimization of protein glycosylation via disuccinimidyl linker for pure glycoconjugate vaccine synthesis	**OP 44 - Canan Ozyurt** DEVELOPMENT of a FLUORESCENCE-BASED NUCLEIC ACID BIOSENSOR for the DETECTION of BACTERIAL PROTEASE ENZYME	**OP 45 - Mohamed Nadjib** Chiral Separation and Absolute Configuration Assignment of Racemic Bioactives Molecules

July 5, 2023			
	Hall A		
09.00-09.45	**Session Chair: Ezel Boyaci** **PL 4 - Janusz Pawlisyzn** Development of Green Analytical Devices for Clinical and Medical Applications		
09.45-10.00	Coffee Break		
	Hall A	**Hall B**	**Hall C**
	Session Chairs: Bobak Mosadegh, Maria Montes Bayon	**Session Chairs: Ellia Psillakis, Serge Rudaz**	**Session Chairs: Antonio Salgado, Lukasz Ciesla**
10.00-10.30	**KL 21 - Firat Guder** Flexible and Stretchable Sensors for Physiological Monitoring and Rehabilitation	**KL 22 - Rafael Lucena** Biopolymer sorptive phases in bioanalysis	**KL 23 - Mamas Prodromidis** Wax screen printed fabric-based colorimetric microfluidic wearable biosensor for the determination of biomarkers in sweat -
10.30-11.00	**KL 24 - Susana Campuzano** What can bioelectroanalysis do for decentralized and sustainable precision medicine?	**KL 25 - Barbara Bojko** Solid phase microextraction - new diagnostic tool in transplant surgery	**KL 26 - Jiri Homola** Plasmonic biosensors for biomedicine
11.00-11.20	**IL 11 - Esen Bellur Atici** Synthesis, analysis, risk-knowledge-data-based process & impurity evaluation of drug substances	**IL 12 - Ezel Boyaci** Recent Advances in Targeted and Untargeted Metabolomics with Solid Phase Microextraction	**IL 13 - M. Asunción Alonso-Lomillo** Sensors on pharmaceutical and biomedical electroanalysis
11.20-11.35	**OP 46 - Verónica Serafín** Electroanalytical biotools to advance food allergy at different molecular levels	**OP 47 - Leon Reubsaet** Smart sampling: next-generation DBS samplers for LC-MS based protein analysis	**OP 48 - Sineenat Siri** Colorimetric aptasensor for paraquat detection based on truncated DNA aptamer and gold nanoparticles
11.35-11.50	**OP 49 - S. Irem Kaya** Development of selective and sensitive MIP-based electrochemical sensor for Quercetin determination from Rubus sanctus, Fragaria vesca extracts and some herbal supplements	**OP 50 - Wei Zhou** Recessed solid-phase microextraction acupuncture needle for in vivo tissue sampling and direct analysis by mass spectrometry via automated microfluidic open interface	

PROGRAM

11.50-13.00	**Lunch and Vendor Seminars** **Sultan Süleyman Ökten**, Regional Sales Manager, EBSCO Information Services Publishing Open Access with American Chemical Society (ACS): Read & Publish		
	Hall A		
13.00-13.45	**Session Chair: Jean-Michel Kauffmann** **PL 5 - Gustavo Rivas** Integrating exfoliation and (bio)recognition properties in carbon nanotubes. Biosensing applications		
	Hall A	**Hall B**	**Hall C**
	Session Chairs: Can Dincer, Jean-Michel Kauffmann, Gustavo Rivas, Suna Timur, Zbigniew Brzozka, Firat Guder	**Session Chairs: Jacques Crommen, Bezhan Chankvetadze, Janusz Pawliszyn, Vladimir Ioffe, Salvatore Fanali, Davy Guillarme**	**Session Chairs: Daniel Quesada-González, Zuhre Senturk, Mamas Prodromidis, Susana Campuzano, Alberto Escarpa, Serge Rudaz**
13.45-13.55	**YS 1 - Vuslat B. Juska** Multiplexed miniaturised microfabricated silicon devices for electrochemical biosensing: from chemical to biological systems	**YS 16 - Timothy Joseph Bushman** Bacterial Espresso? Accelerated Solvent Extraction of Bacterial Cell Pellet as a Method for the Improved Extraction Recovery of Selected Metabolites	**YS 31 - Pakawat Kongpreecha** Development of colorimetric aptasensor for detection of 17β-estradiol in milk based on newly designed aptamers and gold nanoparticles
13.55-14.05	**YS 2 - Dogus Akboga** Living Biosensors with Synthetic Genetic Circuits to Monitor Nanomaterial and Heavy Metal Triggered Toxicity	**YS 17 - Ummugulsum Polat** Application of Magnetic Solid Phase Extraction for Sensitive and Simultaneous Analysis of Glipizide and Pravastatin Drugs in Synthetic Urine Samples with HPLC-DAD	**YS 32 - Kubra Turan** Development of Electrochemical Sensor Based on Glassy Carbon Electrode Modified with CeO_2/PNMA/fMWCNTs composite for Simultaneous Detection of Uric Acid and 5-Fluorouracil
14.05-14.15	**YS 3 - Tugba Nur Bozkurt** A novel decision support system based on feature extraction, feature ranking, feature selection for accurate diagnosis of diseases using medical images	**YS 18 - Tugce Unutkan Gösterisli** A liquid phase microextraction method for extraction and preconcentration of parabens in lipstick samples prior to high performance liquid chromatography – UV detection	**YS 33 - Raghad Alhardan** A novel electrochemical tyrosinase biosensor for the monitoring inhibition of Rosmaniric acid from plants
14.15-14.25	**YS 4 - Alara Karaman** Cell and Microparticle Manipulation Using Acoustic Waves in Microchannels	**YS 19 - Suleyman Bodur** Determination of chloroquine phosphate at trace levels in human serum, saliva and urine samples by gas chromatography–mass spectrometry after vortex assisted spraying based fine droplet formation liquid phase microextraction	**YS 34 - Manolya Mujgan Gurbuz** The first electrochemical sensor for the determination of Ibrutinib in human serum in the presence of anionic surfactant
14.25-14.35	**YS 5 - Orelia Cerlati** Insight into Aβ/membrane lipids/metal ion interplay in the context of Alzheimer's disease	**YS 20 - Sezin Erarpat** Determination of Vitamin B12 in vitamin tablets by high performance liquid chromatography combined with flame atomic absorption spectrophotometry with a novel nebulizer interface supported T-shaped slotted quartz tube	**YS 35 - Zeynep Caglayan Arslan** Detection of Mutations on ctDNA Samples with All-MEMS Photoelectrochemical Sensor

PROGRAM

14.35-14.45	**YS 6 - Meryem Beyza Avci** Portable handheld biosensing platform for point-of-care virus detection in field settings	**YS 21 - Cemil Can Eylem** 18O assisted GC-MS based fluxomics analysis for central carbon metabolism intermediates in colorectal cancer cell line	**YS 36 - Ulfet Erdogan Uzunoglu** Determination of Liposomal Vitamin C Using Polarography
14.45-14.55	**YS 7 - Ayse Necla Akilli** Direct Determination of Surface Proteins in Leishmania	**YS 22 - Seyma Nigiz** Molecular and metabolic characterization of multi-drug resistant isolates of Acinetobacter baumannii	**YS 37 - Pelin Senel** Spectroscopic and electrochemical studies on the interactional of purine nucleoside antimetabolite Clofarabine with double helix deoxyribonucleic acid
14.55-15.20	**Coffee Break**		
15.20-15.30	**YS 8 - Arnon Buntha** Development of fluorescent aptasensor to detect 17β-estradiol in human blood	**YS 23 - Fabian Hammerle** Sophisticated mass spectrometry-based techniques for the identification of novel mycosporine-like amino acids in marine organisms	**YS 38 - Busra Sevim** Evaluation of Thermal and Structural Stability of Pediocin PA-1 Mutants with MD Simulations
15.30-15.40	**YS 9 - Merve Calimci** High-yield and facile genomic DNA extraction method on glass fiber strip for LAMP PCR based pathogenic bacteria detection	**YS 24 - Veselina Adimcilar** Fast and Sensitive Determination of Orotic Acid in Urine Samples with Capillary Electrophoresis Coupled with Contactless Conductivity Detection	**YS 39 - Abdullah Al Faysal** Elucidation of binding interactions and mechanism of tyrosine kinase inhibitor Axitinib with dsDNA via multi-spectroscopic and voltammetric studies
15.40-15.50	**YS 10 - Nina Felli** Hyper-crosslinked β-cyclodextrin polymer as a sustainable sorbent for bioanalytical applications	**YS 25 - Hatice Demirtas** Enhancing Anticancer Potential: Optimization of Niclosamide-Loaded Lipid Nanocapsules for Improved Delivery and Quantification	**YS 40 - Sevilay Erdogan Kablan** A Validated Electrochemical Method for Quantification of Molnupiravir in Capsule Dosage Forms
15.50-16.00	**YS 11 - Nilay Kahya** Controlled release of silver sulfadiazine from CeO_2-loaded chitosan composite films as potential wound dressings	**YS 26 - Ceren H. Bozmaoglu** Solid Lipid Nanoparticles Loaded with Vemurafenib: A Promising Approach for the Treatment of Metastatic Melanoma	**YS 41 - Nilay Bilgin Saritas** Photodegradation of favipiravir and oseltamivir in the presence of microplastics
16.00-16.10	**YS 12 - Meltem Okan** An Upconverting Nanoparticle based Photoelectrochemical Sensor for the Detection of ctDNA	**YS 27 - Lorenzo Antonelli** A new nano-composite material from recycled PLA for magnetic dispersive solid-phase extraction	**YS 42 - Hayrani Eren Bostanci** The Effect of Vitamins on Oxidation in Experimentally Diabetes Conditioned Rats
16.10-16.20	**YS 13 - Semih Calamak** Colloidal Silver Nanoparticle Color Change Determination Using Digitized RGB Intensities as a Smartphone Readout for Sensing Application	**YS 28 - Nevin Ulas Colak** Two-step microwave extraction with NADES and biological activity comparison of Origanum Michrantum and Origanum Minutiflorum Plants	**YS 43 - Husyein Oguzhan Kaya** Electrochemical Biosensors for Healthcare Applications
16.20-16.30	**YS 14 - Fatma Kurul** Advancing Single Cell Growth Measurement: A Plasmonic Functional Assay Platform with Refractive Index Sensing	**YS 29 - Ayse Nur Buke** Determination of The Dissolution of Hypericin From Polymeric Nanoparticles by HPLC Analysis	**YS 44 - Marina Serin** Development of a prototype biosensor to determine the severity of demyelination due to diabetic neuropathy in serum

PROGRAM

16.30-16.40	**YS 15 - Ibrahim Gadashli** Comparison of antibody and lobaric acid small molecule application in the treatment of ovarian cancer at the transcriptome level in the IL-6R / STAT3 / PD-L1 pathway	**YS 30 - Sinem Demir** Drug release profles of Atenolol and Benidipine from pHresponsive polymeric hydrogel matrix	**YS 45 - Ozum Ozoglu** Development of N-doped Holey Graphene-based Aqueous Inks to Construct Flexible Electrochemical Sensors
17.30-18.15	**Ottoman Historical Band and Traditional Dance Show**		
19.30-22.00	**Gala Dinner**		

July 6, 2023		
	Hall A	
09.00-09.45	**Session Chair: Salvatore Fanali** **PL 6 - Maria Montes Bayon** Bioanalytical tools to evaluate the capabilities of iron oxide nanoparticles decorated with cisplatin (IV) as drug delivery systems in cell models.	
09.45-10.00	Coffee Break	
	Session Chairs: Rafael Lucena, Marcello Locatelli	**Session Chairs: M. Asunción Alonso-Lomillo, Gerhard Scriba**
	Hall A	**Hall B**
10.00-10.30	**KL 27 - Elia Psillakis** Designing for a green analytical chemistry future	**KL 28 - Alberto Escarpa** Micromotors in action: smart microsensors swimming in a concept or a futuristic reality?
10.30-11.00	**KL 29 - Caterina Temporini** Challenges and Advances in Extracellular Vesicles Analysis	**KL 30 - Suna Timur** Biofunctional Nanostructures, In vitro Diagnostics Platforms
11.00-11.15	**IL 5 – Bobak Mosadegh** Soft Robotic Sensing Array Catheters for Cardiac Arrythmias	**OP 53 - Aysegul Golcu** Investigation of binding constants of some small molecules to deoxyribonucleic acid in solution medium
11.15-11.30	**OP 52 - Erica Aparecida Souza Silva** Chloroformate derivatization combined with HS-SPME-GC/MS for determination of SCFAs in mice liver: a proof of concept	**OP 55 - Bilal Kizilelma** Carbon based electrochemical immunosensor for malignant tumors marker anti-SOX2 detection
11.30-11.45	**OP 54 - Zahirul Kabir** On the transport of an anticancer drug, leflunomide in human blood circulation as studied by multi-spectroscopic and computational investigations.	**OP 57 - Zeynep Aydogmus** Electrochemical design of two new sensors based on graphene nanoparticles and polymer resin -modified carbon paste electrodes for the determination of Tenofovir and Entecavir
11.45-12.00	**OP 58 - Kemal Cetin** Production of a hybrid structure consisting of polycaprolactone electrospun nanofiber and poly(2-hydroxyethyl methacrylate)-based cryogel for 5-fluorouracil release	**OP 59 - Elif Burcu Aydin** A label-free electrochemical magneto immunosensor for celiac disease
12.00-12.15	**OP 60 - Zeynep Kalaycioglu** The affinity of dopamine and serotonin to serum albumin: Capillary electrophoresis-frontal analysis and in-silico molecular docking approaches	**OP 61 - Ece Ozkan** Molecularly Imprinted Electrochemical Sensor For The Selective and Sensitive Determination Of Venetoclax In Pharmaceuticals and Human Serum
12.15-13.00	Lunch	

PROGRAM

	Hall A
	Session Chairs: Sibel A Ozkan, Bezhan Chankvetadze
13.00-13.20	**IL 14 - Mehmet Ozsoz** CRISPR Powered Electrochemical Biosensors
13.20-13.50	**KL 31 - Salvatore Fanali** Nano-liquid chromatography: recent advances in chiral analysis
13.50-14.20	**Closing Ceremony** **Sibel A. Ozkan, Bezhan Chankvetadze**

POSTER PROGRAM

	MONDAY (3 JULY 2023)	Authors	Presenter
PM-01	Electrochemical Nanosensor Development for the Determination of VEGFR Tyrosine Kinase Inhibitor Drug Axitinib	Ahmet Cetinkaya, S. Irem Kaya, Pelin Senel, Nejla Cini, Esen B. Atici, Sibel A. Ozkan, Mine Yurtsever, Ayşegul Golcu	Ahmet Cetinkaya
PM-02	Simultaneous determination of hydroquinone and catechol using highly conductive ketjen black screen–printed electrodes treated with electrical discharge	Mamas Prodromidis, Maria Trachioti	Mamas Prodromidis
PM-03	SARS-CoV-2 Detection By A Novel QTF Based Immunosensor	Bahar Ince, Mehmet Kavacik, Mehmet Altay Unal, Mustafa Kemal Sezginturk, Sibel Ayşil Ozkan	Bahar Ince
PM-04	Construction of a sensitive electrochemical magneto-biosensor based on functionalized magnetic particles for anti-transglutaminase antibody detection	Muhammet Aydin, Elif Burcu Aydin, Mustafa Kemal Sezginturk	Muhammet Aydin
PM-05	Impact of the solid state on the sterilization of indomethacin	Karyna Krupianskaya, Guy Van Den Mooter, Erik Haghedooren, Ann Van Schepdael	Karyna Krupianskaya
PM-07	The first report for the electrochemical investigation of a new tyrosine kinase inhibitor vandetanib: Its voltammetric determination in biological samples with the presence of anionic surfactant using a carbon paste electrode	Pinar Talay Pinar, Cihat Mete, Zuhre Senturk	Pinar Talay Pinar
PM-08	SERS based exosome detection using paper substrates	Elif Calik Kayiş, Hilal Torul, Sevda Akay Sazaklioglu, Sevda Akay Sazaklioglu, Huseyin Celikkan, Hafize Eda Vatansever, Hafize Eda Vatansever, Ugur Tamer	Elif Calik Kayiş
PM-09	Selective, and sensitive voltammetric detection of antibacterial drug linezolid using designing and fabrication of electrochemical nanosensor TiO_2 nanoparticles and MWCNT-COOH modified glassy carbon electrode in environmental samples	Nida Aydogdu, Ersin Demir, Sibel A. Ozkan	Nida Aydogdu
PM-10	Electrochemical enzymatic biosensor based on a novel conducting polymer and NH_2 functionalized quantum dots for the sensitive detection of catechol	Gulsu Keles, Raghad Alhardan, Sevki Can Cevher, Saniye Soylemez, Ali Cirpan, Levent Toppare, Sevinc Kurbanoglu	Gulsu Keles
PM-11	Disposable electrochemical sensor for the analysis of cholesterol	Olga Domínguez-Renedo, Paula Hoyuelos Manso, M. Asunción Alonso Lomillo	Olga Domínguez-Renedo
PM-13	Development of Lateral Flow Immunosensor for Detection of Growth Hormone: An ongoing study	Eda Gumus, Haluk Bingol, Erhan Zor	Eda Gumus
PM-14	Voltammetric determination of cytotoxic antineoplastic drug Cladribine	Pelin Senel, Ayşegul Golcu	Pelin Senel

POSTER PROGRAM

	MONDAY (3 JULY 2023)	Authors	Presenter
PM-15	A Systematic Protocol for GC-MS and LC-MS based Metabolomics and Lipidomics Analysis from Tissue Samples: By a Holistic Multi-Omics Optimization Approach based on Design of Experiment (DOE)	Cemil Can Eylem, Emirhan Nemutlu, Ayşegul Dogan, Vedat Acik, Selcuk Matyar, Yurdal Gezercan, Suleyman Altintaş, Ali Ihsan Okten, Nursabah Elif Başci Akduman	Cemil Can Eylem
PM-17	Development and Characterization of Mucoadhesive Buccal Film Formulations Containing Tamoxifen	Yagmur Karaman, Eylul Su Saral Acarca, Sibel Ilbasmis Tamer	Yagmur Karaman
PM-18	Chitosan Modified Filter Paper for Colorimetric Based Non-Invasive Detection of Glucose	Emine Yildirim, Tugba Tezcan, Ugur Tamer	Emine Yildirim
PM-19	Synthesis of fluorescent carbon dots from anethum graveolens by hydrothermal carbonization	Buşra Karataş, H. Eda Satana Kara	Buşra Karataş
PM-20	Investigation of Cholinesterase Inhibition, Antimicrobial Activity and Volatile Contents of { Eryngium campestre }	Merve Badem, Seyda Kanbolat, Nevin Ulas Colak, Tugba Subas, Sila Ozlem Sener, Ufuk Ozgen, Rezzan Aliyazicioglu, Sengul Alpay Karaoglu, Ali Kandemir	Merve Badem
PM-21	Synthesis, characterization and optimization of novel phenylalanine-coated copper nanoclusters with high fluorescence intensity	Mehmetcan Bilkay, Hayriye Eda Satana Kara	Mehmetcan Bilkay
PM-22	Investigation of *Asphodelus aestivus* as potential antioxidant, antityrosinase and anticollagenase agent on skin aging	Seyda Kanbolat, Merve Badem, Sila Ozlem Sener, Burcu Erdemir, Sena Sari, Ebru Ozdemir Nath	Seyda Kanbolat
PM-23	Investigation of the use of *Asphodelus fistulosus* as a dermocosmetic agent against skin aging	Merve Badem, Seyda Kanbolat, Sila Ozlem Sener, Hatice Senel, Fatma Arikan, Ebru Ozdemir Nath	Merve Badem
PM-24	A new quartz tuning fork-based immunosensor for analysis of citrullinated vimentin	Meltem Calişkan, Mehmet Altay Unal, Fikret Ari, Mustafa Kemal Sezginturk, Sibel Ayşil Ozkan	Meltem Calişkan
PM-25	Selective and sensitive determination of Tolvaptan by developing molecularly imprinted polymer-based electrochemical sensor	Fatma Budak, Leyla Karadurmus, Ahmet Cetinkaya, Ahmet Cetinkaya, Esen Bellur Atici, Sibel A. Ozkan	Fatma Budak
PM-26	A label-free electrochemical AGR2 immunosensor prepare using a hand-made disposable electrode	Melike Bilgi Kamac, Ayşenur Yilmaz Kabaca, Merve Yilmaz, Muhammed Altun, Mustafa Kemal Sezginturk	Melike Bilgi Kamac
PM-27	Preparation and characterization of MXene-AuNP and MXene-COOH-AuNP modified screen-printed carbon electrodes for use in biosensor	Merve Yilmaz, Melike Bilgi Kamac	Merve Yilmaz
PM-28	A New Drug Candidate Molecule (3-((5-methylpyridin-2-yl)amino) isobenzofuran-1(3H)-one) Synthesis, Characterization and antioxidant activity	Ayşe Daut Ozdemir, Zeynep Tanrikulu Yilmaz, Hakki Yasin Odabaşoglu, Pelin Senel, Veselina Adimcilar	Ayşe Daut Ozdemir

POSTER PROGRAM

	MONDAY (3 JULY 2023)	Authors	Presenter
PM-29	Quantitative Determination of Sunset Yellow (E-110) In Pharmaceutical Formulations by Micro Plate Spectrophotometry After Extraction with Hydrophobic Deep Eutectic Solvent	Donay Yuvali	Donay Yuvali
PM-30	Development of highly sensitive fluorescence aptasensor based on aptamer-functionalized magnetic nanoparticles for 17β-estradiol detection	Wissuta Chinnawongsuwan, Sineenat Siri	Wissuta Chinnawongsuwan
PM-31	Production of spinel ferrite nanoparticles for latent fingerprint visualization	Phadcharapon Phonna, Sineenat Siri	Phadcharapon Phonna
PM-32	New approaches to investigate the antioxidant activity and the analysis of radical reactions course. 1HNMR study	Wojciech Rogóż, Beata Morak-Młodawska, Aleksandra Owczarzy, Karolina Kulig, Małgorzata Maciążek-Jurczyk	Wojciech Rogóż
PM-33	Spectroscopic analysis of 9-amino-5-methyl-12(H)-chino[3,4-b][1,4]benzothiazine chloride interaction with potential proteins carriers. In vitro studies.	Aleksandra Owczarzy, Monika Trzepacz, Wojciech Rogóż, Karolina Kulig, Andrzej Zięba, Jadwiga Pożycka	Aleksandra Owczarzy
PM-34	Electrochemical DNA biosensor applications with newly developed electrode surfaces based on Cu-ZrO_2 nanoparticles	Elifcan Emiroglu Bolukbas, Dilsat Ozkan-Ariksoysal, Sabriye Yusan, Ikbal Gozde Kaptanoglu, Umit Huseyin Kaynar	Elifcan Emiroglu Bolukbas
PM-35	Electrochemical Characterization of Natural and Synthetic Molecules and Investigation of Their Interactions with DNA	Huseyin Oguzhan Kaya, Seda Nur Topkaya	Huseyin Oguzhan Kaya
PM-36	Evaluation of the Electrochemical Characteristics of Triazolopyrimidinone Derivatives as Novel Drugs and Determination of Their Interactions with DNA	Ceylin Bozdemir, Fatma Kurul, Huseyin Istanbullu, Seda Nur Topkaya	Ceylin Bozdemir
PM-37	Circular dichroism study of albumin nanoparticles	Karolina Kulig, Zuzanna Denisiuk, Wojciech Rogóż, Aleksandra Owczarzy, Małgorzata Maciążek-Jurczyk	Karolina Kulig
PM-38	Kidney injury molecule-1 detection by a novel mass-based biosensing system	Berfin Vural, Mehmet Altay Unal, Fikret Ari, Mustafa Kemal Sezginturk, Sibel A. Ozkan	Berfin Vural
PM-39	Production and characterization of a hand-made disposable panel electrode for use in biosensor	Melike Bilgi Kamac, Ayşenur Yilmaz Kabaca, Merve Yilmaz, Muhammed Altun, Mustafa Kemal Sezginturk	Ayşenur Yilmaz Kabaca
PM-40	Investigation of New Potential Bcl-2 Inhibitors via In Silico Approaches	Seyma Simsek, Okan Aykac, Burcin Turkmenoglu	Okan Aykac
PM-41	Selenium nanoparticle characterization and determination in β tc cells by single particle inductively coupled plasma-mass spectrometry	Tugba Nur Akbaba, Cigdem Yucel, Sibel Ilbasmiş Tamer, Orkun Alp, Nusret Ertaş	Tugba Nur Akbaba

POSTER PROGRAM

	MONDAY (3 JULY 2023)	Authors	Presenter
PM-42	Electrochemical determination of enzyme inhibitor thiram based on bimetallic nanoparticles utilizing green electroanalytical nanosensor	Murat Celik, Murat Celik, Burcin Bozal Palabiyik, Cigdem Kanbeş Dindar, Bengi Uslu	Murat Celik
PM-43	An ITO-based biosensor for the detection of Citrullinated Vimentin as an important biomarker for Rheumatoid arthritis	Ecem Uzman, Mustafa Kemal Sezgïnturk	Ecem Uzman
PM-44	Development of a novel EGFR-targeted peptide for targeted delivery of drugs in anaplastic thyroid cancer	Zehra Cagla Kahvecioglu, Samuel Vandecasteele, Marine Bougard, Charlotte Rogien, Fabrice Journe, Sophie Laurent, Sven Saussez, Carmen Burtea	Zehra Cagla Kahvecioglu
PM-46	Immuno-modulatory role of microbiota-derived postbiotics against periodontal diseases	Fadime Kiran, Hikmen Can, Hazal Kibar Demirhan	Fadime Kiran
PM-47	Weisella spp.-derived Postbiotics: A Novel Therapeutic Strategy for Dental Caries	Emine Omeroglou, Fadime Kiran	Emine Omeroglou
PM-48	Enhancement of SERS Signal Sensitivity Using Hybrid Nanoparticles	Turkan Kiral, Ismail Hakki Boyaci, Ender Yildirim, Hilal Torul, Eylul Evran, Ugur Tamer	Turkan Kiral
PM-49	Highly Selective Molecularly Imprinted Polymer-Based Electrochemical Sensor for The Determination of Pazopanib	Seyda Nur Samanci, Ahmet Cetinkaya, Nassim Doufene, Nadia Bounoua, S.Irem Kaya, Esen Bellur Atici, Sibel A. Ozkan	Seyda Nur Samanci
PM-50	ITO-PET coated electrode based biosensor system for determination of kidney injury molecule-1	Elif Ceren Ankara, Sude Aras, Burcak Demirbakan, Mustafa Kemal Sezginturk	Elif Ceren Ankara
PM-51	An ITO-PET based biosensor system for AFP early detection: process optimization studies	Alaaddin Kaya, Yagmur Yigit, Davut Karyelioglu, Burcak Demirbakan, Mustafa Kemal Sezginturk	Alaaddin Kaya
PM-52	Determination of the Therapeutic Efficacy of Atranorin Associated with the Ferroptosis Pathway in Breast Cancer at the Biochemical Assays	Mine Ensoy, Demet Cansaran Duman	Mine Ensoy
PM-53	Determination of the therapeutic efficacy of the drug candidate molecule atranorin at the transcriptomic level as an innovative strategy in the treatment of melanoma cancer	Mine Ensoy, Ibrahim Gadashli, Ayşe Hale Alkan, Demet Cansaran Duman	Ibrahim Gadashli
PM-54	Investigation of potential binders to GPR17 membrane receptor by surface plasmon resonance and grating-coupled interferometry	Gabriella Massolini, Davide Capelli, Marco Rabuffetti, Maria Pia Abbracchio, Ivano Eberini, Daniela Ubiali, Giovanna Speranza, Stefano Capaldi, Enrica Calleri	Gabriella Massolini
PM-55	First voltammetric detection of Karbutylate via different carbon-based electrode	Selva Bilge, Yusuf Osman Donar, Beyza Ozoylumlu, Samed Ergenekon, Ali Sinag	Selva Bilge
PM-56	Metal Affinity-Based Polymeric Drug Nanocarriers	Cansu Ilke Kuru, Fulden Ulucan-Karnak, Sinan Akgol	Fulden Ulucan-Karnak

POSTER PROGRAM

	MONDAY (3 JULY 2023)	Authors	Presenter
PM-57	Preparation and detailed characterization Rod-like CuO nanoparticles/waste masks carbon for voltammetric detection of pazopanib	Selva Bilge, Burcu Dogan Topal, Esen Bellur Atici, Ali Sinag, Sibel A. Ozkan	Selva Bilge
PM-58	Aripiprazole Imprinted pHEMA Cryogel Patches for Neurological Treatments	Merve Calisir, Muhammed Erkek, Nilay Bereli, Adil Denizli	Muhammed Erkek
PM-59	Electrochemical DNA biosensor design for determination of the Interaction between DNA and favipiravir drug used in the treatment of COVID-19	Fadime Mullaahmetoglu, Merve Cenikli, Rabia Ozturk, Dilsat Ozkan-Ariksoysal	Fadime Mullaahmetoglu
PM-60	*Pseudomonas aeruginosa* imprinted polydopamine@graphene-coated pencil graphite electrode for selective bacterial detection	Tunca Karasu, Neslihan Idil, Erdogan Ozgur, Lokman Uzun	Tunca Karasu
PM-61	A Comperative Controlled Release of Lycopene from Three Different Composites	Burcu Akar, Tunca Karasu, Mesut Kaplan, Hatice Kaplan Can, Lokman Uzun	Burcu Akar
PM-62	Study on Molecular Modeling of Some New Adenosine Monophosphate Activated Protein Kinase (AMPK) Activators	Meltem Unlusoy, Sumeyye Guney Kalkan	Meltem Unlusoy
PM-63	Evaluation of Dexpanthenol Bilayer Oral Films	Ozge Inal, Gulin Amasya	Ozge Inal
PM-64	Potential Binding Mechanisms Between Silk Fibroin and Sulfonamide Drugs	Makbule Beyza Sen, Hasan Tahsin Sen, Semih Calamak	Semih Calamak
PM-65	Development of lanthanide probes for mapping ROS levels in brain tissue	Fabrice Collin, Chantal Galaup, C. Fonta, R. Mauricot, Stephane Balayssac, Francois Couderc, L. Gibot, Veronique Gilard, N. Leygue, L. G Nowak, Lucie Perquis	Fabrice Collin
PM-66	Spectrophotometric Analysis of Folic Acid with Dispersive Liquid-Liquid Microextraction-Deep Eutectic Solvents (DESs) Technique	Songul Ulusoy, Remziye Aydin	Songul Ulusoy
PM-67	Amine functionalized multi-walled carbon nanotube-based electrochemical DNA biosensor for evaluation of efavirenz-DNA interaction	Burcu Dogan Topal, Manolya Mujgan Gurbuz, Havva Nur Gurbuz, Aytekin Uzunoglu, Sibel A. Ozkan	Burcu Dogan Topal
PM-68	Multicomponent (Ugi-4MCR, Passerini-3MCR) reactions for the synthesis of adamantane containing bioactive derivatives	Tinatini Bukia, Ana Goletiani	Tinatini Bukia
PM-70	Inkjet Printing of 2D/1D MXene/hCNT Nanoflowers Flexible Electrochemical Sensors for the Detection of Caffeine	Havva Nur Gurbuz, Hasan Huseyin Ipekci, Aytekin Uzunoglu	Havva Nur Gurbuz
PM-71	Accurate and Precise Determination of Pregabalin in Pharmaceutical Formulations using HPLC-UV Methodology	Kenan Can Tok, Ceren H. Bozmaoglu, Mehmet Gumustas, H. Sinan Suzen	Kenan Can Tok

POSTER PROGRAM

	MONDAY (3 JULY 2023)	Authors	Presenter
PM-73	Development of an electrochemical biosensor for the determination of lactate dehydrogenase	Aysen Gumustas, Hilal Torul, Ugur Tamer, Ender Yildirim	Aysen Gumustas
PM-74	Electrochemical behavior of Enzalutamide in the presence of Human Serum Albumin	Nurgul K Bakirhan, Fatima Chenguiti, Merzak Doulache, Sibel A Ozkan	Nurgul K Bakirhan
PM-75	Investigation of electrochemical interaction between olaparib and albumin	Nurgul K Bakirhan, Amina Keciba, Merzak Doulache, Sibel A. Ozkan	Nurgul K Bakirhan
PM-76	Development and Validation of Analytical Method for Quantitative Analysis of Plumbagin: Application to Proliposomal Formulation	Ozay Ozturk, Zerrin Sezgin Bayindir	Zerrin Sezgin Bayindir
PM-77	Electrochemical, spectrofluorimetric & molecular docking approaches to evaluate the interaction of Cinacalcet HCl with calf-thymus dsDNA	Didem Nur Unal, Cem Erkmen, Sevinc Kurbanoglu, Gokcen Eren, Bengi Uslu	Didem Nur Unal

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-01	New developments in coated blade spray-mass spectrometry for high-throughput and rapid analysis	Wei Zhou, Janusz Pawliszyn	Wei Zhou
PT-02	Vertical introducing bio-compatible solid-phase microextraction pin to mass spectrometry using probe electrospray ionization interface for high sensitivity and low matrix effect analysis	Wei Zhou, Janusz Pawliszyn	Wei Zhou
PT-03	Validated LC-MS/MS method for simultaneous quantification of KRASG12C inhibitor sotorasib and its major circulating metabolite (M24) in mouse matrices and its application in a mouse study.	Irene A. Retmana, Nancy H.C. Loos, Alfred H. Schinkel, Jos H. Beijnen, Jos H. Beijnen, Rolf W. Sparidans	Irene A. Retmana
PT-04	Modelling the enantiorecognition of structurally diverse pharmaceuticals on O-substituted polysaccharide-based stationary phases	Pieter De Gauquier, Jordy Peeters, Kenno Vanommeslaeghe, Yvan Vander Heyden, Debby Mangelings	Pieter De Gauquier
PT-05	FPSE-HPLC-UV/Vis method for the favipiravir quantification in biological matrices	Gizem Tiris, Isil Gazioglu, Kenneth G Furton, Abuzar Kabir, Miryam Perrucci, Marcello Locatelli	Marcello Locatelli
PT-06	FPSE-HPLC-PDA quantification of seven antidepressant drugs in post–mortem samples	Marcello Locatelli, Fabio Savini, Miryam Perrucci, Kenneth G Furton, Isil Gazioglu, Antonio Maria Catena, Cristian D'ovidio, Abuzar Kabir	Marcello Locatelli
PT-07	Adulteration detection of corn oil, rapeseed oil, peanut oil, and sunflower oil in Argan oil by FT-NIR and FT-MIR spectroscopy coupled to chemometrics	Meryeme El Maouardi, Mohammed Alaoui Mansouri, Kris De Braekeleer, Abdelaziz Bouklouze, Yvan Vander Heyden	Meryeme El Maouardi
PT-08	Analytical Niche Techniques in Phytochemistry – a Comparison of SFC and CE	Michael Zwerger, Stefan Schwaiger, Markus Ganzera	Michael Zwerger
PT-09	A Rapid Chiral HPLC Method for Determination of Enantiomeric Impurity of Lifitegrast	Merve Ugur, Esen Bellur Atici, Sibel A. Ozkan	Merve Ugur
PT-10	Development and validation of an HPLC method for determination of an eltrombopag precursor and its eleven related substances	Timur Demirhan, Elif Guksu, Yucel Yazar, Sibel A. Ozkan, Esen Bellur Atici	Timur Demirhan
PT-11	Development and validation of stability indicating analytical methods for determination of enzalutamide and related substances	Burcu Oktar Uzun, Pelin Kaygu, Cagan Agtaş, Esen Bellur Atici, Sibel A. Ozkan	Burcu Oktar Uzun
PT-12	Determination of hyaluronic acid concentration by using HPLC-RID	Uzeyir Dogan	Uzeyir Dogan
PT-13	On-site determination of methadone in saliva by ambient ionization mass spectrometry using a polyamide-cotton composite immobilized into a hypodermic needle	Jaime Millán Santiago, Rafael Lucena Rodríguez, María Soledad Cárdenas Aranzana	Jaime Millán Santiago

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-14	Voltammetric quantification studies of the DNA binding of the antineoplastic drug Azacitidine from injection suspension	Pelin Senel, Soykan Agar, Mine Yurtsever, Aysegul Golcu	Aysegul Golcu
PT-15	NMR-based metabolomics studies to investigate TNAP (tissue-nonspecific alkaline phosphatase) functions in brain and liver	Stéphane Balayssac, Eva Drevet Mulard, Lionel G Nowak, Anne Briolay, Laurence Bessueille, Gilles Rautureau, David Magne, Caroline Fonta, Veronique Gilard	Veronique Gilard
PT-16	A simple and rapid LC-MS/MS method development for the quantification of octreotide in cancer patients plasma samples	Elif Damla Gok Topak, Ece Ozkan, Emirhan Nemutlu, Sibel A. Ozkan, Omer Dizdar, Sercan Aksoy, Sedef Kir	Elif Damla Gok Topak
PT-17	Bioanalytical method development and validation of nifuratel in human plasma by LC-MS/MS detection and its application to a pharmacokinetic study	Ezgi Ozen	Ezgi Ozen
PT-18	Mechanism Elucidation Studies of Wound Healing Potential and Determination of HPLC Profile of *Lysimachia verticillaris* and *Lysimachia vulgaris*	Seyda Kanbolat, Merve Badem, Sila Ozlem Sener, Nuriye Korkmaz, Tugba Subas, Rezzan Aliyazicioglu, Ufuk Ozgen	Seyda Kanbolat
PT-19	Combination of the targeted metabolomic profiling and bioinformatics approach for the diagnostics of cardiovascular diseases (MetaboScan system)	Natalia Moskaleva, Kseniia Shestakova, Pavel Markin, Svetlana Appolonova	Natalia Moskaleva
PT-20	Metabolomics investigations of anti-virulence drug-induced toxicity	Mark Savitskii, Natalia Moskaleva, Svetlana Appolonova	Natalia Moskaleva
PT-21	Possible effects of gene polymorphisms on the development of Sjogren syndrome	Ulku Terzi, Ilker Ates	Ulku Terzi
PT-23	Analysis of Residue Solvent and Alcohol in Herbal Extracts by Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID)	Damla Uvez, Onur Kenan Ulutaş, Aysel Berkkan	Damla Uvez
PT-24	Automated optofluidic platform for cell counting	Meryem Beyza Avci, S. Deniz Yasar, Arif E. Cetin	S. Deniz Yasar
PT-25	A Dispersive Liquid-Liquid Microextraction Method for Therapeutic Drug Monitoring of Four Antidepressants Drug in Plasma by GC-MS	Selen Al, Aykut Kul, Olcay Sagirli	Selen Al
PT-26	A New Method for Therapeutic Drug Monitoring of Anxiolytic Drugs in Plasma by LC-MS/MS	Aykut Kul, Selen Al, Murat Ozdemir, Demet Dincel, Olcay Sagirli	Aykut Kul
PT-27	Development and validation of a fast and simple LC- MS/MS method for quantitative analysis of organophosphate flame retardants in human urine	Irem Iyigundogdu, Ayhan Ibrahim Aysal, Ismet Cok	Irem Iyigundogdu

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-28	Box–Behnken experimental design for optimizing the HPLC method to determine venetoclax in human plasma and bioanalytical method validation ICH M10 guideline	Saniye Ozcan, Abeer Elriş, Mazlum Akif Altun, Serkan Levent, Nafiz Oncu Can	Abeer Elriş
PT-29	A novel HPLC method for the quantification of selexipag in pharmaceuticals using Box-Behnken design	Saniye Ozcan, Egemen Guvenc Ogut, Serkan Levent, Nafiz Oncu Can	Egemen Guvenc OGUT
PT-30	Exploring the Performance of Cellulose Tris-3,5-Dichlorophenylcarbamate (CDCPC) as a Stationary Phase for the Chiral Electro-chromatographic Separation of Azole Antifungals	Massimo Giuseppe De Cesaris, Alessandra Gentili, Chiara Fanali, Salvatore Fanali, Bezhan Chankvetadze, Giovanni D'orazio	Massimo Giuseppe De Cesaris
PT-32	Determination of Zofenopril and Hydrochlorothiazide by Capillary Electrophoresis and High Performance Liquid Chromatography in Pharmaceutical Preparations	Buşra Ozyurek, Sadik Onur Ayranci, Arin Gul Dal Pocan	Buşra Ozyurek
PT-33	Quantitative analysis of urinary nucleosides and deoxynucleosides from bladder cancer patients before and after tumor resection	Małgorzata Artymowicz, Wiktoria Struck-Lewicka, Paweł Wiczling, Marcin Markuszewski, Michal Markuszewski, Danuta Siluk	Danuta Siluk
PT-34	Senstive HPLC-DAD Analysis of Phenobarbital by Fabric Phase Sorptive Extraction in Urine Sample	Beyza Bayam, Halil Ibrahim Ulusoy, Ummugulsum Polat, Marcello Locatelli, Songul Ulusoy, Abuzar Kabir	Beyza Bayam
PT-35	Development HPLC Method for Determination of Naproxen Sodium Drug in Urine Samples by Solid Phase Microextraction Techniques	Merve Turkel, Songul Ulusoy, Esra Durgun, Ummugulsum Polat, Halil Ibrahim Ulusoy	Merve Turkel
PT-36	Development of Sensitive and Accurate Analysis Method based on HPLC-DAD after Magnetic Solid Phase Extraction for Bisphenol A Molecule in Beverage Samples	Ayşe Berru Ulukuş, Aslihan Gurbuzer, Ummugulsum Polat, Halil Ibrahim Ulusoy, Gokhan Sarp, Erkan Yilmaz	Ayşe Berru Ulukuş
PT-37	Application of LC-MS and GC-MS techniques for determination of metabolic changes in serum and urine samples from women with polycystic ovary syndrome	Anna Rajska, Magdalena Buszewska Forajta, Szymon Macioszek, Renata Wawrzyniak, Paweł Wityk, Andrzej Berg, Agnieszka Kowalewska, Dominik Rachoń, Michał Jan Markuszewski	Anna Rajska
PT-38	A plant metabolomics approach: Evaluation of the *Momordica charantia* seed and fruit extracts	Duygu Eneş, A. Ahmet Basaran, Bilge Başak Fïdan, Sacide Altinoz, Mustafa Celebier	Duygu Eneş
PT-39	Profiling of carnitine and acylcarnitines in gliomas using solid phase microextraction (SPME) – extended studies	Magdalena Gaca-Tabaszewska, Joanna Bogusiewicz, Paulina Szeliska, Karol Jaroch, Paulina Zofia Goryńska, Krzysztof Goryński, Marcin Birski, Jacek Furtak, Dariusz Paczkowski, Marek Harat, Barbara Bojko	Magdalena Gaca-Tabaszewska

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-40	XCMS And MS-Dial for Q-TOF LC/MS Based Untargeted Metabolomics: Comparison of Data Processing Tools	Nergiz Yilmaz, Buşra Ucar, Bilge Başak Fidan, Ozan Kaplan, Incilay Suslu, Mustafa Celebïer	Nergiz Yilmaz
PT-41	Anti-Cancer Effects ofF Exopolysaccharides Derived From *Lactiplantibacillus plantarum* EIR/IF-1, Isolated From Breastfed Infant Fecal Microbiota	Emine Omeroglou, Fadime Kiran	Emine Omeroglou
PT-42	Development and Easy Applicable Sample Pre-Treatment Procedure for Sensitive Analysis Sibutramine and Fluoxetine in Adulterated Herbal Products	Aslihan Gurbuzer, Ibrahim Narin, Halil Ibrahim Ulusoy	Aslihan Gurbuzer
PT-43	Metabolomics profiling using dual stationary phase columns	Tuba Recber, Ipek Baysal, Samiye Yabanoglu Ciftci, Mehmet Gumustas, Sedef Kir, Bezhan Chankvetadze, Emirhan Nemutlu	Tuba Recber
PT-44	Development of Chromatographic Methodfor Sensitive and Reliable Analysis of Fingolimod And Citalopram Molecules	Esra Durgun, Ibrahim Narin, Halil Ibrahim Ulusoy	Esra Durgun
PT-45	An experimental search to better understand what pooled samples provide to untargeted metabolomics	Emine Koc, Nergiz Yilmaz, Bilge Başak Fidan, Ozan Kaplan, Mustafa Celebier	Emine Koc
PT-46	Could Metabolomics Shed Light on The Potential Connection Between a mRNA Vaccine and Leukemic Hematopoiesis?	Batuhan Erdogdu, Bilge Başak Fidan, Ozan Kaplan, Mustafa Celebier, Ibrahim Celalettin Haznedaroglu	Bilge Başak Fidan
PT-47	Optimization of The Novel Antiviral Drug Molnupiravir by LC-ESI-MS/MS	Yasin Dari, Demet Dincel, Erol Sener, Dilek Dogrukol-Ak	Yasin Dari
PT-48	Simultaneous determination of ruscogenin, neuruscogenin, trimebutine and parabens using HPLC	Gurkan Ozen, Emirhan Nemutlu	Gurkan Ozen
PT-49	Determination of Methylation Levels in the Promoter Region of the Soluble Epoxide Hydrolase Gene in Patients with Diabetic Retinopathy	Esma Ozmen, Seyyid Mehmet Bulut, Serpil Erşan, Kurşad Ramazan Zor, Erkut Kucuk, Ismail Abasikeleş, Durmuş Ayan, Feyyaz Aslan, Ismail Sari	Esma Ozmen
PT-50	Development and validation of dispersive liquid-liquid microextraction followed by micellar electrokinetic chromatography-tandem mass spectrometry for determination of six anticancer drugs in human plasma	Zvonimir Mlinarić, Lu Turković, Miranda Sertić	Zvonimir Mlinarić
PT-51	Light exacerbates local effects induced by pH unfolding in monoclonal antibodies	Elena Rizzotto, Ilenia Inciardi, Elisabetta De Diana, Marina Coppola, Giorgia Miolo, Patrizia Polverino De Laureto	Elena Rizzotto
PT-52	Evaluation of Extractive Probes Congruent with Thermal and Solvent Desorptions for Targeted and Untargeted Analysis	Ayşegul Seyma Kir, Kubra Kahremanoglu, Atakan Kara, Ezel Boyaci	Ayşegul Seyma Kir

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-53	Toward a better understanding of the effect of the light glucose-induced chemical modifications on the structure and biological activity of a monoclonal antibody	Elena Rizzotto, Ilenia Inciardi, Elisabetta De Diana, Luca Menilli, Marina Coppola, Giorgia Miolo, Patrizia Polverino De Laureto	Elena Rizzotto
PT-54	Untargeted Q-TOF LC/MS Metabolomics For Autism Spectrum Disorder	Buşra Ucar, Ozan Kaplan, Bilge Başak Fidan, Incilay Suslu, Dilek Unal, Cihan Aslan, Mustafa Celebier	Busra Ucar
PT-56	Development and Evaluation of HLB/PTFE Thin Film Samplers for Extraction of Selected Pesticides From Complex Samples Prior to Their GC-MS Determination	Yeliz Akpinar, Kubra Kahremanoglu, Ezel Boyaci	Yeliz Akpinar
PT-57	Phenolic and Flavonoid Compounds and Biological Activities of Teucrium brevifolium: in silico and in vitro Evaluation	Anil Yilmaz, Fatih Uckaya, Mehmet Boga, Taner Ozcan, Tuncay Dirmenci, Gulacti Topcu	Anil Yilmaz
PT-58	Controlled Release of Ibrutinib from Collagen Nanobubbles	Sena Piskin, Eda Cinar Avar, Canan Armutcu, Lokman Uzun	Sena Piskin
PT-59	Comparative evaluation of a transdermal patch form drug in the franz cell using two different types of synthetic membranes	Gizem Ersec, Hande Celebi Yatkin, Ediz Yildirim	Gizem Ersec
PT-60	Multi-mycotoxin Analysis in Food Supplements by LC-MS/MS	Engin Bayram, Serdar Colak, Dilek Cerav	Engin Bayram
PT-61	Evaluation of the Matrix Effect of Four Different Sample Matrices for 9 Neurotransmitters by Post-Column Infusion	Saniye Ozcan, Serkan Levent, Nafiz Oncu Can	Saniye Ozcan
PT-62	Alternative Analytical Method and Validation to Pharmacopeia Methods for NDMA and NDEA Related Compounds of Losartan, Valsartan and Irbesartan Drug Products	Humeyra Funda Vardar, Buşra Gulsen, Filiz Demir, Serdar Unlu	Filiz Demir
PT-63	UV/Vis spectrophotometer determination of cadmium in tap water with matrix matching calibration strategy after preconcentration with vortex assisted liquid phase microextraction	Ozan Yagmuroglu, Emine Gulhan Bakirdere, Sezgin Bakirdere	Ozan Yagmuroglu
PT-64	Capillary electrophoresis and GC-FID analysis of omega-3 fatty acids in food supplements	Lucie Perquis, Amélie Icher, Stephane Balayssac, Véronique Gillard, Ghislaine Biasini, Fabrice Collin, Francois Couderc	Fabrice Collin
PT-65	Metabolomic analysis of clinical *E. Coli* strains in urinary tract infection and urosepsis patients	Michal J. Markuszewski, Joanna Raczak Gutknecht, Paweł Wityk, Danuta Dudzik, Wiktoria Struck Lewicka, Małgorzata Waszczuk Jankowska, Beata Krawczyk, Magdalena Fordon, Ewa Sokołowska, Jacek Szypenbejl, Mariusz Siemiński	Michal J. Markuszewski

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-66	Nanostructured Lipid Carrier (NLC) Formulations of Paliperidone for Treatment of Schizophrenia	Omer Yedikaya, Gulin Amasya, Ulya Badilli, Nurten Ozdemir	Omer Yedïkaya
PT-67	Three cyclodextrins mixture in CEKC: a long travel for resolution of 8 diastereoisomers derivative, a short trip to resolution of 4 diastereoisomers and 2 enantiomers derivatives.	Gaelle Grolaux, Julie Charton, Christophe Furman, Emmanuelle Lipka	Emmanuelle Lipka
PT-68	Simultaneous Analysis of Nitrosamines in Pharmaceutical Products by Liquid Chromatography APCI-Triple Quadrupole Mass Spectrometry	Engin Bayram, Serdar Colak, Dilek Cerav	Engin Bayram
PT-69	Separation of enantiomers on novel chiral cyclic sulfoxides in high-performance liquid chromatography using cellulose-based chiral stationary phases with acetonitrile and aqueous-acetonitrile as mobile phases	Iza Matarashvili, Ani Rurua, Mariam Shanidze, Rusudan Kakava, Alessandro Volonterio, Bezhan Chankvetadze	Iza Matarashvili
PT-70	Innovating a novel HPLC method for validated assay of Quercetin in nano drug delivery systems	Aslihan Hilal Algan, Kenan Can Tok, Ayesegul Karatas	Aslihan Hilal Algan
PT-71	Some technical aspects of enantioselective high-performance liquid chromatography tandem mass spectrometry.	Lasha Giunashvili, Saba Jorbenadze, Tamar Khatiashvili, Aluda Chelidze, Alfredo Fabrizio Lo Faro, Simona Pichini, Francesco Paolo Busardo, Tivadar Farkas, Bezhan Chankvetadze	Lasha Giunashvili
PT-72	Optimization of High-Performance Liquid Chromatographic Method for Impurity Analysis in Antituberculotic Drug Isoniazid	Lali Chankvetadze, Iza Matarashvili, Ann Gogolashvili, Gerhard K.e. Scriba, Bezhan Chankvetadze	Lali Chankvetadze
PT-73	Deep eutectic solvent based microextraction method for the preconcentration/extraction of palladium from wastewater samples prior to slotted quartz tube – flame atomic absorption spectrometry measurement	Elif Seda Kocoglu, Ozge Yilmaz, Emine Gulhan Bakirdere, Sezgin Bakirdere	Elif Seda Kocoglu
PT-74	Development an Analytical Method for the Trace Determination of Rhodium in Soil Sample Matrices by Flame Atomic Absorption Spectrometry after Manganese Ferrite Nanoparticle based Dispersive Solid Phase Extraction	Hakan Serbest	Hakan Serbest
PT-75	An innovative and sensitive QTF-based panel biosensor system for the analysis of leptin: A potential biomarker for early detection of obesity.	Burcu Ozcan, Mehmet Altay Unal, Fikret Ari, Mustafa Kemal Sezginturk, Sibel A. Ozkan	Burcu Ozcan
PT-76	A novel QTF-based panel system for the detection of Aflatoxin B1	Burcak Demirbakan, Mehmet Altay Unal, Fikret Ari, Mustafa Kemal Sezginturk, Sibel A.L Ozkan	Burcak Demirbakan

POSTER PROGRAM

	TUESDAY (4 JULY 2023)	Authors	Presenter
PT-77	A novel and high-sensitive Quartz Tuning Fork based sensor system for C1-INH determination	Nur Tarimeri Koseer, Mehmet Altay Unal, Fikret Ari, Mustafa Kemal Sezginturk, Sibel A.Ozkan	Nur Tarimeri Koseer
PT-78	Comparison of Immunopurification Methods for Erythropoietins Analysis Anti-doping Control	Yeşim Somay Selbes, Kamile Elaldi, Emirhan Nemutlu	Yeşim Somay Selbes
PT-79	Integration of Thin Film Extractive Samplers with Surface Enhanced Raman Spectroscopy: Preliminary Investigation for Rapid Pesticides Determination	Silanur Sevgen, Yeliz Akpinar, Ezel Boyaci	Silanur Sevgen

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PLENARY LECTURES

PLENARY LECTURES

PL-001

Recent Challenges and Trends in Enantioselective Analysis of Chiral Drugs

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This presentation shortly summarizes the developments in the last few decades in enantioselective analysis of chiral drugs. The major emphasis is made on separation-based techniques such as gas chromatography (GC), high-performance liquid chromatography (HPLC), supercritical or subcritical fluid chromatography (SFC), nano-liquid chromatography (nano-LC), capillary electrophoresis (CE), capillary electrochromatography (CEC) and lab-on-a-chip devices. The major advantages and disadvantages of each technique will be highlighted [1]. On the other hand, trends in chiral analysis in the last few years, as well as current and future challenges in this field will be discussed. Although state-of-the art enantioselective separation techniques comfortably allow the resolution of enantiomers that was considered very challenging few decades ago, there are still many challenges in this field. First of all, our dream is to see very complex mixtures of stereoisomers separated with the highest efficiency in shortest possible time, with the lowest consumption of mobile phases and minimal impact on the environment. The potential of each technique for solving upcoming challenges is evaluated. Solving problems of the future will require a truly multidisciplinary approach for developing new chromatographic materials with superb thermodynamic and kinetic performance, chromatographic instrument pressure, flow rates and detection speed limits and what is of upmost importance, understanding of enantioselective intermolecular recognition mechanisms. This latter may allow the design of tailor-made enantioselective recognition systems on the molecular level.

Keywords: Enantioselective analysis, chiral drugs, enantioseparation, recognition

Reference:

[1.] P. Peluso, B. Chankvetadze, Application of Enantioselective Liquid Chromatography, in: S. Fanali, B. Chankvetadze, P.R. Haddad, C.F. Poole, M.-L. Riekkola (Editors), Liquid Chromatography, volume 2: Applications (3rd edn.), Chapter 24, pp. 817-866, Elsevier, 2023.

PL-002

(Hi)story of Chromatography: Monoliths

Frantisek Svec

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The first modern monolithic columns emerged nearly 35 years ago, and their advent was the first step toward their current widespread use. Although some ideas about monolithic media appeared earlier, their applicability was not well demonstrated, or they did not yield exciting results. Three groups have made major contributions to the beginning of the current success of monolithic materials. Specifically, Stellan Hjertén described highly compressed pieces of crosslinked poly(acrylamide)-based gel, we invented monolithic disks and columns of porous organic polymers, and Nobuo Tanaka finally came up with silica-based monolithic columns. While silica-based monolithic columns enabled rapid separation of small and medium-sized molecules, their organic polymer-based counterparts excelled at separating proteins and other large molecules. Later, hybrid monoliths, which combine sol-gel technology with free-radical polymerization, and click monolith production were added to the arsenal of available monolith approaches. The well-known advantages of organic polymer-based monoliths include ease of preparation, robustness, high permeability to flow, mass transport mainly by convection, and a wide variety of chemistries. Numerous new chemistries and functionalization methods have been developed to produce porous polymer monolithic columns for diverse applications, including various types of chromatography, including liquid, gas, supercritical fluid and thin-layer chromatography, electrochromatography, solid-phase extraction, microfluidics, sample preparation, enzyme immobilization, and many others, thus confirming the versatility of monoliths. For example, thin monolithic layers are attracting attention because they allow efficient protein separations with very simple means, followed by easy detection by mass spectrometry. Similarly, the use of monolithic columns in supercritical fluid chromatography expands the application space in the "green" technology arena. This presentation will follow the timeline in the development of monoliths and demonstrate several interesting but often less known applications.

Keywords: Chromatography, history, monoliths

PL-003

Trends in Analytical Chemistry

Jean Michel Kauffmann

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In the few recent years there has been a considerable interest in developing new analytical instrumentation and new methodologies for the assay of organic and inorganic compounds in various, more or less complex, samples. Major trends are directed towards the application of miniaturized instrumentation and green analytical methods allowing for selective and sensitive assays to be performed with little or no sample treatment on site and at site. This presentation will provide a global survey of recent developments in the area of instrumentation and chemical analyses with a special focus on applications in the medical and pharmaceutical domains.

Keywords: Analytical chemistry, miniaturization, green chemistry

PL-004

Development of Green Analytical Devices for Clinical and Medical Applications

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Recent world-wide efforts towards implementation of sustainable technologies will have Impact on future practice of analytical chemistry including sampling and sample preparation steps. The current sample preparation techniques used in extraction of compound of interests from complex samples, such as animal tissue involve time-consuming procedures that are not always effective at minimizing matrix interferences. These methods often involve the use of large amounts of organic solvents which lead to hazardous waste. Moreover, they lack automation and high-throughput capabilities. New developments in high throughput Solid Phase Microextraction (SPME) determinations facilitated by matrix compatible coatings and balance coverage phenomena have been demonstrated not only for GC/MS and LC/MS but also with direct MS coupling. These are critical advances that will impact the effectiveness of public medical care and protection. In this presentation, we will describe two alternative approaches for multiclass multi-residue analysis based on solid phase microextraction. The first approach is based on conventional liquid chromatography-tandem mass spectrometry methods (LC-MS/MS). The second approach is based on the emerging direct analysis MS techniques. In both approaches, the main goal is aimed at minimizing matrix effects and organic solvent use, while maximizing sample throughput. The fully automated sample preparation workflow allows for a total extraction time of less than 1 min per sample when 96 extractions are simultaneously conducted, while the direct to MS workflow allows for a total analysis time of less than 1 min per sample with screening in both negative and positive ionization modes in the Coated Blade Spray (CBS) and Microfluidics Open Interface (MOI) method. During the presentation development of micro biocompatible SPME chemical biopsy probes similar to sensor morphology will be described to facilitate direct in-vivo sampling eliminating need for tissue biopsy and therefore promptly providing chemical information required by medical team. All methods developed are characterized by excellent accuracy and precision.

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https://uwaterloo.ca/pawliszyn-group/

Keywords: Sample Preparation, SPME, chemical biopsy, medicine

[PL-005]

Integrating Exfoliation and (BIO)Recognition Properties in Carbon Nanotubes. Biosensing Applications

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The development of biosensors able to meet the current requirements of Clinical Chemistry is one of the most important challenges in the field of electrochemical sensors. Carbon nanotubes (CNTs) have demonstrated to be an excellent material to build innovative and versatile electrochemical (bio)sensing

platforms due to their unique properties.

Different functionalization schemes to reduce the strong tendency of CNTs to form bundles and to improve their compatibility with the solvent have been reported in the last decades. In this regard, we propose "smart" strategies to functionalize CNTs through the rational selection of functionalization agents that simultaneously allow the exfoliation of CNTs and provide them with particular (bio)recognition properties.

Typical examples will be discussed in this presentation, ranging from enzymatic biosensors and genosensors based on the use of glucose oxidase and calf-thymus double-stranded DNA as exfoliation agents and biorecognition elements, to different multipurpose biosensing platforms obtained by the biofunctionalization of CNTs with avidin, concanavalin A and human immunoglobulin G (IgG). The avidin that supports the nanostructures makes possible the anchorage of different biotinylated biomolecules and opens the doors to the development of a myriad of biosensors, like glucose and hydrogen peroxide enzymatic biosensors or genosensors for the extremely sensitive quantification of BRCA 1 gene and SARS-CoV-2 nucleic acids. Concanavalin A-CNTs nanohybrids allow the desing of biosensors through the specific anchoring of glycoproteins while IgG makes possible the successful exfoliation of CNTs at the same time that provides them with interestig immunoaffinity properties.

In summary, the rational selection of biomolecules to exfoliate the CNTs represents a very interesting alternative to build innovative and competitive biosensors without additional steps for the immobilization of the biorecognition layer. The versatility and the efficiency of the resulting architectures paves the way for further developments of simple, label-free, friendly and highly sensitive biosensors with multiple applications for biomarkers detection.

The authors acknowledge CONICET, ANPCyT and SECyT-UNC for the financial support.

Keywords: Electrochemıcal bıosensors, carbon nanotubes, functıonalızatıon, bıomarkers

PL-006

Bioanalytical Tools to Evaluate The Capabilities of Iron Oxide Nanoparticles Decorated With Cisplatin (IV) ss Drug Delivery Systems in Cell Models.

***Maria Montes Bayon*[1], *Lucia Gutierrez Romero*[1], *Borja Gallego Martinez*[2], *René Rodriguez González*[2], *Elisa Blanco González*[1]**

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The positive chemotherapeutic effect of cisplatin in most cancers (e.g., ovarian, prostate, etc.) is hampered by inherent and acquired drug resistance, a multifactorial and still not well characterized process. In fact, several mechanisms have been suggested to participate in conferring platinum-resistant properties to a tumor cell, such as genetic alterations in genes involved in drug uptake (and efflux), DNA repair, autophagy, apoptosis, and cell cycle control pathways [1]. In order to overcome some of these drawbacks, particularly those related to the reduced drug uptake, different combinations of cisplatin (or cisplatin precursors/prodrugs) to nanostructures that permit efficient drug uptake are being widely investigated. Among them, nanodelivery systems including metallic nanoparticles show the capability to incorporate cisplatin (IV) prodrugs. Ultrasmall iron oxide nanoparticles (<10 nm) have been loaded with cis-diamminetetrachloroplatinum (IV), a cisplatin (II) prodrug, and used as efficient nanodelivery system in cell models. Initial results regarding the cellular incorporation of this nanotransporter, by using inductively coupled plasma mass spectrometric (ICP-MS) approaches with single cell sample introduction systems revealed a significantly higher uptake with respect to cisplatin in ovarian cells (A2780). In addition, a controlled release mechanism was playing an important role in the toxicity of the nanotransporter, as observed in the platination patterns of nuclear and mitochondrial DNA.

In this presentation, we will show the extension of the work to different cell models of ovarian cancer and osteosarcoma. To address the effect of the Pt incorporation in the apoptotic cellular status, fluorescent activated cell sorting experiments will be off-line connected to SC-ICP-MS experiments. Therefore, the differences on the Pt-content of the viable/apoptotic/necrotic cells will be also illustrated in order to address possible differences in the mechanisms of actions of the different metallodrugs

assayed. In addition, the proposed nanodelivery system will be tested in novel 3-dimensional cell culture systems which more closely resemble the in vivo biology. Spheroids obtained from scaffold-free techniques including hanging drop microplates using osteosarcoma cell lines will be tested to address the platination level of the cells using the proposed nanocarrier in comparison to cisplatin. This type of experiments, will provide information on the penetration rate of the nanocarrier and should resemble, more closely, the in vivo behavior of the nanotransporter.

Keywords: Cisplatin, nanocarriers, single cell, ICP-MS

Reference:

[1.] Galluzzi, L.; Vitale, I.; Michels, J.; Brenner, C.; Szabadkai, G.; Harel-Bellan, A.; Castedo, M.; Kroemer, G. Systems biology of cisplatin resistance: Past, present and future. Cell Death Dis. 2014, 5, e1257.

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KEYNOTE LECTURES

KEYNOTE LECTURES

KL-001

Teaching the Old Dog New Tricks: Characterization of Sugammadex and its Various Complexes

Szabolcs Béni

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As the first selective reversal agent of neuromuscular blockers, Sugammadex (SGM), - an octacarboxylic acid derivative of the native γ-cyclodextrin - has become a successful cyclodextrin drug in the clinical practice. The success is attributed to the selective and practically irreversible binding of SGM with the anesthetics, avoiding most of the side effects of conventional muscle relaxants. The formation of a particularly strong host-guest complex is driven by the ionic interactions with the cyclodextrin side chains and the tight fit of the aminosteroid backbone of the neuromuscular blockers into the cyclodextrin cavity.

In order to better understand the intermolecular interactions of sugammadex, 1H NMR-pH titrations were carried out and by adapting the microscopic site-binding (cluster expansion) model the complete set of pKa values were determined for the first time. In view of its pH-dependent charge state, chiral capillary electrophoretic method was also developed for the separation of designer drug racemates. Besides, the charge-dependent interaction between remdesivir and SGM has also been characterized.

As to date there is no known antidote available for toxic glycoalkaloids abundant in nature, we have also investigated the interactions of SGM with the water-insoluble phytotoxins solasodine, solanidine, and its glycoside α-solanine, to explore the potential of SGM in new indication such as a phytotoxin antidote.

Concentration-, temperature- and pH-dependence of the complexes were assessed, further proving their exceedingly stable nature at neutral to acidic pH. The particular importance of the ionic interactions for the complex formation was also evidenced by the partial dissociation of the complex under basic conditions. Using through-space magnetic dipolar correlation methods, the structures of the complexes were identified with the positive charge of the aminosteroids oriented towards the carboxyl sidechains of SGM enabling the intermolecular ionic interactions alongside cavity inclusion.

Based on these results, we suggest that SGM has a great potential to meet new indications to expand its application as a selective antidote in the treatment of intoxication by various plant species.

Keywords: Characterization, sugammadex, cyclodextrin

KL-002

Impact of Sterilization on the Chemical Composition of a Selection of Active Pharmaceutical Ingredients

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Ensuring the sterility of life science products plays a pivotal role in the healthcare sector whereby "sterile" refers to the absence of viable microorganisms. Gamma irradiation and ethylene oxide sterilization are two commonly applied methods for the sterilization of medical devices, packaging components and Active Pharmaceutical Ingredients (APIs) of medicinal products.

Dedicated studies on the effects of sterilization processes on APIs remain limited. In this research study, five APIs, frequently used in sterile ophthalmic preparations were subjected to both gamma irradiation and ethylene oxide under different process conditions. The following APIs of GMP quality were selected: dexamethasone, aciclovir, tetracycline hydrochloride, triamcinolone and methylprednisolone. The effect of sterilization conditions on the APIs was evaluated by the assay and related substances test prescribed by the European Pharmacopoeia (Ph. Eur.). It was concluded that exposure to ethylene oxide resulted in compliance with Ph. Eur. for all APIs. While dexamethasone and methylprednisolone did not meet the requirement for the Ph. Eur. after exposure to gamma irradiation, the other three APIs did meet the requirement under the specified irradiation conditions.

Subsequent optimization of sterilization parameters via extrapolation positively influenced the compliance to the Ph. Eur. requirements.

Keywords: Sterilization, gamma irradiation, ethylene oxide, HPLC, assay, related substances

KEYNOTE LECTURES

KL-003

From Analytical to Preparative Scale in Chiral Supercritical Fluid Chromatography: Economy, Challenge, Opportunity

Emmanuelle Lipka

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In drug development, chirality is a key-point and the need to separate the different isomers, as they may have different pharmacological effects, remains. Different reviews have been recently devoted to chiral separation as a witness to this hot topic. The global market for chiral chemicals was valued at USD 39.79 billion in 2015 and is projected to expand at a compound annual growth rate of 13.67% to be USD 96.89 billion by 2023, thus it is reasonable to assume that separative methods both at the analytical and at the preparative scale, are an inescapable necessity. Supercritical fluid chromatography, particularly at the preparative scale, is view as a green technique thanks to the reduced amount of solvent used in the mobile phase replaced by non toxic, non corrosive and recyclable carbon dioxide. Next to this feature, SFC is also a powerful, versatile technique which allow to succeed in very challenging separations. In this presentation we will try to adress some answers to choose the right dimensions columns, the convenient technique or how to increase productivity.

Keywords: Chiral, metalocens, analytical method, enantioseparation

KL-004

New Trends in Quality Control: New Compendial and ICH Guidelines Outlining Quality by Design (QbD) Principles

***Sandra Furlanetto*[1], *Serena Orlandini*[1], *Giuseppe Pieraccini*[2], *Roberto Gotti*[3], *Luca Marzulo*[1], *Benedetta Pasquini*[4], *Amanda Guiraldelli*[5]**

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Quality Control (QC) is critical in the pharmaceutical industry and involves the set-up and use of reliable and robust analytical procedures (AP). The AP lifecycle includes procedure development, validation, transfer, verification, ongoing AP performance monitoring and AP maintenance. International Conference on Harmonisation (ICH) draft guidelines Q14 [1] and ICH Q2(R2) [2] describe the development and validation activities, respectively, that should be carried out during the AP lifecycle used to assess the quality of drug substances and drug products. ICH Q14 outlines the scientific principles for developing, managing, and submitting APs, while ICH Q2(R2) provides guidance on how to ensure that an AP is appropriate for its intended purpose through validation activities (drug quality assurance). USP Chapter <1220> [3] provides a framework that incorporates a holistic view of various aspects of the AP lifecycle to ensure the reliability and accuracy of APs used in pharmaceutical analysis, being driven by Analytical Quality by Design (AQbD) principles.

Although it is not mandatory to adopt AQbD when developing a new AP, the issue of ICH draft guidelines Q14 and Q2(R2) is expected to facilitate the shift from the traditional univariate AP development to the new quality paradigm based on QbD risk-based approach. The possibility to define the Method Operable Design Region (MODR) and to establish analytical control strategies for the procedure can help the industry to ensure fitness for use during the AP lifecycle. MODR may be designed by using systematic approaches for knowledge acquisition and management (e.g., Design of Experiments - DoE) and it is defined as "a combination of analytical procedure parameter ranges within which the analytical procedure performance criteria are fulfilled and the quality of the measured result is assured" [1]. Moving within the qualified MODR may not require regulatory notification, and validation may be performed at the edges of the MODR in order to assure transferability of the procedure.

AP lifecycle also consists of the Analytical Target Profile (ATP) and the use of quality risk assessment tools (e.g., DoE). The main benefits of AQbD implementation include the acquisition of an in-depth AP knowledge and the design of a more robust procedure. In this context, pharmacopeias and regulatory bodies have been encouraging the pharma and biopharma industry to implement AQbD principles to promote continuous quality improvement and help advance quality of medicines.

Keywords: Quality control, ICH, QbD

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KL-005

Analyte Complexation and Chiral Separations in Capillary Electrophoresis

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The importance of the stereochemistry of pharmaceutical drugs is well recognized as stereoisomers often differ in their pharmacological, toxicological and/or pharmacokinetic profile. Consequently, powerful analytical techniques are required in drug development and quality control allowing the accurate and sensitive determination of the stereoisomeric purity of synthetic drugs, natural products or pharmaceutical formulations. Apart from HPLC, CE has become an attractive alternative for this purpose.

In CE the chiral selector is added to the background electrolyte acting as a pseudostationary phase, which is also mobile in contrast to chromatographic methods. Consequently, two stereoselective principles contribute to stereoisomer separations, *i.e.*, the formation of transient diastereomeric complexes between analyte enantiomers and the chiral selector (also referred to as the thermodynamic or chromatographic enantioselective mechanism) as well as the motility of these complexes (electrophoretic enantioselective mechanism). Both principles can cooperate or counteract each other.

The presentation will discuss the effects of analyte complexation and mobility of the analyte-selector complexes on enantioseparations in CE using cyclodextrins as chiral selectors. The application of design of experiments (DoE) in method development for chiral drugs such as dextromethorphan will be addressed. Furthermore, the effect of the substitution pattern of cyclodextrins on enantioseparations will be presented.

Keywords: Capillary electrophoresis, cyclodextrin, complexation, enantiomer migration order

KL-006

Thermodynamics of Liquid Chromatography

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Van't Hoff plots (logarithm of the retention factor, ln k, vs. the reciprocal of absolute temperature, 1/T) are commonly used in chromatographic studies to characterize the retention mechanisms based on the determined enthalpy (ΔH°) and entropy (ΔS°) change of analyte adsorption. In reversed phase liquid chromatography, the thermodynamic parameters could help to understand the retention mechanism. In chiral chromatography, however, the conclusions drawn based on van 't Hoff plots can be deceptive because several different types of adsorption sites (enantioselective and nonselective) are present on the surface of stationary phase. The influence of heterogeneity, however, cannot be studied experimentally.

We can employ two reversed phase columns with different retention mechanisms to show that by serially coupling the columns, the obtained thermodynamic parameters are not related to the results obtained on the respective individual columns. Further and more detailed experiments suggest that the numerical molar thermodynamic values determined from the slope and intercept of the van 't Hoff plots can be erroneous because of the assumptions made compared to the original van 't Hoff equation and because of the significant influence of the chromatographic circumstances. Both experimental and theoretical studies show that a more complex thermodynamic study of retention and selectivity on any type of chromatographic stationary phase is necessary than the one offered by van 't Hoff plots. The use of the Eyring equation – that is due to the transition state theory – is planned to combine microscopic kinetic modeling with the investigation of the thermodynamic characteristics of retention and selectivity.

Experimental results and calculations will be presented for both chiral and achiral separation systems.

Keywords: Liquid chromatography, thermodynamics, Van't Hoff

KL-007

Three/four-Dimensional Chiral HPLC Analysis of Amino Acids in Mammals; Their Origins, Regulation Systems and Diagnostic Values

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D-Amino acids, the enantiomers of L-amino acids widely present in mammals, have been considered not present (at least not significant) in mammals for a long time. However, the current progress of analytical technologies enables the precise determination of trace levels of D-amino acids in biological matrices, and their physiological significance in mammals has gradually been unveiled. In the present study, multi-dimensional HPLC methods have been designed/developed and applied to the analysis of various D-amino acids in mammalian tissues and physiological fluids including human clinical samples to clarify their origins, regulation systems and diagnostic values. For the precise analysis of trace levels of D-amino acids in mammals, multi-dimensional HPLC systems combining two or three different separation modes have been designed and developed. The amino acids are normally derivatized with fluorescence reagents such as 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), and separated as fluorescent organic anions. In the first dimension, the NBD-amino acids were separated/purified by a reversed-phase column as their D plus L mixtures, and the chiral separations were carried out in the final dimension to determine the enantiomers. For the higher selective analysis, an anion-exchange column or a mixed mode column could be integrated between the reversed-phase and chiral separations. Determination of NBD-amino acids by MS or MS/MS following the multi-dimensional LC separations is also effective to perform highly selective analysis of chiral amino acids.

To clarify the origins of D-amino acids in mammals, a mouse strain lacking serine racemase (SRR-KO) was established and utilized to determine the intrinsic levels of D-Ser. In the frontal brain areas (cerebrum and hippocampus), the amounts of D-Ser decreased by the reduction of SRR activity, indicating that D-Ser present in mammalian brain is biosynthesized by SRR. Several D-amino acids were considered to be derived from intestinal bacteria, therefore, the amounts of D-amino acids in the germ-free mice were evaluated. In the plasma and urine of germ-free mice, the amounts of several D-amino acids, especially D-Ala and D-Pro, drastically decreased, indicating that these D-amino acids are intestinal bacterial origin. The intrinsic amounts of D-amino acids in mammals are also regulated by the degrading enzymes, D-amino acid oxidase (DAO) and D-aspartic acid oxidase (DDO). In the plasma of mice lacking DAO activity, the amounts of D-Ala, D-Pro, D-Leu and D-Ser significantly increased. In the plasma of mice lacking DDO activity, the amount of D-Asp significantly increased, indicating that the enzymatic regulation is also the key to control the intrinsic D-amino acid amounts in mammals. Alterations of D-amino acid levels were observed in relation to the diseases in mammals. Especially, the plasma levels of D-Ala, D-Asn, D-Pro and D-Ser clearly increased by the progression of chronic kidney disease. These results indicate that D-amino acids are present and regulated in mammals with biological significance. Multi-dimensional HPLC systems developed in the present study are useful tools for the precise determination of D-amino acids in complicated biological samples, and further studies using various biological/clinical samples are ongoing.

Keywords: D-Amino acids, chiral separation, multi-dimensional HPLC

KL-008

Cell-on-a-chip Microsystems – Alternative in Vitro Models to Study Effectiveness of Medical Therapies

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One of the main goals of cellular engineering is to develop advanced, three-dimensional (3D) cellular and tissue models that mimic the physiology of tissues in vivo [1]. Thanks to the use of microsystems, it is possible to imitate the spatial growth of cells, the complex composition of the extracellular matrix or to control intercellular interactions in laboratory conditions.

The intensive development of miniaturization, which has been ongoing for several years, has given the opportunity to use modern technological solutions in chemical and biological research. Miniature Lab-on-a-chip systems are one of the modern microfluidic technologies that enable the creation of advanced 2D and 3D cell cultures in laboratory conditions [1]. Flow-through cell culture has a significant advantage over conventional macroscopic cell culture techniques and can contribute to improving the state of knowledge in many areas of medicine, biology and chemistry.

A number of applications of cellular engineering, developed in our research group will be present within this keynote lecture, i.e. the development and testing of new drugs and the study of the effectiveness of various combinations of anticancer therapies (2), the Islet-on-chip system to create both culture of pancreatic islet cells and test pathogenic or therapeutic agents (3,4).

Keywords: Cells-on-a-chip, in-vitro, medical therapies

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KL-009

Disposable Sensors For Next-Generation Point-of-Care Diagnostics

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Disposable sensors are low-cost and easy-to-handle sensing devices for short-term or single-shot measurements. Over the last decade, they have become increasingly important for different applications, including from environmental, forensic, pharmaceutical, agricultural, and food monitoring to diagnostics, especially the point-of-care testing and wearables. In this talk, first a short introduction to disposable sensors will be given. Afterwards, a broad spectrum of different biosensing approaches for next-generation on-site testing will be presented: (i) Multiplexed on-site therapeutic drug monitoring of antibiotics from invasive and non-invasive samples toward personalized antibiotherapy, (ii) CRISPR-powered electrochemical biosensors for nucleic-acid-amplification-free, simultaneous and on-site detection of multiple RNAs and other biomolecules for COVID-19 management, (iii) wearable microfluidic immunosensing devices for lab-on-a-bird applications and beyond, (iv) low-cost electrochemical paper-based wearable sensors that can be integrated to any type of facemask for wearable and continuous monitoring of breath biochemistry and/or testing of the infectious diseases such as coronaviruses from exhaled breath, and (v) light-controlled dynamic bioassays using optogenetic switches (OptoAssays) for wash- and pump-free point-of-care diagnostics.

Keywords: Therapeutic drug monitoring, CRISPR diagnostics, wearable breath analysis, optically controllable bioassays

KL-010

Size, Charge, and N-glycosylation Variant Analysis of the Anti-COVID-19 Monoclonal Antibody Bamlanivimab by Capillary Gel Electrophoresis

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Coronavirus Disease 2019 (COVID-19) is caused by a betacoronavirus called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). The receptor-binding domain (RBD) of the spike protein expressed on the surface of the virus plays a key role in the viral entry into the host cell via the angiotensin-converting enzyme 2 (ACE2) receptor. Neutralizing monoclonal antibodies having the RBD as a target have the ability to inhibit ACE2 receptor binding, therefore, they prevent SARS-CoV-2 infection representing a promising biopharmacological strategy. Bamlanivimab is the first anti-spike neutralizing monoclonal antibody, which got an emergency use authorization from the FDA for COVID-19 treatment. Albeit, bamlanivimab is primarily a neutralizing mAb, some of its effector function related activities might play a role in its mode of action possibly affected by their N-linked carbohydrates at the conserved Fc

region, potentially influenced by the manufacturing process. Capillary gel electrophoresis methods such as SDS-CGE and cIEGF are widely accepted in the biopharmaceutical industry for the characterization of therapeutic antibodies. In this paper we introduce a capillary gel electrophoresis based workflow for i) size heterogeneity analysis to determine the presence/absence of the non-glycosylated heavy chain (NGHC) fragment (SDS-CGE); ii) capillary gel isoelectric focusing for possible N-glycosylation mediated charge heterogeneity determination, e.g., for excess sialylation and finally, iii) capillary gel electrophoresis for N-glycosylation profiling and sequencing. Our results have shown the presence of negligible amount of non-glycosylated heavy chain (NGHC), while 25% acidic charge variants were detected. Comprehensive N-glycosylation characterization revealed the occurrence of >8% core-afucosylated complex and 17% galactosylated N-linked oligosaccharides, suggesting possible ADCC effector function besides the commonly considered neutralizing mode of action.

Keywords: Monoclonal antibody, COVID-19, glycosylation

KL-011

New Chemometric-Based Strategies for Pharmaceutical and Biomedical Analysis

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The present communication aims at providing an overview of how chemometrics and, in particular, recently developed algorithms and chemometrics-based analytical strategies, can help dealing with complex problems both in the pharmaceutical and bioanalytical fields.

In the first case, the potential of the application of spectroscopic techniques in combination with different chemometric methods will be illustrated through representative examples. The relevance of these methodologies is due to the fact that spectroscopy (in particular, NIR) combined with different data analytical tools can lead to effective, high performing, fast, nondestructive, and sometimes, online methods for checking the quality of pharmaceuticals and their compliance to production and/or pharmacopeia standards. Selected examples will include the possibility of nondestructively quantifying the enantiomeric excess of APIs [1,2] or the amount of dexamethasone in pharmaceutical formulations by coupling infrared spectroscopy and chemometric calibration [3]. Moreover, the possibility of developing a quantitative structure–activity relationship (QSAR) model for the determination of gut permeability of 228 pharmacological drugs at different pH conditions (3, 5, 7.4, 9, intrinsic) will also be presented [4].

On the other hand, the role of chemometric techniques in the omic field will also be discussed. In this context, a part of the communication will be devoted to presenting and discussing a general chemometric framework for building and validating models for the omics (mostly, metabolomics) both for exploratory and predictive purposes, focusing, in particular, on the aspect of biomarker discovery. In particular, the different strategies for data processing, data integration and validation will be extensively presented and compared.

Across all the examples, the advantages of combining information from two (or more) matrices, when available, to build the final chemometric model (i.e., of multi-block data analysis or "data-fusion") will be stressed [5].

Keywords: Chemometrics, strategies, pharmaceutical, biomedical

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KL-012

Interest Oriented Mesoporous Silica Films for the Electrochemical Detection of Biologically Relevant Species

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Nanoporous silica thin films consisting of highly ordered and vertically aligned nanochannels have received considerable research interest in the past years, notably because they guarantee great molecular accessibility and fast mass transport [1-3]. With nanochannels of monodisperse diameters in the 2-3 nm range, they are also characterized by size and charge selectivity at the molecular level, giving them properties of selective permeability [4-6]. When deposited onto electrode surfaces, they are very

promising for various applications, in particular in electroanalysis and sensors [2,3,7-9].

This keynote lecture will summarize and highlight (i) the main synthesis strategies to get oriented mesoporous silica films on electrodes; (ii) their basic permselective properties and factors likely to affect them; (iii) the way to functionalize them to give the corresponding organic-inorganic hybrid membranes; (iv) their applications in the field of electrochemical analysis and sensing with emphasis on preconcentration electroanalysis [3,10], protection against (bio)fouling [10,11], electrocatalysis [12,13] and genosensing [14], with a special focus on the detection of biologically relevant molecules.

Keywords: Thin films, mesoporous silica, modified electrodes, sensors, biosensors

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KL-013

From Untargeted Metabolomics to Longitudinal Targeted Monitoring in Clinics

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Chronic kidney disease (CKD) is a growing health concern affecting millions of people worldwide, partly due to an aging population. CKD is characterized by progressive damages to kidneys that impairs their ability to function efficiently, leading to a range of complications including anemia, cardiovascular disease, and end-stage renal disease. Thanks to an untargeted metabolomic study, the first aim was to evaluate metabolic alterations related to CKD progression. As no single technique offers a holistic monitoring of all metabolites in a biofluid, the use of multiple analytical platforms was used. Reversed-phase chromatography (RPLC) and hydrophilic lipophilic interaction chromatography (HILIC) coupled to high resolution mass spectrometry (HRMS) were considered as the complementary techniques for their coverage of apolar and polar metabolites, respectively.

More than 250 annotated compounds were investigated thanks to the fusion of datasets generated from multiple platforms using an in-house database. After multivariate data analysis (network PCA), identified biomarkers whose relative plasma levels changed with the degree of CKD progression, were evidenced. Genome-scale network modeling confirmed both tryptophan metabolism and nucleotide interconversion as relevant pathways potentially associated with disease severity. Metabolites, related to these pathways, were further considered as target compounds to improve CKD prognostic. The biomarkers selection was completed by published literature evidencing stratification of CKD severity considering specific biological pathways, such as kynurenine or indole acid biosynthesis. To confirm these results and develop a longitudinal follow-up of a new cohort of patients, a straightforward internal calibration approach, allowing the absolute quantification of the pattern was then developed to be implemented as a daily approach in a clinical environment.

Clinical LC–MS/MS assays traditionally require calibration curves to be generated in the same matrix as the study sample. In the case of endogenous compound quantification, as no bank matrix exists, the multi-targeted internal calibration (MTIC), as an extension of the isotope dilution approach, offered the best possibility to design specific, sensitive, accurate, and fast solutions. The proposed strategy, that requires no calibration curve building, starts with a micro sampling collection procedure, followed by a protein precipitation with a solvent containing several stable isotope labeled (SIL) standards as internal calibrants. After evaporation and reconstitution, the sample is directly injected. The method was validated in terms of repeatability, intermediate precision, and trueness for 16 biomarkers and applied to human plasma samples. The progression of CKD in patients at different

stages of the disease was confirmed, including a clear discrimination with healthy individuals and allowing the long-term follow-up of each patients thanks to an absolute concentration of biomarkers

Keywords: Metabolomics, clinical analysis, LC-MS, targeted monitoring

KL-014

Chirality post COVID: Challenges, Adaptations, and Future Prospects

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The research and teaching landscape post-COVID has changed and will continue to change. There needs to be a playbook that can guide academics about appropriate responses. It is all about the rapid design process and the implementation of adaptive responses to the challenges higher education is facing. This presentation explores the pandemic's profound effects on Chirality research and industry, shedding light on the challenges faced, adapting strategies employed and the long-term implications. It begins with a snapshot of our own Chirality research in Australia, particularly enantioselective chromatography using chiral monoliths, disruptions faced by the sudden imposition of lockdowns, travel restrictions, departure of international students and social distancing measures. These measures led to laboratory closures, interrupted supply chains and hindered collaborations impacting workflow and scientific productivity.

Keywords: Chirality, enantioselective chromatography, monoliths, pharmaceuticals

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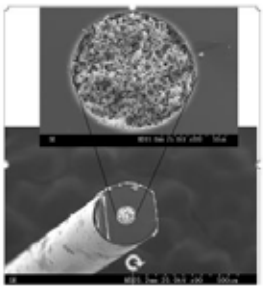

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KL-015

The Story of the 100-Year Path of Voltammetry, Starting with Drops of Mercury and Reaching the Mysterious Shores of the Brain

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Voltammetry, a method at the core of electroanalytical chemistry, investigates and evaluates the current-potential relationship obtained at a given working electrode. If the dropping mercury is used as working electrode, the analytical technique is called as polarography, which is a special branch of voltammetry. Last year, 2022 was the 100th anniversary of the first paper published by Czech Jaroslav Heyrovský on the development of polarography [1]. For this discovery, he was awarded Nobel Prize in 1959 as the first electrochemist [2]. Polarography, which dominated for about the first 50 years, is considered to be the ancestor of today's modern voltammetric techniques [3]. Later, depending on the important developments in computer technologies and microelectronics, the design and use of new electrochemical devices has created a revolution in voltammetry. The first steps forward began with the transition from the dropping mercury electrode to solid electrodes. Later, the use of various electrode modifications made it possible to detect analytes even at sub-nanomolar concentrations [4,5]. In addition to these developments, voltammetry has started to be used as a very powerful tool in neuroscience to solve the mystery of the brain since the redox (oxidation/reduction) process is very basic for living organisms [6,7]. In light of the above information, this presentation will focus on the story of the 100-year path of voltammetry from the drop of mercury to the mysterious shores of the brain.

Keywords: Polarography, voltammetry, history, application in brain research

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KL-016

Electrochemistry/mass Spectrometry – A Versatile Tool to Mimic the Metabolism of Drug Substances

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The elucidation of the metabolic fate of drug candidates in the human body is one of the major challenges in pharmaceutical research. The main route of drug elimination is an enzymatic biotransformation. These are frequently initiated by oxidation reactions ("phase I metabolism"), which are catalyzed by enzymes of the cytochrome P450 superfamily. With a strongly increasing number of novel chemical entities in recent years, rapid screening techniques that provide both reliable and easily accessible information about the biotransformation of a drug candidate are required. This is particularly valid for reactive metabolites, which are important to assess possible liver toxicity of new drug candidates. Therefore, the development of new methods for rapid screening of drug candidates in early phases is required.

Electrochemistry (EC) is one of the classical methods to induce oxidation reactions. Typical cells for electrochemistry coupled to mass spectrometry are amperometric and coulometric cells, both of which are associated with different advantages and disadvantages.

It seems obvious to employ EC as simulation technique in drug metabolism studies. Electrochemical flow-through cells coupled on-line to analysis techniques such as electrospray mass spectrometry with or without previous liquid chromatographic separation have nowadays become the technique of choice for these simulation experiments, since they may provide exhaustive information about the nature of the electrochemically generated metabolites.

The detection of reactive metabolites using conventional in vivo and in vitro techniques is hampered because the intermediately formed reactive species are prone to covalent binding to cellular macromolecules. In contrast, the on-line coupling of an electrochemical reactor with liquid chromatography/mass spectrometry (EC/LC/MS) allows the direct detection of reactive metabolites of pharmaceuticals, which are all known for readily binding to cellular macromolecules after metabolization by cytochrome P450 enzymes.

The respective experimental approaches, applications and possible future trends are presented in this work. Particular focus is directed to the electrochemical cells with different dimensions and working electrode materials and to further coupling reactions of the obtained reactive metabolites with trapping agents as glutathione or with biomacromolecules.

Keywords: Electrochemistry /mass spectrometry, metabolism, liquid chromatography, drug analysis

KL-017

Rapid Purification and Separation of Antibody Drugs Based on Epitope-Mimetic Peptide Recognition Technology

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Rapid purification and separation of antibody drugs are essential for their drug development, production, therapeutic drug monitoring as well as in vivo biotransformation analysis. How to effectively recognize and enrich target antibodies from complex biological matrices and eliminate the interference from mismatched impurities or endogenous proteins remain a major challenge. In response to these urgent needs, we developed a series of approaches based on epitope-mimetic peptide recognition strategy. For example, a variety of epitope-mimetic peptide modified organic polymeric monoliths were designed to specifically recognize Trastuzumab, Rituximab and

Infliximab in cell culture media or plasma. In order to further improve its affinity for target antibody, a dual-site recognition technique was developed by introducing two specific ligands modified nanocomposites, *i.e.* an epitope-mimetic peptide and an aptamer. The proposed dual-sites binding materials can recognize different key sites in the variable region of Trastuzumab, offering better selectivity and adsorption capacity. For realizing high-throughput and high-speed purification, epitope-mimetic peptide modified magnetic membrane roll column or membrane were also developed for different purpose. The former can combine with online automated protein purification system to reduce the burden of lab-scale antibody purification, while the latter can significantly speed up the pretreatment of biologic samples.

Keywords: Antibody drugs, epitope peptide, affinity purification, monolith

KL-018

Innovative Chromatographic Strategies to Improve the Characterization of Oligonucleotides

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The emergence of new DNA or RNA-based therapies is opening up new perspectives for the treatment of genetic diseases. Among them, therapeutic oligonucleotides are enjoying growing success due to their high specificity for their target and their improved pharmacokinetic properties. Thus, although considered by regulatory agencies as small molecules, they also share characteristics with therapeutic proteins. Oligonucleotides are therefore a new class of pharmaceutical compounds requiring specific considerations.

In order to ensure the safety and efficacy of these new therapeutic molecules, their characterisation is essential and requires adapted and robust analytical methods. Reverse phase liquid chromatography with added ion pairing agents (IP-RPLC) is the reference method for the analysis of oligonucleotides, and HILIC is gaining in popularity.

In the present work, various strategies will be exposed to improve sensitivity, throughput, and selectivity when analyzing therapeutic oligonucleotides in IP-RPLC and HILIC modes. The goal of this presentation will be to highlight i) the importance of bioinert columns to limit adsorption of oligonucleotides, ii) the interest to use alternative column chemistries to improve selectivity, iii) the possibility to work with ultra-short columns of only a few mm to achieve high throughput separations, and iv) the use of pressure as an additional parameter to tune selectivity.

Keywords: Oligonucleotides, ion pairing reversed phase, ultra-short columns, adsorption, HILIC

KL-019

Electromembrane Extraction of Peptides

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Electromembrane extraction (EME) is a green microextraction technique for acids, bases, and ionic analytes [1]. Extraction is accomplished across a liquid membrane and into aqueous acceptor under the influence of an electrical field. The liquid membrane comprises 2-10 µL of organic solvent (membrane solvent), which is immobilized in the pores of a polymeric support membrane. The acceptor is an aqueous buffer. EME is unique in the way that the mass transfer is controlled by the electrical field. For extraction of bases, the negative electrode is placed in the acceptor, while the positive electrode is in the sample. Sample and acceptor are neutral or acidic, and basic analytes are extracted as cations. Acids are extracted as anions, the electrical field is reversed, and sample and acceptor are kept neutral or alkaline.

EME provides high selectivity, and this is controlled by the chemical properties of the liquid membrane, by the direction and magnitude of the electrical field, and by pH. Sample volumes can range from 0.05-10 mL, and EME may provide substantial pre-concentration. Since acceptors are aqueous, they can be injected directly in LC-MS. Finally, the consumption of organic solvents and chemicals are extremely low, and EME is a green approach to sample preparation. EME was commercialized recently [2], and it has potential for automation and high-throughput analysis.

This presentation will focus on EME of peptides. Because most peptides are relatively polar species with multiple charges, transfer across the liquid membrane requires the presence of an ionic carrier. Extraction efficiency is increasing with the concentration of ionic carrier in the liquid membrane, but systems with high concentration are unstable due to electrolysis. Therefore, the chemical composition of the liquid membrane has to be carefully balanced. The presentation will discuss the fundamentals of EME, and recent progress in our laboratory related to extraction of peptides.

Keywords: Sample preparation, microextraction, electromembrane extraction, peptides

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KL-020

Current state-of-the-art Ion Mobility and Mass Spectrometry-based Analyses of Pharmaceuticals and Biomolecules

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In the biological applications such as the analysis of peptides, proteins, oligonucleotides, biomolecule complexes, pharmaceuticals and post-translational modifications mainly in proteomics, soft ionization mass spectrometric techniques Matrix-assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS) and Electrospray Ionization-Mass Spectrometry (ESI-MS) have been used frequently in recent years. However, the Ion Mobility-Mass Spectrometry (IM-MS) technique provides detailed information on ions' size, shape, and conformational properties in addition to conventional mass spectrometric data. Mass spectrometry is one of the key analytical technologies on which the emerging "-omics" approaches are based. It may provide detection and quantization of thousands of proteins and biologically active metabolites from a tissue, body fluid or cell culture working in a "global" or "targeted" manner, down to ultra-trace levels. In many configurations, additional tandem MS analyses (MS/MS) are feasible. In MS/MS mode, one mass analyzer is used to select a single ion that is subsequently transferred into a collision cell, where it collides with gas molecules such as argon (e.g., collision-induced dissociation, CID) causing the ion fragmentation. The multiple fragment ions are then analyzed in the second-stage mass analyzer enabling information on structural features of the parent ion purposes such as drug discovery, diagnostics and bio-analyses.

The rising performance of MS technologies is expected to bear more fruit in the request for a better understanding of human diseases, leading to new molecular biomarkers, hence affecting drug targets and therapies. Omics based MS methods especially for proteins offering both top-down and bottom-up approaches enable analysis of large biomolecules such as biotherapeutics and/or their targets to determine primary and higher order structure. This is essential for determining the structure function relationship of proteins and the mechanism of action of their related drugs. Coupled MS/MS is especially useful for this, as it utilizes the information provided by sequential fragmentations to give insight into higher order structure. Monoclonal antibodies (mAbs) are types of protein biotherapeutics that can be analyzed and characterized by these techniques during development and industrial manufacturing.

Protein-based biopharmaceuticals generally have much more complex structural features than small molecule drugs. These proteins have a Y-shaped structure and have a molecular weight of about 150 kDa. Four polypeptide chains in its structure are connected by disulfide bonds from the hinge regions, inside and outside the chain. In addition to its advantages such as high sensitivity, accuracy, analysis speed, and low sample consumption compared to other analytical methods, mass spectrometry can provide detailed data on the properties of mAbs such as structural features, post-translational modifications, and precise molecular mass determination during their production steps. IM-MS technique provides detailed conformational properties, besides conventional mass spectrometric analysis data. In this communication, MALDI-TOF/TOF-MS/MS, High Resolution LC-ESI-MS/MS and TIMS-TOF-MS results will discuss for the analyses of biomolecules and mAb-based biopharmaceutical drugs.

Acknowledgment: This study is supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) with the project number: 120Z532.

Keywords: Ion mobility mass spectrometry, pharmaceuticals, biomolecules

KL-021

Flexible and Stretchable Sensors for Physiological Monitoring and Rehabilitation

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Flexible and stretchable devices can conform to the body, extent and shrink with it natural movements, making them uniquely suited for a large range of application in healthcare. In this

lecture, our latest work in wearables, and stretchable devices will be presented including textile based sensors, polymer composites and their applications in health monitoring and rehabilitation.

Keywords: Sensor, monitoring, flexible

KL-022

Biopolymer Sorptive Phases in Bioanalysis

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Simplification and miniaturization have been two main driving forces in the development of sample preparation. Although both aspects were initially proposed due to their undisputed positive effect on analytical performance, their implementation has permitted the development of greener approaches. In other words, sustainability has underlain the evolution of sample preparation in the last years. Returning to nature on the development of new sorptive material (SM) is an interesting strategy in this field for many reasons. On the one hand, there are myriads of natural resources that can inspire the design of new functional materials, increasing the versatility of the approaches. On the other hand, using natural substances reduce the environmental impact of synthetic approaches. In this communication, the role that biopolymers may play in preparing novel and effective SM in sample preparation will be discussed, emphasizing the applications in the bioanalytical field. The term "biopolymer" is wide enough to involve substances of different nature including, for example, polysaccharides, proteins, or DNA. All of them have been proposed as sorptive phases or substrates in sample preparation.

The abundance of (ligno)cellulosic materials makes them especially attractive since the resulting SM, as long as the synthetic approaches remain simple, are globally affordable. Paper, cotton, wood, and cork are examples of these materials extensively used in sample preparation. These materials can be used raw, but they can be easily modified to boost specific interactions with the analytes. Also, their different shapes (planar, fibrous, or tips) permit the development of different extraction and instrumental workflows. In this communication, the synthesis of SM based on (ligno)cellulosic substances will be discussed in detail to provide a general overview of their potential application. The different extraction workflows, most of them pretending a high sample throughput, will be discussed, and the simplification of the analytical procedure by the direct combination of SM with instrumental techniques (both spectroscopic and spectrometric) will be considered.

In the last two years, our group has also scrutinized the potential of polydopamine as a sorptive phase. This bioinspired polymer, able to interact with different analytes, can be easily deposited over different surfaces (stainless steel, cellulose). Polydopamine-based sorptive phases have been integrated into electrospray emitters for the direct combination of sample preparation and mass spectrometry. This combination will be also outlined.

Keywords: Natural materials, sorptive phases, polymers

KL-023

Wax Screen Printed Fabric-Based Colorimetric Microfluidic Wearable Biosensor for the Determination of Biomarkers in Sweat

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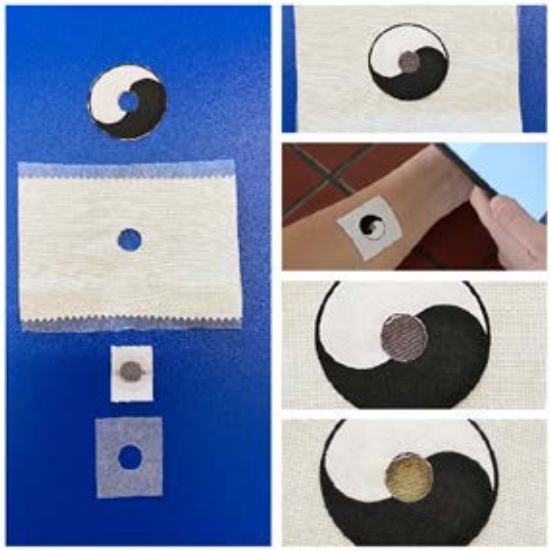
Wearable sensors for healthcare monitoring offer a non-invasive, real-time analysis of biological samples. Fabric (cloth) is a very promising material for the development of wearable (bio)sensors due to its low cost, ability to transport fluid via capillary forces, flexibility, high tensile strength and durability, and biocompatibility. Hence, cloth is an ideal material for the development of economical and user-friendly diagnostic devices. The fabrication of cloth-based microfluidics has been implemented with various methods, such as weaving, wax-transfer printing, manual rubbering with solid wax through a screen, paper-aided wax printing, photolithography and stitching.

Here, we present the formulation of a screen-printing compatible wax-based thixotropic ink and the fabrication of low-cost microfluidics based ink on a 95:5 cotton:elastane cloth with an automatic, high throughput screen printing technique.

We have developed colorimetric wearable fabric-based (bio) sensors for the determination of different biomarkers in sweat. Sweat chloride, urea and pH are essential biomarkers, since they constitute indicators for cystic fibrosis, kidneys' malfunction, and dehydration. Research on the wax-based ink composition was accomplished by preparing inks in different solvents, thixotropic

polymer solutions, and wax types and quantities.



Sweat analytes were determined taken as a measure the change in the RGB color intensity and the L*a*b* color coordinate system of the assay zones. The proposed colorimetric procedure for the determination of chloride ions depends on the chemical reaction between the chloride ions and silver chromate that causes the discoloration of silver chromate. The detection range was 20-80 mmol/L chloride which covers the normal range of chloride ions in sweat. For the determination of urea and pH, a shared approach was followed, based on the color change of a pH indicators blend. In the case of urea, enzymatically produced ammonia, due to the hydrolysis of urea in presence of urease, causes pH changes that relate linearly with the concentration of urea in sweat. The color was measured by using the mobile app ColorGrab, that obtains live responses from the RGB channels, or by the color analysis of images obtained by a scanner or a mobile phone using the open access software ImageJ.

Our future goal is to expand the application of our fabric-based wearable biosensors at other important biomarkers, thus enabling health experts and untrained users to monitor and manage health issues in a facile way outside the laboratory.

Keywords: Fabric microfluidic analytical devices, point of care, biosensors, medical diagnostics

KL-024

What Can Bioelectroanalysis Do for Decentralized and Sustainable Precision Medicine?

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In the era in which we look for personalization in material things, consumer products, movie services, music, etc., it is no wonder that the quest for individualization in medicine, comprising both the diagnosis and monitoring of diseases, as well as the applied therapy, is also gaining momentum.

This, coupled with the unsettling context in which we currently find ourselves, beginning to see the light after the COVID pandemic, and with Brexit, the Russian invasion of Ukraine and the economic recession as a backdrop, make it more important than ever to reimagine research and implementation in our society of precision medicine, which will guarantee us all a longer and higher quality life, a more rational use of society's resources and the right and opportunity to participate in our own self-care.

And this will undoubtedly be driven by the identification of biomarkers of different omic levels as well as by the development of disruptive technologies, both for their multidetermination and for analysing their clinical potential, that are affordable, easy to use, versatile in design, use and application, sustainable, and capable of providing answers in decentralized environments in the shortest possible time.

The unprecedented evolution of electrochemical bioplatforms in recent years, which we have closely followed and to which we have been fortunate enough to contribute to, has positioned them as very promising technologies capable of meeting many of these challenging demands.

With all this in mind, this lecture will present the most remarkable opportunities offered by selected electroanalytical biotools, recently proposed by our research group and collaborators, which have shown pioneering applications to investigate and implement cancer, Alzheimer, and COVID-19 precision medicine by interrogating candidate molecular markers at different omic levels in clinical specimens or identifying and confirming the diagnostic value of new molecular signatures of

autoantibodies.

In particular, the most relevant aspects of disposable bioplatforms for single or multiplex detection, developed both in integrated formats or assisted by the use of magnetic microcarriers, and exploiting ingenious assay formats and amplification strategies easily implementable in any environment for the determination of miRNAs, methylations, and single-point mutations in nucleic acids, and antibodies or autoantibodies against antigens of different nature identified by proteomics (viral, circulating or exosomal tumor antigens, peptides and proteoforms) and produced by attractive technologies (HaloTag, phage display and directed mutation) will be critically discussed. All these bioplatforms demonstrate compatibility with translation to the clinic in terms of simplicity, sensitivity and reliability, competitiveness with available methodologies in terms of sustainability, multiplexed and/or multiomic character and applicability in any environment and by any user, and have successfully dealt with the analysis of samples from selected patient cohorts.

Keywords: Electroanalytical biotools, precision medicine, muktiplexed, multiomics, sustainability.

KL-025

Solid Phase Microextraction - New Diagnostic Tool in Transplant Surgery

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An ongoing organ shortage has led to rapid growth in the number of patients on organs waiting lists. The major contributing factor to organ shortage is impaired donor tissue quality due to the aging of the population and pre-existing diseases. The suboptimal grafts are particularly susceptible to ischemia-reperfusion injury (IRI). To enable utilization of these graft novel preservation methods are under development or undergo clinical trials. Among them, machine perfusion at hypo- or normothermia (HMP and NMP, respectively) is showing superior effects over the standard static cold storage (SCS). Still, in-depth knowledge on biochemical changes occurring in the grafts during these procedures as well as after organ transplantation is missing in order to understand short and long term clinical outcomes associated with particular preservation strategy and time of storage. A parallel problem is lack of accurate methods of assessing graft quality and estimating donor risk. The decision is mainly based on the donor's medical history, visual assessment, examination results and pretransplant biopsies in some cases.

As very minimum invasive sampling method, SPME was used for temporal metabolomic profiling of kidney grafts. The sampling was performed in three porcine autotransplantation models of renal donation after circulatory death (DCD) using different preservation strategies: 8-hour SCS group, 8-hour NMP group, and 8-hour HMP group. SPME sampling was performed in vivo prior to kidney procurement; after 1 h and 2 h of warm ischemia; after 1 h, 3 h, 5 h, and 7 h of perfusion; in vivo immediately after reperfusion, and in vivo on postoperative day 3. The results of comprehensive metabolomic and lipidomic LC-HRMS analysis revealed that preservation temperature has more pronounced impact on the profile of kidney than the preservation method's mechanical characteristics. However, alterations of some metabolites suggest that hypothermia might be associated with IRI, mitochondrial dysfunction, pro-inflammatory effect, and oxidative stress.

Another application of SPME was focused on evaluation of liver metabolism and biliary tract function depending on the organ preservation method and degree of warm ischemia time. The analyses were performed in high-throughput manner using thin film microextraction protocol on bile samples collected from porcine model donors with mild (heart beating donor, HBD) and moderate warm ischemia (DCD). The grafts were subjected to static cold storage or normothermic ex vivo liver perfusion before transplantation. As previously, number of metabolites discriminating particular preservation methods and characterizing alterations in subsequent donor types (degree of ischemia) were identified. Bile acids were recognized as compounds of particular importance and potential biomarkers of monitoring liver graft function.

In conclusion, SPME coupled to LC-HRMS and LC-MS/MS enabled temporal profiling of kidney and bile metabolome and lipidome, as well as identification of biochemical pathways altered by preservation conditions and time. These proposed strategies can fill the gap in diagnostic portfolio and significantly improve the process of decision making about transplantation or rejection of given donor graft.

Acknowledgment: The studies were funded by National Science Centre, grants 2017/27/B/NZ5/01013 (kidney study) and 2021/41/B/NZ5/00860 (liver/bile study).

Keywords: Solid phase microextraction, transplantation, graft assessment, metabolomics, lipidomics

KL-026

Plasmonic Biosensors for Biomedicine

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Optical biosensors hold promise for applications in numerous important areas, such as molecular biology, medical diagnosis, environmental monitoring, food safety, and security. Surface plasmons are special modes of electromagnetic field that can be excited at the metal-dielectric interface and allow for high confinement of the electromagnetic field at the metal surface. Affinity biosensors based on optically excited surface plasmons (often referred to as plasmonic biosensors) represent the most advanced optical label-free biosensor technology. In the past two decades, plasmonic affinity biosensors have become the main tool for the real-time investigation of biomolecular interactions. Plasmonic biosensors have also been researched for the detection of chemical and biological species. However, their penetration in clinical applications has remained rather limited [1, 2].

In this presentation, we introduce plasmonic affinity biosensors, discuss the main challenges in the development of plasmonic biosensors for medical diagnostics and present selected advances in plasmonic biosensor research that aim to address some of these challenges. In particular, we cover advances in plasmonic nanostructures, sensor instrumentation, transport of target molecules in microfluidic systems, functional coatings, and assays for the detection of analytes in complex biological media. Examples of medical applications of advanced plasmonic biosensors are also presented. The first example is related to the development of plasmonic biosensors for the diagnosis of Myelodysplastic syndromes (MDS - a group of hematological malignancies with a risk of progression into acute myeloid leukemia). In particular, a new approach to the detection of MDS-related microribonucleic acids (miRNAs) is described and demonstrated to be able to detect miRNAs in blood plasma at physiologically relevant (sub-fM) concentrations [3]. The second example describes the use of a plasmonic biosensor to quantify interactions between selected MDS-related proteins immobilized on the surface of the plasmonic imaging sensor and blood plasma and shows that this interactomic approach can help discriminate among different MDS subgroups and healthy donors. The third example is concerned with the biosensor-based study of the role of pregnancy associated plasma protein A2 (PAPP-A2) in prognosis of patients with renal system disorder.

Keywords: Plasmonic, biosensors, medicine

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KL-027

Designing for a Green Analytical Chemistry Future

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The basis of a sustainable analytical laboratory depends on analytical instruments, processes, materials, and solvents that are designed following principles that make them conducive to life. Important inherent properties of processes applied as well as materials, solvents and instruments used, need to be considered from the earliest stage of design to address whether they are depleting versus renewable, toxic versus benign, or persistent versus readily degradable. Products, consumables, devices and instruments will need to integrate the principles of green analytical chemistry [1] and green sample preparation [2] under an expanded definition of performance that includes sustainability considerations. This transformation will require the best of the traditions of science and innovation coupled with new emerging systems thinking and systems design that begins at the molecular level and results in a positive impact on the global scale.

Keywords: Green analytical chemistry, green sample preparation, sustainable analytical chemistry

Lierature:

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KL-028

Micromotors in Action: Smart Microsensors Swimming in a Concept or a Futuristic Reality?

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Micromotors represent one of the most exciting horizons in micro and nanotechnologies. The utilization of self-propelled micromotors in (bio)chemical assays has led to a fundamentally new approach where their continuous movement around the sample and the mixing associated effect, all this as a collective behavior, greatly enhances the target-receptor interactions and hence the performance of the bioassay [1-3].

In our lab, we are focusing on the design and development of micromotors which are constituted by (nanostructured) layers (tubular-based shape) and particles (Janus-based shape) that confer them self-propulsion using (photo)-catalytic propulsion and magnetic guidance with compatibility in biological media due its tremendous significance, as it has been critically reviewed [4-6]. They also smartly incorporate nanomaterials and molecular recognition-based functionalization to obtain sensitivity and exquisite selectivity on board using electrochemical and fluorescence detection approaches. Also, we have explored the coupling of micromotors even with electrochemical microfluidics. In our experience, we humbly found that micromotor technology is an attractive alternative to performing fast, reliable bioassays and diagnostic testing, especially when an extremely low volume of samples is available, or the analysis must be performed in a micro-size environment.

This Keynote will discuss selected micromotors-based bioassays with potential in diagnostics and some future directions. But ultimately, we try to answer the talk title's central and disturbing question.

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Keywords: Micromotors, microsensors, bioassay

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KL-029

Challenges and Advances in Extracellular Vesicles Analysis

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Extracellular vesicles (EVs) are membrane-derived nanoscale vesicles released from cells into the extracellular space, with important roles in cell-cell communication involved in regulating several biological processes. EVs are formed by a lipid bilayer decorated with marker proteins, and can carry proteins, RNA and cofactors. Their molecular composition is representative of the cells from which they originated, for this reason EVs have a high potential as disease markers. In addition EVs represent a new frontier in biotechnological therapy and administered as drugs in what are called "cell free therapies", but also as carriers of synthetic drugs loaded into them, then used as nanoparticle systems for the drug delivery, thanks to their innate biocompatibility and bioavailability.

Comprehensive understanding of the physiological and pathological functions of EVs requires enormous efforts in purification, characterization, visualization and content determination.

Their characterization involves: (1) isolation of specific EV populations with high efficiency and purity, using methods that preserve their integrity; (2) complete characterization of size, morphology and/or specific markers with low sample consumption; (3) identification of content, even in individual subpopulations.

Given the structural complexity of these sub-cellular entities, the analytical platforms dedicated to their characterization employ complementary techniques, including different separation

approaches (dimensional and affinity chromatography, capillary electrophoresis), spectroscopy, spectrometry, microscopy and microfluidics. These methods have specific characteristics and intrinsic criticalities, which make them difficult to be exhaustive if applied isolated. Indeed, the complete characterization of these molecular entities requires the combination of multiple analytical approaches, and increasingly new analytical techniques have been used and are being developed to overcome these challenges.

The first part of this presentation will be dedicated to overview both well established and innovative, complementary analytical methods for the characterization of EVs, with special focus on EV-based therapeutic products and specifically derived biopharmaceuticals.

In the second part of the lecture, a case study will be described where samples deriving from cell supernatants obtained from healthy human cell lines will be characterized with a combination of complementary analytical approaches. EVs and soluble proteins have been characterized to explore their potential use in therapy and rationally design a recombinant bioproduct.

Keywords: extracellular vesicles, biologicals, analytical characterization

KL-030

Biofunctional Nanostructures, In vitro Diagnostics Platforms

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Healthcare technology has recently been converting from centralized medical laboratories or hospitals to point-of-care (POC) diagnostic devices together with the advancements in micro- and nanotechnologies as well as cloud computing. The need for a continuous, real-time monitoring of specific health conditions motivates the reduction of the gap between the available healthcare facilities and the demand, particularly in developing countries due to inadequate healthcare budgets [1, 2]. POC diagnostic platforms have the following properties such as affordable, sensitive and specific, user-friendly, rapid and robust, equipment-free, and deliverable to those in need, namely 'ASSURRED' technologies [3]. Compared to controlled laboratory-based techniques, POC gives rapid and accurate results without being costly, thus, it is preferable especially in resource-limited areas [4]. Nanoparticles can be surface-modified and mostly coloured, due to surface plasmon resonance properties, to provide visibility, practicality, and efficiency in colorimetric analysis [5]. They can be also used as a signal enhancer molecule in electrochemical measurements [6] and using a proper surface modification, these structures could become carriers, targeting agents, or detection interfaces on different sensing platforms [7]. Due to the lack of integration and automation of the designs, most of the POC designs has either not been commercialized or widely established in the market. Both to automate and integrate the POC diagnostics to medical world, smartphone technology emerges as the obvious choice. There emerges a term 'mobile health (mHealth)' which aims to provide an immediate resource for clinical decision by the healthcare professionals, prescription information and other medical treatments for a better personalized healthcare [8, 9].

Keywords: *In vitro* diagnostic, Point of Care diagnostic (POC), nanoparticles, smartphone sensing

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KL-031

Nano-Liquid Chromatography: Recent Advances in Chiral Analysis

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The separation and analysis of chiral compounds is an important topic in various research and application fields such as biomedicine, agrochemical and food chemistry, the pharmaceutical industry, drug analysis, forensics, etc. The chirality originates from, e.g., the presence of one or more asymmetric centers in the compound's structure. Enantiomers possess quite similar physical-chemical properties; however, they could exhibit different bioactivity, e.g., one could be more active than its antipode. In some cases, one enantiomer could be even dangerous causing problems for human health. Therefore, the analysis of chiral compounds is a challenging issue for some studies, e.g., food safety and security, drug formulation control, metabolic processes, etc. Some analytical techniques have been widely employed for the analysis of enantiomers, among them, the most used include high-performance liquid chromatography (HPLC), gas chromatography (GC), supercritical liquid chromatography (SFC), etc. In addition to the mentioned techniques, chiral compounds have also been separated employing miniaturized ones such as electromigration (capillary electrophoresis-CE and capillary electrochromatography-CEC) and nano-/capillary-liquid chromatography (nano-LC/CLC) [1, 2]. The advantages of miniaturized techniques over conventional ones include higher chromatographic efficiency, higher mass sensitivity, lower consumption of solvents, shorter analysis time, etc. In nano-LC/CLC, the chiral compound separation is usually obtained by the direct resolution method where a chiral stationary phase is trapped on the capillary column. Examples of CSP used with this technique include silica modified with polysaccharides or with glycopeptide antibiotics or cyclodextrins. In addition, monolithic materials containing chiral selectors (CSs) have also been applied.

Detection is usually performed with UV detectors; however, complex matrices are investigated, and the sensitivity is not adequate due to the low volumes injected (10-100 nL). To improve the sensitivity some approaches have been done, e.g., the focusing method, sample pre-concentration, and coupling with mass spectrometry (MS). The use of MS, in addition, to increasing the sensitivity due to the features of MS and the low flow rate used, offer the possibility to characterize the analyzed compounds.

In this communication advantages and disadvantages of using miniaturized techniques for chiral separations will be illustrated taking into mind the most important parameters influencing enantioresolution. Some examples related to the use of different particles type, mobile phases, and samples (herbicides, pharmaceutical compounds, and nutraceuticals) will also be proposed and discussed.

Keywords: Chiral separations, nano-liquid chromatography, pharmaceuticals, pollutants, chiral selectors

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PBA2023

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INVITED LECTURES

INVITED LECTURES

IL-001

Integrating Experimental and Computational Approaches to Study Enantioselective Recognition in Liquid-Phase Enantioseparations

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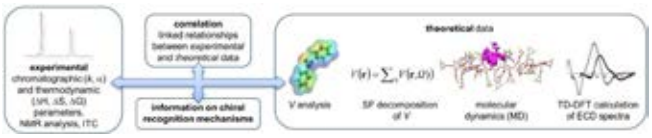
Noncovalent interactions play a key role in enantioseparation science, where they are the essential elements of the code by which chiral selector (the agent which discriminates between two enantiomers of a chiral compound) and selectand (each enantiomer which is recognized) interact with each other to result the enantiomer distinction and physical separation [1,2]. Although computational treatment of large multi-phase real-life systems is still in its infancy, in the last few years application of molecular modeling methods and techniques to enantioseparation science have been providing useful information to understand the molecular bases of enantioselective recognition occurring in liquid-phase enantioseparation processes [1-3]. On the other hand, a modern attitude to enantioseparation science needs to be founded on multidisciplinary approaches to disclose the molecular bases of mechanisms controlling selector-selectand affinity and enantioselection, going beyond trial-and-error approaches. In this presentation, advancements and open issues in integrating experimental and computational approaches to inspect chromatographic enantioselection will be described. In particular, features and applications of four computational approaches used in the field of enantioseparation science will be discussed: *a)* electrostatic potential (V) analysis to investigate in detail the shape of both analyte and selector [4]; *b)* the Bader-Gatti electron density source function (SF) approach, suitably extended to the V field, to evaluate the atomic contributions to local V [5]; *c)* molecular dynamics to simulate the binding modes of the enantiomers with the polysaccharide-based selectors [6]; d) time-dependent density functional theory (TD-DFT) calculations to obtain theoretical electronic circular dichroism (ECD) spectra for absolute configuration assignment.



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IL-002

Not Only Reverse Phase… A Need to Use Alternative HPLC Techniques for Pharmaceutical Analysis

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The most popular type of HPLC is reversed phase (RP), which is very flexible, robust and allows full control, including "fine tuning", especially, for sophisticated gradient methods. However, one may face a case, where RP HPLC is not the optimal choice – or even inappropriate for some specific separations, due to various reasons.

We present a case study, where RP HPLC method appeared to be incapable of ensuring appropriate method performance characteristics. An alternative HPLC type had to be found – and a new method developed.

A dissolution method for a novel formulation comprising two drug substances employed HPLC method adopted from the method for assay and impurities testing. This method includes a long gradient since the drug substances have a very different

lipophilicity, and in the original methods all the components, including numerous impurities and degradation products must be reliably separated.

The method, although including a long run, worked also for monitoring dissolution profile of the drug product.

The problem occurred when the drug product was subjected to alcohol damping study. In the cases, where alcohol content was high (above 10%), the strength of the diluent of the sample (dissolution medium containing ethanol) was much higher than the strength of the eluent (mobile phase, especially at the earlier stages of the gradient), the peaks, mainly, the one which eluted earlier, were deteriorated.

This could not be improved by diluting the sample with water since the concentration was very low – and the sensitivity of the method could have been lost.

Both drug substances are weak bases, having different pKa. Therefore, a decision was taken to develop an alternative method using a mixed-mode column HPLC (a combination of cation exchange with reverse phase in one column).

The method was successfully developed – being unaffected by the high alcohol content (up to 40%) in the sample solution. The method is isocratic and, as an added bonus, has a much shorter run time, than the previous one.

Keywords: HPLC, reverse phase, ion exchange, mixed mode chromatography, strong / weak solvent

IL-003

NMR Studies on the Elucidation of the (Unusual) Structure of Selector-Selectand Aggregates Between Daclatasvir and Some Cyclodextrins

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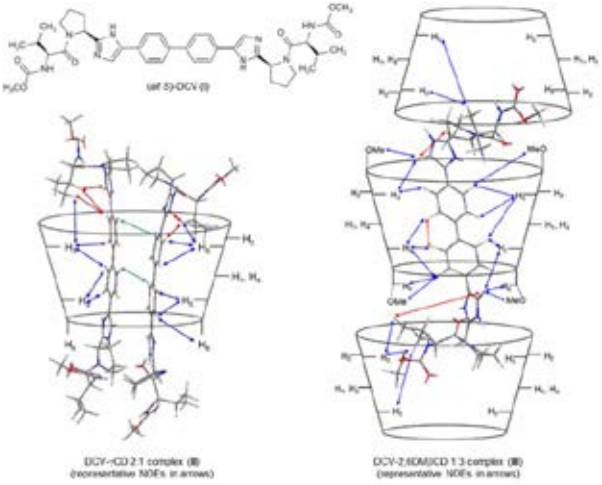
NMR spectroscopy is particularly suited to describe the structure of analyte-chiral selector supramolecular aggregates (complexes) in conditions very similar to those used in CE runs. Detailed information on the structure of those complexes may help to explain the outcome of chiral analyses by CE [1]. In some attempts to determine the chiral purity of the anti-hepatitis C drug daclatasvir (DCV, I) by CE, a plateau between two electrophoretic peaks was observed when certain cyclodextrins (CDs) were used as the chiral selectors. Remarkably, this anomalous behavior was also seen when single enantiomer DCV was analyzed. ^{1}H, ROESY and DOSY NMR experiments demonstrated the formation of high order supramolecular aggregates, alongside with "normal" 1:1 complexes, when γ-CD was used as the chiral selector. These higher order entities (2:1 complexes, II) were constituted by two stacked DCV molecules, positioned inside the γ-CD cavity [2]. With 2,6-di-O-Me-β-CD and other single isomer methylated β-CDs, however, a different kind of higher order aggregates in which one DCV molecule was inserted into two or even three cyclodextrins (1:2 and 1:3 complexes, III) were seen [3].



Keywords: Daclatasvir, Cyclodextrins, Supramolecular aggregates, Nuclerar Magnetic Resonance (NMR), Structure elucidation

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INVITED LECTURES

IL-004

Adavances in Solid Phase Extraction: The Evolution of Carbon-Based Sorbents

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Sample preparation is an indispensable step in the analysis of substances present in traces and ultra-traces in complex matrices, such as food, environmental, or biological samples. It is the most delicate phase of an analytical procedure because it is easily subject to errors, can occupy up to 80% of the time dedicated to an analysis and involves the most polluting steps of an entire method, still today mainly based on Brown Chemistry. In recent decades, this sector of Analytical Chemistry has been at the centre of an unceasing research activity, aimed at identifying new sorbent materials, developing alternative extraction devices and preparing special solvent systems, with the ultimate goal of exacerbating the "figures of merit" of a method and, when possible, to make a green transition.

This talk offers an idea of the rapid transformation that has taken place in this sector in the last twenty years, focusing the attention on carbon-based sorbents that, more than other materials, have revolutionized solid phase extraction (SPE, solid phase extraction). From graphitized carbon black to carbon nanotubes, this transition has expanded the SPE frontiers, ranging from the conventional SPE on cartridge to new extraction devices and modes (for instance, stir disk-SPE, rotating disk-SPE), from microparticle carbonaceous sorbents to carbon-based composite sorbents and free-standing paper-like materials such as buckypaper. This communication illustrates peculiarities, advantages and limitations of such sorbents and relative methods of execution as well as some of their relevant applications, among which the study of the fate of natural oestrogens in the environment.

Keywords: Solid phase extraction, graphitized carbon black, carbon nanotubes, nanocomposite sorbents, buckypaper

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IL-005

Soft Robotic Sensing Array Catheters for Cardiac Arrythmias

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This work presents the design and fabrication of a novel cardiac mapping catheter that integrates soft robotic actuators and stretchable electronics to enable whole-chamber mapping for the intended goal of diagnosis and treatment of atrial fibrillation and ventricular tachycardia. The novel catheter design aims to overcome the limitations of existing mapping technologies, such as speed of mapping time, increased sensor contact, and/or spatiotemporally resolved cardiac electrograms. We describe the fabrication methods of both the soft robotic actuators and stretchable electronics using a laser cutting that can be done in any laboratory. To evaluate the device's performance, in vitro tests were performed using 3D printed heart models to assess the soft robotic design to achieve conformability. Subsequently, in vivo studies were conducted on live pigs to evaluate the device's performance in a beating heart model. Preliminary results demonstrate the successful integration of soft robotic actuators and stretchable electronics into a minimally invasive catheter that could be delivered percutaneously. The device exhibited excellent navigation capabilities, acquiring electrical signals from both the left atrium and left ventricle. These findings highlight the potential of this innovative catheter for improved diagnosis and treatment of cardiac arrhythmias. Future work will explore strategies to perform whole-chamber mapping with commercial mapping systems.

Keywords: Robotic, sensing, array, cardiac arrythmia

INVITED LECTURES

IL-006

Recent Results in Food Sample Preparation Employing Deep Eutectic Solvents

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The need to assess the quality and safety of food has led to an important growth of literature on this issue. In this field, several studies show that sample preparation, taking into account the extraction, isolation and enrichment of analytes, greatly affects the reliability and accuracy of the analysis. Conventional extraction techniques are expensive, laborious, time-consuming and based on the use of a large amount of organic solvents that generate waste and pollute the sample and the environment. In 1988, the advent of green analytical chemistry, led to the search for green and fast solutions for the extraction and determination of endogenous and exogenous molecules in various matrices. Deep eutectic solvents (DESs) are new generation green solvents that have found great applicability for this purpose. DES are non-toxic, inexpensive, and easy to prepare showing good biodegradability, biocompatibility, and sustainability.

These green solvents are formed by a molecule that acts as hydrogen-bond acceptor (quaternary salt etc.), and molecules that act as hydrogen-bond donor (amines, carboxylic acids, carbohydrates, etc.). The obtained mixtures show a lower melting point than their individual components and resulted to be liquid at room temperature. Depending on the molecules that compose them, DESs are characterized by different polarities and can be selected for the extraction of different analytes.

These new generation solvents have been applied in different extraction techniques, such as solid-liquid, liquid-liquid and matrix solid phase dispersion extraction (MSPD), for the recovery of bioactive molecules from food products and food by-products, showing a high extraction efficiency. In some cases, DESs were more efficient than the organic solvents traditionally used. Adding a certain quantity of water to the DESs, with the aim to reduce their high viscosity, resulted to improve the extraction repeatability.

DESs have also been widely used in the determination of contaminants in food, both as extractive solvents and as a means to increase yield. It was reported that adding a small amount of DES to the dispersing material, during the homogenization phase of the MSPD technique, increased the extractive yield of contaminants including pesticides.

The results related to the use of DES in sample preparation will be presented for: i) extraction and analysis of endogenous molecules like phenolic compounds from food and food by-products of the agro-food chain; ii) determination of contaminants like acrylamide and pesticides in food products.

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Keywords: Sample preparation, deep eutectic solvent, green extraction, HPLC-MS

IL-007

Lateral Flow Tests: Signal Enhancement Methods and Future Trends for Diagnostics

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The last global outbreak caused by the "Severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) demonstrated the need and the potential of "rapid diagnostic tests", academically known as lateral flow tests (LFTs). These types of biosensors are portable, cheap to produce, equipment/battery-free and easy-to-use, even by untrained users. However, during the outbreak the weaknesses of LFTs were also exposed: the detection limits achieved with these tests, if compared with other techniques as PCR, were higher, which entailed a relatively high number of false negatives, especially during the first days of infection.

The objective of this presentation is to understand the working principle of LFTs, the mechanism in which the signal is produced and some strategies to enhance it, in order to compensate the weaknesses of these tests. In addition, the future trends of this technology will be discussed, including their application for molecular assays and how, in a near future, LFTs could compete with PCR tests.

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Keywords: Lateral flow, Nanotechnology, Molecular diagnostics

Lateral flow tests as the diagnostic tools for the future, combining nanotechnology and molecular (DNA-based) technologies.

IL-008

Development of Enantioselective High-Performance Liquid Chromatography-Tandem Mass Spectrometry Method for Quantitative Determination of Methylone and Some of Its Metabolites in Oral Fluid

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Methylone is a member of the large group of synthetic cathinones known as new psychoactive substances (NPS). It became one of the most frequently used recreational drugs of abuse in the last decade. MET is the b-keto analog of 3,4-methylenedioxymethamphetamine (MDMA) and produces similar pharmacological effects to MDMA. Here, an enantioselective high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the first time for quantitative determination of the recreational drug of abuse methylone and its major metabolites in oral fluid.

The simultaneous chemo and enantioseparation of methylone and its major metabolites was performed on a polysaccharide-based chiral column based on amylose tris (5-chloro-3-methylphenylcarbamate) as chiral selector (Lux i-Amylose-3) with methanol containing 0.4 % (v/v) aqueous ammonium hydroxide as mobile phase. The time required for enantioselective analysis of methylone, and its 2 major metabolites was 15 minutes.

This method was applied for the enantioselective determination of methylone and its metabolites in oral fluid and enantioselectivity in metabolism and pharmacokinetic of the parent compound and metabolites was observed.

Keywords: Chiral HPLC, enantioselective metabolism, enantioseparation, methylone and metabolites

IL-009

Trends in Analytical Strategies for the Determination of Pharmaceuticals

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Pharmaceutical consumption by human and animals for the treatment of diseases and/or improvement of their health is increasing all around the world. After the metabolization of pharmaceuticals in human and animal bodies, drug active compounds and their metabolites are excreted to environment and sewage treatment facilities (Madikizela et al., 2020). Due to insufficient elimination of these chemicals in wastewater treatment plant, pharmaceuticals have been detected in soil, ground water and surface water (Fatta-Kassinos et al., 2019). Pharmaceuticals have also tested for health care and forensic science. Urine (Moeller et al., 2008), blood and hair are generally used for drug testing (Citti et al., 2018; Raharjo & Verpoorte, 2004). For these reasons, various analytical methods have been introduced to literature

for the determination of different pharmaceuticals in biological and environmental matrices. Liquid chromatography (LC) and gas chromatography (GC) are main chromatographic methods used for the separation and detection of drug active compounds (D'Atri et al., 2019). However, complex sample matrices can affect the experimental results if a proper sample preparation is carried out for interference elimination, preconcentration and chemical conversion of analyte for its separation/detection (Moein et al., 2014). Heavy sample matrices and low analyte concentration in samples lead to researchers to apply preconcentration/separation methods such as solid phase extraction (SPE) with different adsorbents, liquid phase microextraction (LPME) and some microextraction methods (Ansari & Karimi, 2017). In addition, accuracy and precision of the analytical methods have great importance to assess bioavailability, toxicokinetic and pharmacokinetic effects of pharmaceuticals and their metabolites (Karnes & March, 1993). Isotope dilution methods provide high accurate analytical results along with small uncertainties and well-known error sources (de Leenheer & Thienpont, 1992). There are various studies for the detection of pharmaceuticals by isotope dilution based analytical methods. Chloroquine (Erarpat et al., 2021), cyanocobalamin (D'Ulivo et al., 2017) and carbamazepine (Teo et al., 2016) are some examples found in literature for the application of isotope dilution methods for pharmaceuticals.

Keywords: Chromatography, pharmaceutical, preconcentration, isotope dilution.

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IL-010

Emerging Challenges for Analytical Chemistry in the Pharmaceutical and Biomedical Analysis

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The main cause of poor and ambiguous results obtained from the bioanalytical process is sample pretreatment, especially in pharmaceutical and biomedical analysis because it involves handling complex sample matrices.

Sample preparation has been recognized as a major step in the chemical analysis workflow. As such, substantial efforts have been invested in recent years to simplify the overall sample preparation process. Major focusses of these efforts include, miniaturization of the extraction device; minimizing/eliminating toxic and

hazardous organic solvent consumption; eliminating sample pre-treatment and post-treatment steps; reducing the sample volume requirement; reducing extraction equilibrium time, maximizing extraction efficiency.

The extreme complexity of biological samples such as whole blood, plasma, serum, urine, and saliva demands a simple, fast and robust sample preparation process prior to the instrumental analysis. Conventional sorbent-based sample preparation techniques including solid phase extraction and its different modifications often involve protein precipitation, solvent evaporation and sample reconstitution as the integral part of sample preparation workflow.

Undoubtedly, it is a daunting task to process biological samples in the field of pharmaceutical and biomedical analysis because the analyst may encounter substantial analyte loss, or the overall analysis may take too much time. Nowadays, we are increasingly inclined to use green solvents for the environment, but without sacrificing analytical performance and selectivity. To all the characteristics mentioned above must be added the difficulty of taking samples because they can involve more or less invasive procedures. For these reasons, we can now also find in the literature the use of less conventional matrices (or originally "confined" to the pharmacotoxicological and forensic fields) as alternative biological samples and new techniques that do not require substantial pretreatment of the sample, such as the fabric phase sorptive extraction (FPSE) or the use of direct analysis techniques.

The presentation will be divided into two distinct parts: firstly, some applications will be described, while in the second part the sample preparation techniques for pharmaceutical and biomedical analyzes will be evaluated, in particular focusing the attention on the most recent techniques such as microextraction in solid phase (SPME), packed sorbent microextraction (MEPS), liquid-dispersive liquid microextraction (DLLME), and fabric phase sorptive extraction (FPSE).

Keywords: Pharmaceutical and biomedical analysis, sample preparation, green procedures, (micro)extraction procedures, biological matrices

IL-011

Synthesis, Analysis, Risk-Knowledge-Data-Based Process & Impurity Evaluation of Drug Substances

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During the synthesis of active pharmaceutical ingredients (APIs), process-related impurities or degradation products may form. As per the general guidelines recommended by International Conference on Harmonisation (ICH Q3A) to qualify the drug substance, the amount of acceptable level for a known and unknown impurity should be less than 0.15% and 0.10%, respectively. [1] As a general rule, the in vivo response to a mutagenic carcinogen is proportional to the (daily) dose and duration of dosing. According to ICH M7, limits can be based on the LTL (less-than-lifetime) principle in that higher exposures (based on specific multiples of a lifetime limit) to a mutagenic impurity over periods of ≤ 10 years are less likely to produce a carcinogenic response in patients than lifetime exposure. A limit based on chronic (lifetime) administration can be considered a virtually safe dose for a carcinogenic compound (threshold of toxicological concern (TTC): 1.5 µg/day). According to ICH M7, alerting structures (class 3) having no mutagenicity data should be controlled at or below acceptable limits (TTC: 1.5 µg/day). [2,3] In order to meet the stringent regulatory requirements, impurities should be identified and their amounts should be controlled carefully. Impurities detected during the process development studies of APIs and potential impurities should be identified, synthesized, and characterized and the mechanism of their formation should be clarified.

One of the most important steps in designing, developing, and optimizing the synthesis of an API is the control of impurities formed during the reactions. The key is to determine the structure of the impurities and then clarify how they are formed. The simplest option here is to adjust the reaction conditions (reagents, solvents, temperature, concentration, order of addition, etc.) to prevent or minimize the formation of these impurities.

In this concept, the process development stages of an immunomodulatory imide drug (IMiD), a class of immunomodulatory drugs that adjust immune responses containing an imide group; synthesis, analysis, and risk-knowledge-data-based process/impurity evaluation will be presented. Analytical studies carried out in this work supported our synthetic process development and optimization studies and

enabled us to see critical points of the process and impurities that need to be followed and controlled. With the knowledge of the impurities and their formation pathways, the synthetic process was optimized to eliminate and/or minimize the formation and carry-over of the impurities to the final drug substance. Also, setting tight and justifiable impurity limits for the starting materials and control of potential impurities including the genotoxic/mutagenic ones resulted in the production of highly pure API on a commercial scale. The product manufactured by using this drug substance is commercially available in Türkiye and European markets and has been submitted to US FDA.

Acknowledgement: The author is thankful to DEVA Holding A.Ş., İstanbul/Türkiye, for supporting this work and the Scientific and Technological Research Council of Türkiye (TUBITAK-TEYDEB Project No: 3160043 and 3170813) for the financial support.

Keywords: Drug substances, synthesis, analysis, impurity evaluation, fate studies

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IL-012

Recent Advances in Targeted and Untargeted Metabolomics with Solid Phase Microextraction

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Investigating roles, mechanisms, and the effect of triggers on biological systems are among the most intriguing research lines that attract scientists from diverse disciplines. In this line of research, although in vivo investigation would provide more insightful knowledge about the system, only several methods are applicable for in vivo noninvasive monitoring of metabolic changes in the biological systems.

Solid phase microextraction (SPME), a sample preparation method that can integrate the sampling and sample preparation in a single step, is one of the most important techniques applicable to in vivo conditions. The sampling principle of this technique depends on the equilibrium established between the free concentration of the analyte in the matrix and the extraction phase [1]. Alternatively, pre-equilibrium sampling based on mass transfer kinetics through the boundary layer surrounding the extractive phase can be used to extract from dynamic systems. Based on the sampling approach, various calibration strategies were developed to enable the determination of both total and free concentrations of a given analyte without any biospecimen removal from the system under investigation. Besides, when the extraction is performed with non-depletive samplers, the integrity of the physiological equilibrium of the studied system is preserved. Furthermore, SPME can take different shapes based on the demands of the study, and up to date, it has been prepared in fiber, arrow, micro tip, coated blade, coated mesh, flexible self-supported thin films, or thin films on a support, and it has been utilized for numerous applications where standard approaches cannot be used [2].

In this presentation, SPME will be in the spotlight, and its power for untargeted and targeted metabolomics investigation in various bioanalytical applications, including in vivo investigations and clinical diagnostic studies will be discussed. In addition, novel extractive phases, samplers and instrumental coupling strategies will be discussed to give the perspective for new developments.

Keywords: Metabolomics, SPME, sample preparation

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IL-013

Sensors on Pharmaceutical and Biomedical Electroanalysis

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The growing interest for the development of chemical sensors, which transform the chemical information of a sample

into an analytically useful signal, is evidenced by the more than 210,000 papers collected in the Web of Science databases. It is noteworthy that 42% of them are electrochemical sensors, in which the transducer is an electrode, and that of these, 35% correspond to electrochemical biosensors, in which the receptor is a biological element. These sensing platforms have been used to detect parameters across various industries, including biomedical analysis and pharmaceutical analysis.

The evolution of electrochemical sensors, characterized by reaching low detection limits with low-cost and portable equipment, has been very notable since the first works based on the use of hanging mercury drop electrodes. These devices have been gradually replaced by sensors with great analytical potential manufactured through partially or fully automated mass production, which reduces production costs. Their surface can be easily modified by nanomaterials and/or recognition elements to obtain a great effectiveness in electron transfer and a higher degree of selectivity. Bioreceptors and artificial matrices, such as molecularly imprinted polymers, have been used for this selective recognition of the analyte [1–4]. The present communication will illustrate, by different examples carried out in our research group, how miniaturized sensors and biosensors have been advantageously applied in the analysis of target compounds in pharmaceutical and biomedical fields. Challenges and opportunities facing the design and operation of these electrochemical devices will be discussed.

Keywords: Sensors, biosensors, electrochemistry, pharmaceutical samples, biological samples

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ORAL ABSTRACTS PROCEEDINGS

YS-001

Multiplexed Miniaturised Microfabricated Silicon Devices for Electrochemical Biosensing: From Chemical to Biological Systems

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The science of making tiny devices via miniaturization of the systems has been an emerging field in the microelectronics industry since 1950s. Since then, continues research and development in the field targets achieving to manufacture the smallest structures in order to meet the necessary criteria required in the microelectronics. Such excellent devices have been gaining attention of the researchers from several research fields including biosensors, microfluidics, photonic crystals, etc. In particular, the miniaturization of the systems has been critical for electrochemical sensing applications due to the benefits of the scaling down approach such as rapid establishment of steady state currents, decreased signal-to-noise ratio, increased sensitivities, etc.

In this study, we will demonstrate several routes for successful fabrication of silicon based electrodes and multiplexed chips. These devices were manufactured in Tyndall cleanroom facilities by microfabrication engineers. Here we demonstrate how to make chips, how to use them efficiently for electrochemical applications of sensing systems.

We have established several routes based on photolithography, deposition and etching to fabricate devices with varying size and dimensions1-4, Figure1. Each design of devices were studied with morphological characterization techniques such as scanning electron microscopy (SEM), and then electrochemical methods. Following this, we have developed electrochemical sensing systems; chemical and biological.

One of the important aspects of using such small scaled devices is the electrochemical reproducibility of the ultramicro surfaces which clearly affects the analytical performance of the developed devices. Therefore we investigated the effect of several cleaning protocols on the electrochemical characteristics of the ultramicro multiplexed device, which has a 1 µm-width and 45 µm-length5, Figure 2. We have developed and optimized the most efficient cleaning protocol for these devices and we assessed the surface morphology of the gold surface before/after cleaning via atomic force microscope (AFM). We discovered that the established cleaning protocol increases surface roughness which may lead a redox-active surface

Fabricated devices were used for miniaturization of hydrogen bubble template to explore the scaling-down limits of in situ template, Figure 3. Copper deposits (CuFoam) were used for glucose electro-oxidation. This device showed excellent electroanalytical performance toward glucose and studied in human serum samples. Furthermore, it is used as a chemical oxygen demand on-chip platform.

We have designed and fabricated further miniaturized chip for immunosensor development. These devices were modified with gold foam in order to increase the surface area of micro electrodes. Gold depositions (AuFoam) were used as a matrix for the development of affinity based biosensors. In order to overcome the non-specific binding problem we first developed a universal antifouling surface (Figure 4). Developed surface provides an easy-to-use platform due to rapid preparation and abundant functional groups for bioconjugation. We have immobilized antibodies on the surface via several crosslinking approaches and selective immobilization techniques (Figure 5). Developed universal platform has applied with anti-Haptoglobin and anti-cortisol antibodies. Both sensors are studied on-chip. We have studied the detection protocol in un-diluted fetal bovine serum in order to demonstrate the efficiency of the developed anti-biofouling on-chip platform.

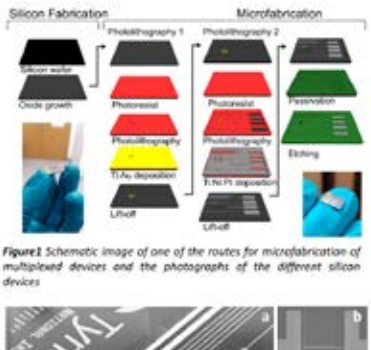

Figure1 *Schematic image of one of the routes for microfabrication of multiplexed devices and the photographs of the different silicon devices*

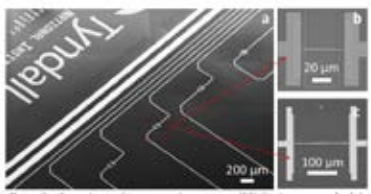

Figure2 *Scanning electron microscope (SEM) images of (a) multiplexed device, (b) individual sensing electrode with 1 µm width and 45 µm length, (c) individual sensing electrode with 1 µm width and 178 µm length*

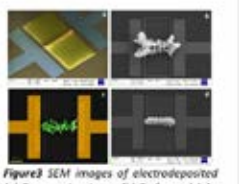

Figure3 *SEM images of electrodeposited (a) Cu nanostructures, (b) Cu foam, (c) Au nanoneedles and (d) Au foam*

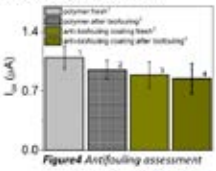

Figure4 *Antifouling assessment*

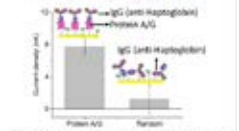

Figure5 *Immobilisation protocols applied onto antifouling coating*

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YS-002

Living Biosensors with Synthetic Genetic Circuits to Monitor Nanomaterial and Heavy Metal Triggered Toxicity

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The evaluation of cytotoxicity and biocompatibility are critical aspects in developing novel biomaterials. Such testing is necessary for any clinical application to understand better and prevent potential issues that newly produced materials may cause in patients[1]. Synthetic biology lays the groundwork for utilizing cells as biosensors- also called living biosensors, in environmental monitoring, disease diagnosis and therapy, and bioproduction of valuable substances[1]. To date, great success in the laboratory has been achieved, enabling cost-effective, user-friendly, renewable, and portable prototyping for field deployment[1]. Nevertheless, there are still issues with specificity, sensitivity, multiplexing, and reaction time, which are essential for precise cytotoxicity evaluation. To address these issues, first, we have demonstrated using the engineered heat-shock response (HSR) mechanism as a powerful candidate to build ordered genetic circuits to report nanomaterial-triggered toxicity[2]. Unlike recent reports utilizing the HSR mechanism as stress-indicating biosensors, we integrated promoter engineering strategies. We tested our engineered promoters against nanomaterials and heat shock, as our sensors output a GFP signal concerning the promoter strength. Our optimized genetic circuits could report the toxicity of quantum dot nanoparticles in 1 hour[2]. The work's second part utilized a neural network-based architecture dependent on Long-Short Term-Memory (LSTM) to decrease the response time of a complex genetic circuit engineered for gold ion detection[3]. Thanks to Long-Short Term-Memory (LSTM)-based networks, we were able to decrease the ON/OFF status of a complex sensor with the initial engineered HSR-based promoter to 30 min with 78% accuracy and over 98% in 3 hours. We also demonstrated that the network could classify the sensor output (raw fluorescence data) into pre-defined gold concentration groups with 82% precision in 3 hours[3]. In the last part of the work, we have constructed another recombinase-based heavy metal biosensing system to detect cadmium ions and characterize optimal conditions for a plan based on recombinases. By describing optimal conditions for a system based on recombinases, we have maximized the response of a cadmium-responsive recombinase-based sensor while maintaining the signal at toxic concentrations[4].

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Keywords: Nanomaterials, heavy metals, toxicity, synthetic biology, living biosensors

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YS-003

A Novel Decision Support System Based on Feature Extraction, Feature Ranking, Feature Selection for Accurate Diagnosis of Diseases Using Medical Images

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Abstract

The continuing shortage of medical professionals around the world steadily increases their workload while preventing patients from accessing appropriate, prompt, and affordable health services. Therefore, it becomes more and more important to develop appropriate decision support systems that will reduce the workload of medical professionals while ensuring that patients receive appropriate and prompt health services. This study proposes a new approach for rapid and accurate diagnosis of diseases using medical images obtained through different medical imaging modalities. The proposed approach is based on the combined use of feature extraction using AlexNet and ResNet-101 neural networks, feature ranking with two-sample z-test, and feature selection with binary genetic algorithm. From this respect, the proposed approach is an innovative decision support system that provides rapid and accurate classification of medical images using much fewer features and can be implemented on low-cost hardware. Performance of the proposed approach was evaluated using three different datasets by implementing k-Nearest Neighbours (KNN) and Support Vector Machine (SVM) classifiers with 4-fold cross validation. Obtained results show that the proposed approach is very successful in highly accurate classification of medical images despite utilizing much fewer features leading to significantly lower computational cost.

Keywords: AlexNet, ResNet-101, Feature vector extraction, Feature ranking, Feature selection, Machine learning, Classification, Decision support systems.

I. Introduction

The world population is growing rapidly. As a consequence of this growth, the number of people suffering from genetic and chronic diseases is also increasing rapidly. However, the number of medical professionals around the world is not increasing in proportion to the number of patients. Therefore, patients cannot receive appropriate, prompt, and affordable health services while on the other hand the workloads of physicians increase considerably in many countries. Hence, it becomes more and more important to develop systems that will reduce the workloads of medical professionals while ensuring that patients receive appropriate and prompt health services.

Artificial intelligence (AI) is a branch of science that uses various methods and algorithms to enable computers to do human-specific thinking, idea generation, and decision-making. With the development of Convolutional Neural Network (CNN) structures, data in the image form have become effectively analysable and classifiable. In this way, the working area of artificial intelligence has greatly expanded.

A CNN is a multilayer neural network. Thanks to its dense layers, a CNN can quickly analyze big data and make highly accurate decisions for a specific purpose. Readers who want to examine the working mechanism of CNNs in more detail can refer to the relevant resources in the literature (Chen and Jain, 2020).

The biggest challenge encountered in the practical applications of CNN and similar AI models is the computational cost caused by the excess number of parameters in the structures of these models. In most applications, especially in image classification applications, the use of GPU-based hardware becomes mandatory. The processing capacity and cost of the physical hardware required to realize such models are quite high, constituting a major obstacle to the spread of these systems. To overcome this difficulty, it is of critical importance to develop innovative artificial intelligence methods and approaches that can be implemented on low-cost hardware platforms while exhibiting high diagnostic performance.

Feature ranking is a preprocessing method that uses statistical scoring to rank the features of a dataset in the order of significance. Feature selection is another preprocessing method used to eliminate problems such as the length of processing time and cost, especially in the classification of big data, and to alleviate the computational load.

In this study, the methods of feature extraction, feature ranking, and feature selection are used consecutively. In this way, an innovative decision support system is proposed that may be used on low-cost hardware to enable fast and accurate classification of medical images using much less features. Thanks to the presented system, it becomes possible to represent medical images with very few numerical data (features). The decrease in the number of features allows for the classification of feature vectors that represent the image data with much lower dimensions by using machine learning models with much lower computational complexity rather than classifying the image data with very high dimensions by using CNN and similar complex deep learning models.

II. Method

Three different datasets are included in this study. The following are the properties of the datasets used:

Dataset 1 (D1): This dataset contains a total of 5,232 chest X-ray images, taken from healthy and pneumonia individuals, including 1,349 normal and 3,883 pneumonia cases. To create a balanced dataset, we use a total of 2400 randomly selected X-ray images, including 1200 pneumonia and 1200 normal images in this study (Kermany et al., 2018).

Dataset 2 (D2): This dataset contains publicly available dermatological images of malignant and benign skin lesions, provided by the ISIC archive (www.isic-archive.com). In our study, we use a total of 2400 images, 1200 malignant and 1200 benign, randomly selected from this dataset.

Dataset 3 (D3): This dataset contains a total of 780 breast ultrasound images divided into three classes as normal, benign, and malignant. Since we focus on binary classification, we use a total of 648 breast ultrasound images, 437 benign and 211

malignant, in our study (Chen and Jain, 2020).

The approach presented in this study consists of three stages: feature extraction (FE), feature ranking (FR) and feature selection (FS). In the first stage, feature vectors of medical images were obtained by using AlexNet and ResNet-101 networks. As a result of passing the images of the datasets D1, D2 and D3 through AlexNet and ResNet-101, six new datasets consisting of numerical data representing the related image dataset were obtained. The sizes of these new datasets are 2400x4096, 2400x4096, 648x4096 for AlexNet, 2400x2048, 2400x2048, 648x2048 for ResNet-101, respectively. In addition, feature vectors obtained from AlexNet and ResNet-101 were concatenated into a single vector so as to obtain new datasets of dimensions 2400x6144, 2400x6144, 648x6144 for D1, D2 and D3, respectively. In the second stage, Two-Sample z-Test method was separately applied to all datasets obtained in the feature extraction stage. Thus, the number of features to be included in the classification step has been reduced by approximately 10%. The formulation of the two-sample z-test is as follows:

$$z = \frac{(\bar{X}_1 - \bar{X}_2) - (\mu_1 - \mu_2)}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \qquad (1)$$

In the last stage, a binary genetic algorithm-based feature selection method using a crossover operator based on the logic OR process was applied separately to all datasets obtained in the feature extraction and feature ranking steps. When the feature selection process is complete, the input datasets are further reduced by approximately 90%. Table 1 shows the number of features obtained after all the processing steps applied to D1. Similar results were obtained for D2 and D3 as well.

The flow chart of the presented approach is shown in Figure 1 for D1.

III. Experimental Results

To evaluate the success of the presented approach, all numerical datasets belonging to D1, D2 and D3, which were created in the feature extraction, feature ranking, feature selection process described in the method section were classified using KNN and SVM classification methods with 4-fold cross validation. The accuracy and sensitivity of the results obtained were evaluated. Due to page restriction, only the the results of D1 are presented in Table 2. Readers who would like to examine how the accuracy and sensitivity values are calculated can refer to the relevant resources in the literature (such as Rokhana et al., 2020).

Table 2 shows the results obtained by classifying the datasets generated by independently applying (I) feature extraction, (II) feature extraction + feature ranking, (III) feature extraction + feature selection and (IV) feature extraction + feature ranking + feature selection processes to D1. When these results are examined, it is seen that the accuracy and sensitivity values increase gradually, although less features are used in each processing step. Similar results were obtained for D2 and D3 as well. The results in these tables show that the proposed approach is very successful in highly accurate classification of medical images while utilizing much fewer features leading to significantly lower computational cost.

In order to demonstrate the superiority of the proposed approach, the classification results of the proposed approach were compared with the results available in the literature obtained by using CNN networks (Kermany et al., 2018; Rokhana et al., 2020; Al-Dhabyani et al., 2019). Comparisons show that the proposed approach significantly outperforms CNN networks used for the same purpose.

All these results indicate that the presented approach is a decision support system that can be successfully used to perform diagnosis quickly and with high accuracy. Moreover, it can be implemented on low-cost hardware thanks to its very low computational complexity.

IV. Conclusion

In this study, a novel classification approach is proposed that enables the classification of medical images with high accuracy through the combined use of feature extraction, feature ranking and feature selection. The performance of the proposed approach is evaluated using KNN and SVM classifiers. The obtained results prove that the proposed approach constitutes a decision support system that can be used effectively for fast and highly accurate classification of medical images with much fewer features. In addition, the results show that the proposed approach is superior to the existing methods in the literature in most cases and can provide higher classification performance.

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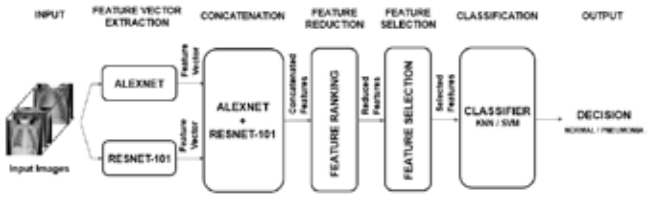

Fig. 1: Flow chart of the presented approach

Tab. 1: Number of features obtained after applying each method to D1.

	FE	FE+FR	FE+FS	FE+FR+FS
AlexNet	4096 (100%)	3651 (89.1%)	409 (9.9%)	365 (8.9%)
ResNet-101	2048 (100%)	1862 (90.9%)	204 (9.9%)	186 (9.0%)
AlexNet + ResNet-101	6144 (100%)	5582 (90.8%)	614 (9.9%)	558 (9.0%)

Tab. 2: Accuracy and sensitivity values of all datasets created for D1.

			AlexNet		**ResNet-101**		**AlexNet + ResNet-101**	
			Knn	Svm	Knn	Svm	Knn	Svm
D1	**I**	Acc	93.71	96.21	93.79	97.33	94.42	97.75
		Sen	97.39	96.52	96.79	98.07	97.85	98.16
	II	Acc	93.67	96.04	94.17	97.08	94.04	97.50
		Sen	97.39	96.25	97.74	97.07	97.42	97.98
	III	Acc	96.08	98.04	96.00	97.13	96.42	98.83
		Sen	99.19	98.08	97.37	97.50	98.94	99.24
	IV	Acc	96.25	98.50	96.58	97.21	96.38	98.88
		Sen	98.88	98.67	98.03	97.58	98.85	99.08

YS-004

Cell and Microparticle Manipulation Using Acoustic Waves in Microchannels

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Microparticle and cell separation is an important field in bioengineering. Several biomedical devices are used in microparticle and or bioparticle (cells, bacteria, viruses etc.) separations. The conventional technologies that are used include centrifugation or porous membrane separation. These methods are commonly used methods for microparticle and bioparticle separation in conventional biomedical devices. In the last couple of decades, microfluidic devices were introduced and, in some instances, improved means of separation of bioparticles are observed with these microfluidic biomedical devices.

A microfluidic channel enables a sensitive means of manipulation and/or separation of bioparticles. There is a need for a force to move the microparticles inside the microfluidic circuit. This force used for manipulation/separation can be a dielectrophoretic, magnetic, fluid drag or an acoustophoretic force. Compared to the other forces acoustic forces generated in a microchannel offers some advantages such as ease of manufacturing of the chips used for microparticle separation, hardware simplicity and high throughput in processing of bioparticles. Also, unlike conventional methods acoustophoretic methods have the capability to separate particles of similar size and similar density from each other due to their differences in acoustic properties.

An acoustophoretic microfluidic device for micro and bioparticle separation manipulates/separates particles due to an acoustic standing wave generated inside the microchannel. The required standing acoustic wave in the microchannel is achieved by inducing elastic waves inside the chip material which shares a common boundary with the microfluidic channel. The acoustic wave inside the microchannel generates an acoustophoretic force on the microparticles which changes with different sizes as well as different acoustic properties (compressibility) of the microparticles that are being manipulated/separated.

In this study, a numerical model of different types of cancer cells is generated using a finite element analysis approach. Using numerical analysis, the motion of different types of cells is simulated under the effect of acoustic and fluid drag forces. Using finite element and boundary element numerical analyses at the

biomedical device level, the capability of an acoustophoretic device in separating cancerous and non-cancerous cells will be demonstrated. Using the presented numerical analysis tool, the factors which affect cell separation will also be investigated.

In the final part of the study, the capability of acoustophoretic devices, which are manufactured from different materials such as silicon, PMMA, glass and PDMS, in manipulation of MDA-MB-231 breast cancer cells inside the microfluidic channel will be demonstrated with experimental studies.

Keywords: Cell manipulation, Acoustophoresis, Microfluidics

YS-005

Insight into *Aβ*/Membrane Lipids/ Metal Ion Interplay in the Context of Alzheimer's Disease

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Alzheimer's disease (AD) is the most common cause of dementia in the elderly, and a public health concern since the number of cases will be doubled in Europe by 2050, without any curative treatment to date. Thus, better understanding how this disease works should help for developing novel therapeutic strategies. One hallmark of AD is the presence of senile plaques in the brain of AD patients, composed of the amyloid β (Aβ) peptide – mainly 40 and 42-residue long – aggregated into fibrils, along with metal ions such as copper (II)[1]. In the presence of a physiological reductant such as ascorbic acid (vitamin C), the Aβ-Cu^{2+} complex is able to generate reactive oxygen species (ROS), which could be a major contribution to oxidative stress in AD[2]. In addition, it has been shown that Aβ40/42 is capable of specifically binding membrane phospholipids with a relatively high affinity, resulting in either the promotion of its aggregation at the surface or in its insertion into the membrane bilayer by creating ion-like channel structure leading to the disruption of the membrane integrity[3],[4]. In this study, we mainly focused on the *Triumvirat* Aβ peptide/ copper ions and membrane lipids to better understand how they interact together under oxidative stress conditions. A membrane model system was developed, composed of a mixture of 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (PLPC) and 1-palmitoyl-2-linoleoyl-sn-glycero-3-phospho-L-serine (PLPS), and its interaction with Cu^{2+} or Aβ-Cu^{2+} was studied. A variety of analytical techniques was used for the characterization of the system, in particular the oxidative damages undergone by lipids and Aβ, like UPLC-MS, UV-spectrophotometry or NMR spectroscopy.

Keywords: Amyloid β, copper ion, ROS, lipid membrane model, oxidative stress

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YS-006

Portable Handheld Biosensing Platform for Point-of-Care Virus Detection in Field Settings

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Abstract

The development of point-of-care devices for rapid and accurate diagnosis of viral diseases in resource-poor settings is crucial for global health. This study introduces a lightweight and portable biosensor that utilizes a plasmonic chip integrated into a lens-free imaging framework. The biosensor employs nanohole arrays on the chip to detect viruses in a label-free manner.

By monitoring the diffraction field patterns of the nanohole arrays under LED illumination, the biosensor successfully detected H1N1 viruses at medically relevant concentrations. To facilitate analyte binding, an affordable and user-friendly sample preparation kit was developed to prepare the surface of the plasmonic chip. The biosensor features a user-friendly Python-based graphical user interface that allows end users to capture and process diffraction field images and access virus information. With highly sensitive nanohole arrays and a lens-free imaging framework, the biosensor achieved a limit of detection as low as 10^3 $TCID_{50}$/mL. The handheld platform, weighing only 70 g and measuring 12 cm tall, eliminates the need for bulky instrumentation, making it suitable for resource-poor settings. Furthermore, the biosensor can be easily adapted for the detection of different viral diseases such as COVID-19 or influenza by coating the plasmonic chip surface with specific antibodies. Overall, this biosensor provides a rapid, accurate, and portable diagnostic solution that can help prevent the spread of viral diseases.

Keywords: Plasmonics, label-free biosensing, point-of-care diagnostics, nanotechnology, lens-free imaging

I. Introduction

Rapid diagnostic methods were required once the WHO declared the COVID-19 pandemic. In this study, we developed a portable biosensor for the point-of-care detection of viral diseases that combines plasmonic label-free sensing technology with lens-free computational imaging. We have successfully demonstrated label-free detection of the 2009 swine flu strain of the H1N1 influenza virus, also known as the swine flu. In order to improve the performance of the biosensor, we created a sample preparation kit that enables effective surface modification of the plasmonic sensor chip with antibodies specific to H1N1 influenza viruses. The sample preparation kit was designed to minimize consumable usage while maximizing the sensing signal obtained with the handheld biosensor. Furthermore, we developed Python™-based software that facilitates the acquisition and analysis of diffraction field images, providing operators with essential virus information. The platform employs simple optics consisting of cost-effective components, which can be easily aligned within a dark environment without the need for complex optical or mechanical instrumentation.

II. Material and method

Plasmonic chips were fabricated on a wafer-scale and prepared to desired dimensions using a wafer cutter. A protective polymer layer was applied to nanoholes, which was later removed with piranha cleaning (3:1 sulfuric acid: hydrogen peroxide). Piranha cleaning not only eliminated contaminants but also rendered the gold (Au) surface hydrophilic, facilitating analyte attachment through physisorption and preventing leakage during incubation. Analyte attachment has a two-step process: incubation with 200 µg/mL protein A/G, binding to the Au surface through physisorption, followed by incubation with 100 µg/mL protein IgG, binding to protein A/G through its Fc region. Between incubations, the chip surface was rinsed with PBS and deionized water to remove unbound protein. H1N1 viruses, obtained from the Department of Medical Microbiology at Ege University, were used. Ten-fold serial dilutions of the virus in sterile PBS were incubated on the chip surface and washed with sterile PBS. Virus immobilization was performed in a Biosafety Level 2 (BSL-2) laboratory. The incubation time was chosen as 1h for each analyte. The plasmonic chip was illuminated with a broadband white LED light source. Transmitted light was collected by an objective lens and coupled to a spectrometer with a 0.09 nm spectral resolution. Experimental data were smoothed using a Savitzky-Golay filter.

III. Results and discussion

The biosensor (Fig. 1A) consists of three components: (i) a plasmonic chip; (ii) an LED light source; and (iii) a CMOS camera (Fig. 1B). The sample preparation kit (Fig. 1C) divides the chip into sensor and control compartments, with the sensor region coated with H1N1-specific antibodies. The spectral variations within the nanohole response enhance the match between the EOT signal and LED response, resulting in increased diffraction field intensity in the presence of captured viruses (Fig. 1D).

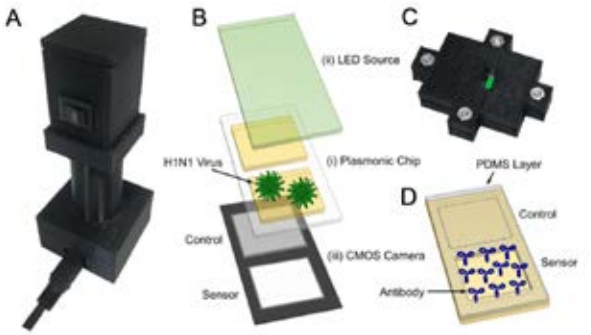

Fig. 1: Schematic illustration of the handheld plasmonic biosensor and sample preparation kit.

The spectral readout in our biosensor relies on monitoring the diffraction field intensity of the plasmonic mode. Our sample preparation kit (Figure 2A) enables targeted analyte binding only to the critical chip surface area. The kit consists of two PDMS sealing layers and two PLA locking caps (Figure 2B), allowing controlled incubation (Figure 2C) while maintaining separate sensor (blue) and control (yellow) regions on the plasmonic chip.

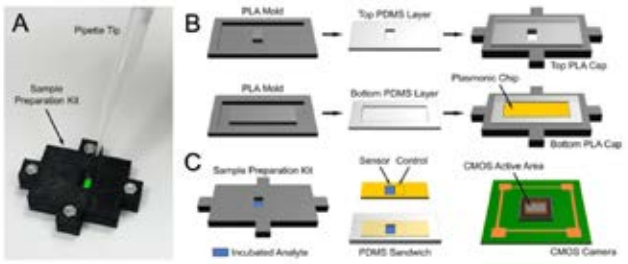

Fig. 2: Sample Preparation Kit

The biosensor operates by capturing diffraction field images of the plasmonic chip using a CMOS camera and monitoring spectral variations within the nanohole response upon analyte binding. Figure 3A illustrates the components, including the CMOS camera housed and the battery-powered LED light source. The chosen LED source has a narrow bandwidth close to the nanohole response, while the CMOS camera exhibits high quantum efficiency across the relevant spectral range. Figure 3B shows the EOT response of the plasmonic chip, the power output of the LED source, and the quantum efficiency of the CMOS camera.

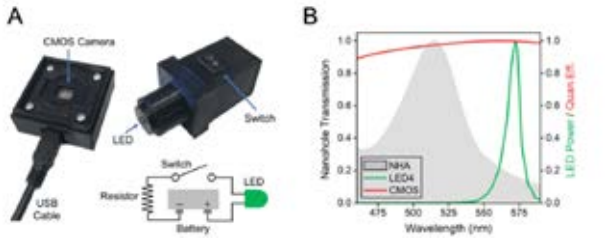

Fig. 3: Optical instrumentation of the portable biosensor

The biosensor enables label-free virus detection through analyte attachment on the plasmonic substrate. A cleaning procedure ensures a chemically favorable chip surface, followed by surface modification using a sample preparation kit. Fig. 4A illustrates an H1N1 virus captured on the sensing surface, confirmed by SEM image (Fig. 4B) and EOT response (Fig. 4C). The addition of protein A/G (200 µg/mL) and protein IgG (100 µg/mL) shifts the EOT response to ~519 nm (red) and ~532 nm (blue), respectively. This high protein concentration ensures sufficient sensing signals even for very low virus concentrations, as demonstrated with an H1N1 virus titer of 10^7 $TCID_{50}$/mL, shifting the EOT resonance to ~551 nm (green).

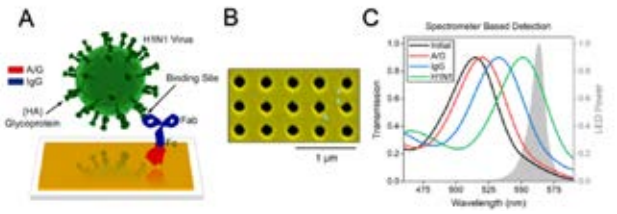

Fig. 4: Label-free detection of H1N1 influenza virus

In our biosensor, the LED spectrum (gray curve in Fig. 5C) was positioned at longer wavelengths compared to the nanohole EOT response (black curve in Fig. 5C). Fig. 5A shows distinct diffraction field intensity differences between control and sensor regions (Fig. 5B). Fig. 5C demonstrates intensity changes in the sensor region, while the control region remains unchanged.

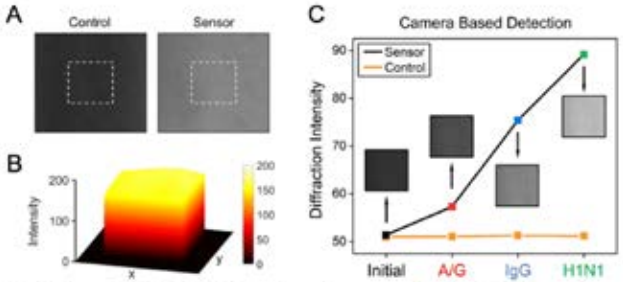

Fig. 5: Using CMOS diffraction images for label-free detection

Fig. 6A (black curve) and 6B (blue curve) show the EOT response of nanoholes coated with control and H1N1 antibodies. After virus incubation, the EOT response shifted to longer wavelengths (Fig. 6B, green curve) for virus binding, confirming the biosensor's specificity for H1N1 since no spectral variation was observed for the control antibody-coated surface (Fig. 6A, dashed red curve).

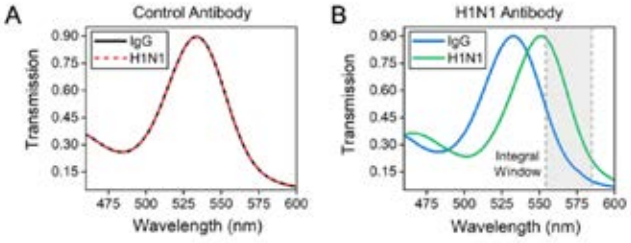

Fig. 6: Detection limit of the portable platform

Additionally, a Python™-based GUI was developed for plasmonic chip evaluation using the CMOS camera.

IV. Conclusion

This article presents a handheld biosensor utilizing a plasmonic chip with nanohole arrays integrated into a lens-free imaging framework. The biosensor records diffraction field intensities using a CMOS camera and LED light source tuned to the plasmonic mode. It successfully detects H1N1 viruses at medically relevant concentrations. A sample preparation kit was developed to minimize consumable usage while maintaining sensitivity. The biosensor also features a user-friendly GUI for camera control and provides sensing information based on diffraction field images. Traditional diagnostic methods for human H1N1 influenza, such as real-time PCR and antigen/antibody tests, have limitations. PCR provides sensitive and specific results but requires several hours. Antigen tests offer rapid results but may yield false negatives at low virus levels, while antibody tests lack accuracy. Our lightweight, field-deployable biosensor offers accurate sensing data, making it a cost-effective alternative for pathogen-based disease diagnosis compared to traditional methods.

Acknowledgements: A.E.C. acknowledges TUBITAK 2232 – Career Integration Fellowship (Project No. 119C002), and BAGEP Award of the Science Academy, Turkey.

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YS-007

Direct Determination of Surface Proteins in Leishmania

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Leishmaniasis is a neglected tropical disease caused by the Leishmania parasite, primarily seen in developing and underdeveloped countries. Due to immigration to our country, the effective population of the disease has increased recently. The lesion, which has visceral and cutaneous forms, can be lethal when it acts on internal organs. Surface proteins are the most crucial part of the parasite and host interaction. The parasite attaches to the host cell through surface proteins, enters the cell, multiplies, suppresses the immune system, and allows many other biological functions. It is the most critical research part in vaccine and biomarker discovery. Generally, cell surface biotinylation and cationic colloidal silica beads with which the surface is coated are used to analyze surface proteins. These methods break down the cell and are more open to contaminant proteins from the cytoplasm and nucleus. In addition, the high hydrophobicity of plasma membrane proteins reduces their solubility in water, while their embeddedness in the membrane makes it difficult to identify.

Instead of the commonly used cell surface analysis methods in this study, a faster and less experimental workflow, aiming to cut only plasma membrane proteins without breaking the cell, was tried in leishmanial species (L.Tropica, L.Infantum, L.Major, L.Donovani). This method aims to digest plasma membrane proteins by treating the cell surface with a proteolytic enzyme for a short time. Thus, it is expected to contain fewer contaminants and unwanted proteins.

As a result of this method analysis with the Fusion Orbitrap Mass Spectrometer, the rate of surface protein defined in 4 different species was 9.34% in L.Tropica, 7.55% in L.Major, 7.9% in L.Infantum, 7.52% in L.Donovani. Consistent with the literature and candidates for biomarker ISCL, KMP-11, Leishmanolysin, PSA-2, ABC transporter, and lanosterol 14α demethylase, proteins were identified.

Keywords: Surface Proteins, Leishmania, Mass Spectrometry based Proteomics

YS-008

Development of Fluorescent Aptasensor to Detect 17β-Estradiol in Human Blood

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In females, 17β-estradiol (E2) is an essential sex hormone that plays a vital role in the reproductive system. However, dysregulation of E2 has been linked to several disorders, including infertility, osteoporosis, and menopausal symptoms. Thus, the development of a sensitive and selective detection system for E2 is significant for medical applications. Hence, this study aims to develop an E2 detection system based on a DNA aptamer coupled with SYBR Green I (SGI) fluorescence dye that can quantitatively detect E2 levels in clinical samples. In this aptasensor, the aptamer can specifically bind to E2 and cause a release of intercalated SGI from the secondary structure of the aptamer, resulting in a change in fluorescence intensity that can be measured to quantify E2 concentration. The optimal conditions for E2 detection were determined, including aptamer concentration, SGI concentration, pH, buffer, and reaction time. The developed E2-aptasensor was found to have a low limit of detection (LOD) of 3.0 pM and showed selectivity towards E2 as compared with other compounds with similar structures (chloramphenicol, diethylstilbestrol, bisphenol A, progesterone, and genistein). Also, the developed aptasensor exhibited efficiently detected E2 spiked in human serum with recovery rates of 99.5-105.5 % and relative standard deviations of 5.18-9.71 %. This finding indicates the development of a novel and efficient aptasensor for the detection of E2, which employs the DNA aptamer and SGI. This aptasensor has the potential to be applied as a promising tool for detecting E2 in biomedical applications.

Keywords: Aptamer, Estrogen, Fluorescence, Serum, SYBR Green

YS-009

High-yield and Facile Genomic DNA Extraction Method on Glass Fiber Strip for LAMP PCR Based Pathogenic Bacteria Detection

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High-yield and facile DNA extraction from biological samples to detect pathogens is of great importance for Point of Care (POC) applications. Recently, glass fiber-based extraction method has become popular due to their ability to efficiently capture and retain DNA molecules. In our study, we demonstrated a simple DNA extraction process using glass fiber without centrifuge and any complex equipment reducing time required for sample processing and analysis for POC applications. The genomic DNA was isolated from S. aureus bacteria spiked reference blood sample using glass fiber strip and performed colorimetric LAMP PCR test. Glass fiber strip was fixed on glass slide. The blood samples spiked with *Staphylococcus aureus* bacteria culture was tested on the proposed material. The sample was incubated with lysis buffer and proteinase K solution. After drying pre-treated glass fiber, the lysate was dropped on the strip and washed several times by ethanol solution to remove undesired molecules originated from biological samples on the strip, DNA was released from the fiber using elution buffer. Nanodrop UV absorbance spectrophotometer and colorimetric LAMP PCR measurements were carried out for the evaluation quantity and quality of DNA isolate. The higher (~five times) DNA yield from glass fiber than the commercial spin column-based isolation kit was found via spectroscopic results. The limit of detection was determined as 10 cfu/ml of S.aureus bacteria in blood sample for colorimetric LAMP PCR. As a result of *Enterococcus faecalis* and *Escherichia coli* bacteria spiked blood samples for specifity evaluation of colorimetric LAMP, there wasn't seen any cross reaction for that samples.

Our work offers a simple, efficient and relatively inexpensive method based on glass fiber for isolating DNA and PCR based detection of pathogens.

Acknowledgement: The authors acknowledge The Scientific and Technological Research Council of Turkey (TUBITAK) with the project no: 221Z056 for funding.

Keywords: Glass fiber, DNA extraction, pathogen bacteria, LAMP PCR

YS-010

Hyper-crosslinked β-Cyclodextrin Polymer as a Sustainable Sorbent for Bioanalytical Applications

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In recent years, there has been remarkable development in the field of analytical chemistry concerning the study of sorbent materials that can meet GAC (Green Analytical Chemistry) criteria maintaining high analytical standards. Notably, the promotion of sustainability is a central aim, both for the synthetic protocol and the sorbent's composition, along with its versatility for different applications. In this regard, β-cyclodextrin-based nanosponges (β-CDNSs) represent the perfect candidate as they are harmless, biodegradable, biocompatible, and inexpensive materials characterized by both hydrophilic and lipophilic properties. The synthesized β-CDNSs, in fact, are hyper-crosslinked polymers obtained by the reaction "in mass", i.e., without the aid of organic solvents, of β-CD (a natural starch degradation product) with citric acid used as a crosslinker, in the presence of a catalyst. The resulting material maintains the ability of β-CD to form inclusion complexes with nonpolar organic molecules and, at the same time, exhibits insolubility in water, which is essential for its use as a sustainable sorbent. The β-CDNS, following characterization performed by thermal analysis (TGA, DSC), IR spectroscopy, BET/BJH analysis, and SEM imaging, was exploited for a bioanalytical application consisting in the dispersive solid phase extraction (dSPE) of cholesterol from human plasma. Cholesterol is a naturally occurring sterol in the human body that performs several key functions that have also been recognized to have a predictive association with cardiovascular disease. Therefore, monitoring cholesterol in plasma is important to assess the risk of such diseases and intervening early to prevent complications. The excellent results obtained from both characterization data and recoveries (around 80 %) showed that the analyte adsorption is driven by the formation of inclusion complexes dependent on logP and analyte size: in fact, the high logP of cholesterol (7.11) allows the sorbent to easily trap the analyte molecules in the hydrophobic cavities; moreover, the size of the internal cavity of the β-CD units corresponds to the dimensions of cholesterol (6.2 Å), thus resulting in an efficient acceptor of this sterol.

Keywords: Nanosponges; cyclodextrins; dispersive solid phase extraction; cholesterol; human plasma

YS-011

Controlled Release of Silver Sulfadiazine from CeO_2-Loaded Chitosan Composite Films as Potential Wound Dressings

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Hydrogels are effective wound dressings due to their capacity to absorb wound exudate and maintain a moist environment for healing and with their film-forming properties, they can form a physical barrier against microbial contamination of the wound. In recent years, studies on new types of biopolymer wound dressings have intensified due to their biodegradable and biocompatible properties. Chitosan is a widely used biopolymer. An advantage of chitosan over other biopolymers is its antibacterial property. During the healing process, it is necessary to protect the wound against microorganisms that may cause infection. However, the antibacterial property of chitosan is weak, and the development of composite materials with additives to increase its antibacterial power is a common research area. In recent years, CeO_2 nanoparticles have attracted attention in biomedical applications with unique reversible transitions between Ce^{3+} and Ce^{4+} states, which is the basis of the antibacterial activity properties of these particles. The incorporation of CeO_2 NPs into biopolymer films has been shown to increase the antibacterial properties of the films [1]. Cerium oxide nanoparticles have been shown to work successfully in the healing of diabetic wounds [2,3]. Silver sulfadiazine (SSD) is an antibacterial drug with a broad spectrum of activity against microorganisms. SSD has been widely used for the treatment of burn wound infections. SSD in the cream form requires multiple daily applications. Cleaning the cream residues also damages the wound every time. The aim of this study is to provide a controlled release of SSD from chitosan films doped with SSD and CeO_2 nanoparticles. The hydrogels were characterized using Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction spectroscopy (XRD), and Field emission scanning electron microscopy (FESEM). The hydrogels were analyzed for porosity, gel content, Water vapor transmission, swelling, water retention, mechanical and antibacterial tests were performed for demonstrating that it has the properties of a wound dressing material. Controlled release experiments of SSD were comparatively examined from biopolymer films with and without CeO_2.

Keywords: Biopolymer, nano-ceria, film

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YS-012

An Upconverting Nanoparticle Based Photoelectrochemical Sensor for the Detection of ctDNA

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Cancer is the most common cause of death and its early diagnosis hold great importance by making it more likely to be treated successfully. Therefore, the key of increasing the survival rate is through diagnosing cancer at early stages. Circulating tumour DNA (ctDNA) originate from apoptotic and necrotic cancer cells and are secreted into circulation through the death of these cells. Detection through ctDNAs allow a non-invasive approach in any sensing platform. For this study, initially an electrochemical platform was chosen for being a highly sensitive technique. Specifically, single stranded DNA (ssDNA) modified screen printed gold working electrodes were exposed to their complementary strands and non-complementary DNA samples with varying mismatches and their Electrochemical Impedance Spectroscopy (EIS) responses were recorded. The system was found to be responding concentrations down to pM range based on complex Randles circuit. Next, photoelectrochemical design was tested to check if this range could be drawn down to lower concentrations. For this, $NaYF_4$:Yb,Er upconverting nanoparticles (UCNPs) were synthesized and employed The UCNPs absorb Near Infra-Red (NIR) radiation and emit visible light. They are known for their large Anti-Stokes shift and usage as photosensitizers in phototherapy. The carboxyl functionalized $NaYF_4$:Yb,Er particles were synthesized at relatively low temperature of 180°C in a Teflon autoclave. Transmission Electron Microscopy images showed particles to have cubic shape with 10 nm diameter. Existence of carboxyl groups was approved by Fourier Transform Infrared Spectroscopy. Their absorbance

and emission spectra were recorded and compared with those in the literature. The X-Ray Diffraction pattern indicated particles to be in α-phase. In the system proposed, target DNAs were labelled with as-synthesized UCNPs. After the hybridization of the two strands, the electrode surface was illuminated with a 980 nm laser and energy transfer mechanism was triggered upon the excitation of the particles. The photocurrent produced shows that changes at μA level can be detected in the proposed system that work with a low-energy triggered mechanism.

Keywords: Electrochemistry, photoelectrochemistry, upconverting nanoparticles, ctDNA

YS-013

Colloidal Silver Nanoparticle Color Change Determination Using Digitized RGB Intensities as a Smartphone Readout for Sensing Application

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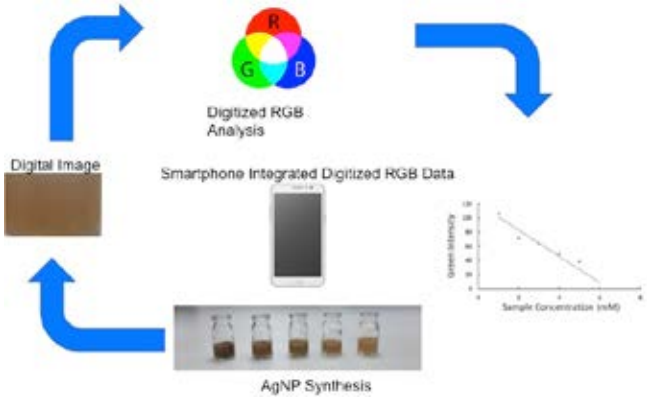
Colorimetric sensors have been widely used in point-of-care (POC) based systems due to their simplicity, low cost, and ease of use. Silver nanoparticles (AgNPs) have significant potential as a colorimetric sensor because of their excellent plasmonic properties in the visible region resulting in bright colors ranging from yellow to brown. In addition, colloidal AgNPs solutions are known to be easily functionalized with any analyte, including biomolecules (antibodies, enzymes, or nucleic acids), drugs, and other metals. The AgNP-based colloidal colorimetric sensor's operation is based on the binding of the target analyte to the AgNPs. This binding causes AgNP aggregation, which changes their optical properties such as color absorbance and fluorescence, which can be measured using sophisticated laboratory equipment. In this study, AgNPs were produced using an efficient chemical reduction process under varied alkaline environments and their optical characteristics and color change were studied using UV-Vis spectroscopy. Afterwards, the UV-Vis spectroscopy data were correlated with the smartphone-integrated RGB (Red-Green-Blue) analytical approach in order to developed a smartphone-based analytical method to detect the color change of AgNPs. In this approach, digitized RGB values were collected using pixel-based data point method to obtain color variations among different AgNPs solutions. The proposed smartphone-integrated RGB method showed that green values represented a significant change in terms of pixel densities compared to red and blue colors (R2 = 0.96). In addition, green pixel densities showed accurate quantitative results for the 50-200 mM NaOH concentration range. These results showed that using digitized RGB data as a smartphone readout provides various benefits, including miniaturization and real-time monitoring of AgNPs-based colloidal colorimetric sensors.

Keywords: Silver nanoparticle, RGB analysis, smartphone-based sensors

Figure 1



Digitized RGB intensities as a smartphone readout

YS-014

Advancing Single Cell Growth Measurement: A Plasmonic Functional Assay Platform with Refractive Index Sensing

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Abstract

Accessing and accurately quantifying cell growth on adhesive substrates is essential for the study of cellular biophysical properties and the evaluation of therapeutic responses to drug therapies. However, conventional optical techniques often suffer from low

sensitivity and variable reliability across different cell types, while microfluidic technologies are limited in their applicability to adherent cells due to their reliance on cell suspension.

In this study, a novel plasmonic functional assay platform has been developed for real-time measurement of individual cell weight and growth. To validate its capabilities, the growth profile of MCF-7 cells was investigated, focusing on the effects of serum starvation and the activity of ornithine decarboxylase (ODC), a key enzyme involved in cell proliferation that degrades under low osmolarity conditions inhibiting growth. The study successfully demonstrated significant differences in the growth profiles of MCF-7 cells and their ODC-overproducing variants, indicating enhanced resistance to the negative effects of low osmolarity. Additionally, the platform evaluated the therapeutic behavior of MCF-7 cells in response to difluoromethylornithine (DFMO), an ODC inhibitor, and identified a DFMO-resistant subpopulation capable of surviving DFMO treatment. The plasmonic functional assay platform provides valuable insights into intracellular activities and therapeutic behaviors of cancer cells. The rapid determination of cell growth kinetics through this platform has great potential for advancing cell biology research and enhancing therapeutic strategies.

Keywords: Plasmonic biosensing, label-free detection, cell growth profiling, functional assay platform, cellular metabolism analysis, nanohole arrays

I. Introduction

Examining individual cell responses to external stimuli can provide a more precise understanding of dynamic processes crucial for cell growth and molecular interactions. By focusing on single cells rather than large populations, it becomes possible to uncover variations in biophysical properties and identify small subpopulations of cancer cells that exhibit resistance to standard treatments (Housman et al., 2014).

In this study, a plasmonic platform is utilized for measuring cell growth as an alternative to optical and microfluidic technologies (Cetin et al., 2021). The platform monitors the effective refractive index near the surface of the plasmonic substrate to determine changes in cell mass. By analyzing spectral variations in the optical response of the plasmonic substrate, the relationship between cell growth and accumulated mass can be inferred (Giner-Casares et al., 2016) followed by chemical growth leading to anisotropic nanoparticles. The resulting plasmonic substrates show a broad plasmon band covering a wide part of the visible and near-infrared (NIR). The platform provides precise mass data from cells adhered to a sensing surface, eliminating the need for sample preparation and promoting their growth in a cell culture environment. Furthermore, the platform enables the evaluation of cell susceptibility or resistance to external stimuli or therapeutic agents by monitoring variations in their growth profiles. It holds potential for both basic and clinical research purposes, allowing for the investigation of single-cell signaling and the detection of secreted factors.

II. Material and method

- **Plasmonic chip preparation**

The plasmonic chip consists of nanohole arrays on a 120 nm aluminum film with a silicon dioxide coating. It is supported by a silicon nitride interlayer on a fused silica substrate, enabling a well-defined and isolated plasmonic mode.

- **Experimental setup**

The experimental setup involves illuminating the plasmonic chip with a white LED source, collecting the transmitted light with a microscope, and connecting it to a multichannel spectrometer as shown in Figure 1. Mechanical effects are minimized using a vibration isolator and motorized translation stage. The transmission response of nanohole arrays is smoothed with a Savitzky-Golay filter, and cell cultures are kept in a controlled environment.

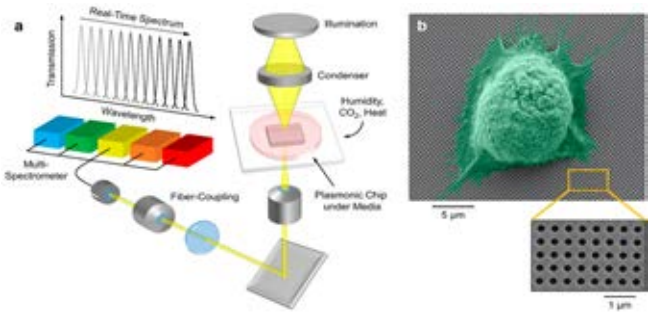

Fig. 1: The Plasmonic Cell–Growth Profiling Platform (A). Scanning electron microscopy (SEM) of MCF-7 cells adhered to a plasmonic chip surface (B).

- **Cell culture and plasmonic chip preparation for cell seeding**

The MCF-7 cell line was maintained in RPMI 1640 growth medium supplemented with 10% fetal bovine serum, 10% penicillin, and 1% non-essential amino acids, while the plasmonic chip surface was coated with a polymer layer for protection. After removing the polymer layer, the silicon dioxide surface of chip facilitated cell seeding at a density of 1×10^4 cells/mL for growth profiling after 24 hours.

- **Preparation of a drug-resistant (DR) cell line**

DFMO concentrations were incrementally increased over 4 weeks to cultivate drug-resistant MCF-7 cells. After 28 weeks, resistant cells were obtained with comparable growth to wild-type cells. DR cells were maintained with periodic 0.1 mM DFMO addition during passages.

III. Results and discussion

- **Principle of the cell-growth profiling platform**

The cell-growth profiling platform utilizes a highly sensitive plasmonic chip with nanohole arrays to monitor changes in cell mass. By measuring the transmission response and considering collective spectral variations, the platform accurately determines cell growth rates. Integrated with a cell culture incubator, it ensures healthy cell proliferation and eliminates the effects of cell migration on measurements.

- **Quantifying heterogeneity in cell-growth profiles with the plasmonic platform**

The Plasmonic Platform monitors real-time changes in cell mass, enabling accurate assessment of heterogeneity in cell-growth profiles (Figure 2). Mapping the Spectral Index of Refraction (SIR) against the initial Spectral Index (SI) values creates a 2-dimensional growth profile, identifying cells with different masses and accumulation rates. Precise measurements are achieved by separating cells on the chip surface and normalizing SIR by SI, while excluding doubling cells or clusters ensures reliable mapping of the growth profile of the cell population.

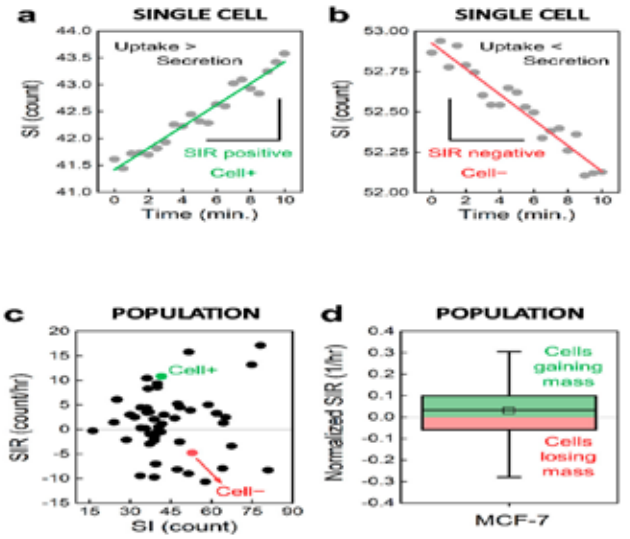

Fig. 2: The capability of the plasmonic platform to assess heterogeneity in growth profiles. It uses SI measurements to track the change in mass for cells gaining (A) or losing (B) mass over time. The SIR vs SI data (C) is presented as a population map, and (D) it is depicted as a 1-dimensional box plot with normalized SIR values.

- **Analyzing the effect of serum starvation on cell growth using a plasmonic platform**

The platform explores intracellular pathways through the impact of starvation on cell growth. It observes reversible cell cycle arrest at the G1 phase during serum starvation. Growth profile changes are monitored using SIR vs SI data. Starvation reduces cell growth, which is restored gradually upon reintroduction of growth factors. Normalized SIR profiles reveal cell cycle arrest and recovery.

- **Using plasmonic platforms to analyze the effect of intracellular metabolism on cell growth**

on cell growth, focusing on ODC. DFMO-induced decreases in normalized SIR profiles are concentration-dependent. DFMO-resistant cells exhibit resistance to the effects of DFMO on cell growth. The platform assesses cell differences and therapeutic responses, with applications in evaluating standard-of-care drugs.

- **Investigating cellular response to hypoosmotic stress through plasmonic platform analysis of polyamine metabolism**

The cell-growth profiling platform assessed the influence of hypoosmotic stress on cell growth and ODC enzyme activity. Low osmolarity significantly reduced cell growth, while drug-resistant cells exhibited higher growth rates than wild-type cells. The platform effectively distinguished between sensitive and resistant cells, highlighting the role of ODC activity in cellular response to stress.

- **Uptake of exogenous polyamines recovers ODC inhibition**

Polyamines are vital for cell growth. Low osmolarity affects polyamine synthesis, but adding putrescine rescues cell growth. Cells with high ODC activity benefit more from putrescine, while DR cells adapt better to low osmolarity due to their high ODC activity. WT cells are more reliant on putrescine for survival.

- **Enhanced recovery after hypoosmotic shock in cells with high ODC activity**

Cells with high ODC activity, known as DR cells, exhibit superior adaptation to hypoosmotic shock. Compared to WT cells, DR cells demonstrate faster recovery of their growth profile after returning to normal osmotic conditions.

IV. Conclusion

A label-free plasmonic functional assay platform capable of real-time determination of cell growth profiles. It has wide-ranging applications in investigating cell biophysical properties, identifying intracellular pathways, and monitoring dynamic cell activities. The platform enables the characterization of drug resistance and phenotypic subpopulations by analyzing cell responses to specific treatments. By assessing growth profile heterogeneity, it provides insights into different cell metabolisms and cell cycle-dependent pathways. Although the platform has strong growth-profiling capabilities, further optimization is required for practical use. Potential improvements include adapting it for suspended cells using binding agents, enhancing spectral variations for low refractive index changes, automating position determination, and increasing throughput by performing simultaneous measurements. These advancements would facilitate single-cell analyses, enabling the evaluation of population heterogeneity in shorter durations for faster test

results. The technique holds potential for applications in various contexts, including tumorigenic growth, metabolic disorders, and personalized drug therapies by providing therapeutic profiles for cancer patients.

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YS-015

Comparison of Antibody and Lobaric Acid Small Molecule Application in the Treatment Of Ovarian Cancer at the Transcriptome Level in the IL-6R / STAT3 / PD-L1 Pathway

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Due to the difficulty of drug apply and toxic effects in ovarian cancer treatment methods, drug resistance, and low response rate to treatment, further research is needed for the treatment of ovarian cancer. Immunotherapy applications such as immune checkpoint blockade show promise in cancer treatment. Further investigation of checkpoint blockade in ovarian cancer, mainly programmed death protein (PD-1) and ligand 1 (PD-L1), IL-6R, and STAT3 pathways, is very important in terms of finding an effective treatment solution. In this study, it was aimed to investigate the anti-proliferative effect of lobaric acid (*LA*), a lichen secondary metabolite with many biological activities, and its potential in cancer immunotherapy at the transcriptomatic level on human ovarian cancer. The anti-proliferative effects of drug candidate small molecule *LA* on OVCAR3, SKOV3, A2780 human ovarian cancer cell lines, and OSE normal epithelial cell have been investigated by xCELLigence Real-Time Cell Analyzer. *LA* does not show cytotoxic effect on OSE cells, but it has been determined that it has an anti-proliferative effect on ovarian cancer cells. As *LA* showed the highest anti-proliferative effect on OVCAR-3 ovarian cancer cells, the synergistic effect of IC_{50} concentration of *LA* with *atezolizumab* (*ATE*, PD-L1 targeted mab) and *tocilizumab* (*Toci*, IL-6R targeted mab) was also determined by xCELLigence analysis. Moreover, the inhibition potential of the IL-6R/STAT3/PD-L1 pathway was determined at the mRNA level by the application of lobaric acid on ovarian cancer cells. For qRT-PCR analysis, six target genes (*CD-274, STAT1, STAT3, JAK1, JAK2, IL-6R*) were used for *LA, ATE, Toci, LA+ATE, LA+Toci, Control* samples and *ACTB* gene was used as housekeeping. The study was performed in two repetitions using stu-t test statistical analysis method and $2^{-\Delta\Delta Ct}$ method. As a result of anti-proliferative effect, IC_{50} concentration of *LA, ATE,* and *Toci* were determined 11.7, 10.7 0.021 µM, respectively. According to qRT-PCR results, there was a 14.47-fold decrease in expression level of *CD-274* (PD-L1) gene with *LA* treatment, 24.59-fold in *ATE,* and 48-fold in *Toci*, a monoclonal antibody targeting PD-1/PD-L1. In the *LA+ATE* and *LA+Toci* groups, 185.46 and 92.09-fold decrease in gene expression was found. In addition, *STAT1, STAT3, JAK1, JAK2, IL-6R* genes were suppressed in *LA, ATE, Toci, LA+ATE, LA+Toci* samples. As a result, it was revealed that *LA* and *LA*+monoclonal antibody combinations suppressed the IL-6R/STAT3/PD-L1 pathway in OVCAR-3 ovarian cancer cells. In Conclusion, results were obtained for the first time that lobaric acid will show less toxicity than ovarian cancer targeted chemotherapeutic drugs, and that it can target IL-6R/STAT3/PD-L1 pathways differently or sinergistly than monoclonal antibodys. LA could be considered as a promising drug candidate molecule for ovarian cancer in cancer immunotherapy applications.

Keywords: Lobaric Acid, ovarian cancer, IL-6, STAT3, PD-L1.

YS-016

Bacterial Espresso? Accelerated Solvent Extraction of Bacterial Cell Pellet as a Method for the Improved Extraction Recovery of Selected Metabolites

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Bacterial secondary metabolites have been a fruitful source of numerous biologically active compounds (Zhang et al. 2015). However, drug discovery from bacteria can be time-consuming and resource intensive. Organic solvents used in bacterial metabolite extraction are also costly to dispose of and can pose a hazard to human and environmental health (Barragán-Martínez et al. 2012, Pena-Pereira et al. 2015). New technologies like next-generation sequencing, molecular networking, and ultra-performance-liquid-chromatography have enabled a renaissance of bacterial natural product discovery (Traxler et al. 2015). Why then, have the techniques used to extract bacterial compounds remained relatively unchanged for the past 50 years (Katz and Baltz 2016)? Therefore, an extraction technique which substantially increases the yield of specialized metabolites per unit of biomass, as well as being more efficient than traditional methods would be an improvement in most experimental designs. Here we introduce the utilization of accelerated solvent extraction or ASE in the extraction of two distinct specialized metabolites, undecylprodigiosin and ectoine, from the model organism Streptomyces coelicolor A3(2).

The ASE method was optimized using five key variables, sample preparation, solvent choice, temperature, static time, and number of cycles. Preliminary results of ethyl acetate extraction indicate a significant advantage to ASE when compared to lyophilization and subsequent sonication of the biomass in undecylprodigiosin recovery when ethyl acetate is the solvent.

Keywords: Bacteria, Metabolites, Extraction, ASE

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YS-017

Application of Magnetic Solid Phase Extraction for Sensitive and Simultaneous Analysis of Glipizide and Pravastatin Drugs in Synthetic Urine Samples with HPLC-DAD

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Use of antidiabetic and antilipidemic drugs have increased in recent years due to prevalence of chronic disease. So, importance of analysis in these drug ingredients have been taking attention in order to follow their therapeutic and toxicological effects. Therefore, developing of new analytical techniques is a challenge detection of drugs at trace levels in biological samples (Sher,2019). Drug active substances in this study are pravastatin sodium (PRV), which is used in the treatment of hyperlipidemia and hypercholesterolemia, and glipizide (GLP), an oral antidiabetic contains sulfonylurea group. Sample pretreatment methods used in pharmaceutical analysis provide both an enrichment of target molecules and a separation of matrix components. So, sensitive and selective determinations can be performed with a conventional instrumental system. Magnetic solid phase extraction(MSPE) was preferred for easy applicable, cheap, fast, and selective properties and applied prior to HPLC-DAD determination step. (Capriotti et. al.,2019)

Melanin coated Fe_3O_4 magnetic nanoparticles (Fe_3O_4@TEOS-Melanin) were newly synthesized and used as solid

phase sorbent for sensitive extraction of these compounds. Characterization of magnetic particles was carried out by Scanning Electron Microscopy(SEM), Raman Spectroscopy, X-ray Diffraction(XRD), and Fourier transformation infrared(FTIR) techniques. Experimental variables in MSPE were studied and optimized including pH, adsorption time, desorption eluent and desorption time. HPLC conditions for analysis of GLP and PRV were pH 3.0 (0.02 M KH_2PO_4):acetonitrile (65:35) by using phenyl hexyl column (250mm, 4,6mm) with 1.0 mL min-1 flow rate. After validation procedures, the linear concentration range for GLP and PRV molecules was obtained in the range of 10.00-750.00 ng mL^{-1}, limit of detection (LOD) and limit of quantification (LOQ) were 3.03 and 9.09 ng mL-1, respectively. The developed method showed satisfactory reproducibility with relative standard deviation (RSD %) less than 3.5% in triplicate measurements by using model solutions containing 100 ng mL-1 of GLP and PRV. Accuracy of the method was tested by means of recovery test were studied and acceptable results were obtained in the range of 95.3-103.2 %. Finally, the developed method was successfully applied to synthetic urine.

Keywords: Glipizide, Pravastatin Sodium, MSPE, HPLC

YS-018

A Liquid Phase Microextraction Method For Extraction and Preconcentration of Parabens in Lipstick Samples Prior to High Performance Liquid Chromatography – UV Detection

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The potential for parabens to disrupt the endocrine system and their relation to cancer has led to considerable debates about their impact [1]. As a result, it is crucial to analyze cosmetic products for the sake of human health and safety. In this study, a highly precise and sensitive liquid phase microextraction method for determining the presence of five types of parabens at trace levels using high performance liquid chromatography. The method's parameters were optimized to increase the extraction efficiency of the analytes. The analytes were eluted using a mobile phase containing a 50 mM ammonium formate aqueous solution (pH 4.0) and acetonitrile (60:40, v/v) and were detected using a UV detector at a wavelength of 254 nm. The method's performance for methyl, ethyl, propyl, butyl, and benzyl parabens was determined, and the analytes had detection limits between 0.33 and 0.78 µg kg-1. The developed method was used to analyze four lipstick samples. The paraben levels in the samples were quantified using matrix-matched calibration standards and found to be in the range of 0.11-1.03%.

Keywords: Paraben, lipstick, high pressure liquid chromatography, dispersive liquid-liquid microextraction

YS-019

Determination of Chloroquine Phosphate at Trace Levels in Human Serum, Saliva and Urine Samples by Gas Chromatography–Mass Spectrometry after Vortex Assisted Spraying Based Fine Droplet Formation Liquid Phase Microextraction

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The COVID-19 pandemic which firstly detected in Wuhan, China in the last month of 2019 [1] has led to millions of death toll all around the world. In these years, various drugs were studied or produced to combat with the pandemic [2]. Chloroquine was an important medication in the prevention and treatment of malaria

[3] and this drug was shown as candidate drug due to its antiviral properties for the treatment of SARS-CoV-2 symptoms [4]. It is also a cost-effective drug, but can increase the risk of heart attack for humans [5] and long-term negative effects on aquatic animals [6]. For this reason, accurate and sensitive analytical methods for the detection of chloroquine at trace levels in human serum, saliva and urine samples have become more important to protect human health and environment from negative impacts of this drug. In this study, chloroquine phosphate was extracted from the biofluid samples by vortex assisted spraying based fine droplet formation liquid phase microextraction (VA-SFDF-LPME) method and detected by gas chromatography–mass spectrometry (GC-MS). The proposed VA-SFDF-LPME method consumed only microliter levels of extraction solvent for each sample due to the usage of lab-made spraying system. Analytical performance studies were also performed for the developed system and limit of detection/limit of quantitation, dynamic range and enhancement in detection power of GC-MS system were found as 2.8/9.2 µg/kg, 9.9–1003.9 µg/kg and 317 folds, respectively. Spiking experiments were conducted to assess the applicability and accuracy of the developed method and recovery results were varied from 90.9% to 114.0% for the selected samples [4].

Acknowledgements

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Keywords: Chloroquine, gas chromatography-mass spectrometry, vortex assisted spraying based fine droplet formation liquid phase microextraction, human urine, human serum, human saliva.

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YS-020

Determination of Vitamin B12 in Vitamin Tablets by High Performance Liquid Chromatography Combined with Flame Atomic Absorption Spectrophotometry with a Novel Nebulizer Interface Supported T-Shaped Slotted Quartz Tube

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Hyphenated systems consisting of chromatographic and spectroscopic methods have drawn attention among scientists [1]. Liquid chromatography-mass spectrometry (LC-MS) is a useful hyphenated system, but it has intensive cost and signal depression problems [2]. Elemental detection systems such as atomic absorption [3] and atomic fluorescence [4] have been also used in literature. Among them, flame atomic absorption spectrophotometry (FAAS) is well-known as its easy practice and low cost [5]. However, it suffers from low nebulization efficiency and short residence time of atoms [6]. Slotted quartz tube (SQT) was produced to solve these problems in FAAS system [7]. In this study, high performance liquid chromatography (HPLC) and FAAS combined by a novel nebulizer interface (NI) supported T-shaped slotted quartz tube (T-SQT) was firstly designed and used for the determination of Vitamin B12 in vitamin tablets. Vitamin B12 was separated on anion exchange column and then the eluent was directly transferred to flame region by the help of NI-T-SQT system. Vitamin B12 was detected in FAAS system via cobalt atom in its molecular structure. Under the optimum experimental conditions, limit of detection/limit of quantitation and linear range were calculated as 1.6/5.3 mg/kg (as Co) and 4.7–92 mg/kg (as Co), respectively. The developed HPLC-NI-T-SQT-FAAS system was evaluated in terms of applicability and accuracy. For this purpose, two vitamin sample solutions were spiked to different concentrations of Vitamin B12 and matrix matching calibration strategy was performed to calculate percent

recovery results. Acceptable recovery results (92.9–112.4%) were achieved by the developed system that proved the applicability and accuracy of the HPLC-NI-T-SQT-FAAS system [8].

Keywords: Vitamin B12, flame atomic absorption spectrophotometry, high performance liquid chromatography, nebulizer interface, T shaped slotted quartz tube

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YS-021

18O Assisted GC-MS Based Fluxomics Analysis for Central Carbon Metabolism Intermediates in Colorectal Cancer Cell Line

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Central carbon metabolism is a primary energy source for all living creatures and is regulated by many enzymes. Modifying these enzymes and intermediates can aid in understanding disease mechanisms and plays a critical role in cancer including colorectal cancer [1-2]. Colorectal cancer was reported as the second most prevalent cancer in women and the third most common cancer in males [3]. Recent studies indicated that the characterization of metabolic phenotypes should consider metabolite levels and turnover/flux rates [4-5]. Therefore, flux rates in addition to metabolic levels are critical for completely analyzing metabolic dynamics and the functions of pathways. The use of stable isotopes ^{13}C, ^{15}N, and ^{18}O allows for the simultaneous measurement of metabolite levels and turnover rates. ^{18}O-based stable isotope labeling has gained popularity due to its short labeling time and ability to monitor a larger metabolomic network than other stable isotopes. In this study, for the first time, ^{18}O-based stable isotope labeling GC-MS analysis was developed to determine the turnover rates of central carbon metabolism intermediates and better understand colorectal cancer cell line dynamics. The method parameters including labeling time, the selection of m/z values for single ion monitoring (SIM), and chromatographic conditions were optimized. Finally, the developed method was applied to CaCo-2 cell lines, and turnover rates of inorganic phosphate, glucose-6-phosphate, glycerol-3-phosphate, pyrophosphate, monomethyl phosphate, 3-phosphoglyceric acid and phosphatidylcholine was found significantly altered in CaCo-2 compared to FHC cell line.

Keywords: Fluxomics, ^{18}O stable isotope, GC-MS, CaCo-2, FHC

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YS-022

Molecular and Metabolic Characterization of Multi-Drug Resistant Isolates of *Acinetobacter Baumannii*

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Since a comprehensive understanding of the resistance mechanisms in multidrug-resistant *Acinetobacter baumannii* (MDRAB) can enlighten possible strategies to tackle the dissemination of antimicrobial resistance. The aim of this study was to investigate the relationship between genotypic resistance patterns of MDRAB isolated from various clinical samples and metabolic pathways.

Total of 100 *A. baumannii* strains were collected in 2019 from Hacettepe University hospital clinical bacteriology laboratory. MALDI-TOF MS was used for bacterial identification. Antibiotic susceptibility profiles were determined by the disk diffusion and broth microdilution method. MDR was defined as resistance to at least one antibiotic in three or more antimicrobial groups. Resistance to all tested antimicrobials, including carbapenems and colistin was classified as pandrug-resistant (PDR) isolates. Biofilm formation was measured by crystal violet staining method. Resistance genes (*bla*OXA-23, *bla*OXA-51, *bla*OXA-24, *bla*OXA-58, ISAba1, *aac*(3)-I, *aac*(6')-Ib, *arm*A and *ade*B were characterized by PCR. Clonal relatedness of the PDR isolates was determined by AP-PCR.

Gas chromatography/Mass spectroscopy (GC/MS) based untargeted metabolomics analysis was performed to understand phenotype differences between resistant and sensitive isolates. After derivization process metabolites were analyzed with GC/MS system between 50-650 m/z value in positive mode. Raw MS data was evaluated in MS-DIAL platform. Fiehn retention index database was preferred for metabolite identification. Statistical analysis and pathway enrichment analysis was carried out in Metaboanalyst 5.0 platform.

All of the strains were resistant to ceftazidime, imipenem, meropenem, ciprofloxacin and piperacillin/tazobactam (100%) while most strains were susceptible to colistin (86%). All strains were positive for *bla*OXA-51. Ninety-one (91%) out of 100 strains had the *bla*OXA-23 gene. However, all strains were negative for the *bla*OXA-58 and *bla*OXA-24. Eighty-nine (89%) strains carrying IS element (ISAba1). The *arm*A gene were detected at higher abundance of 71% compared with the other aminoglycoside resistance genes [*aac*(3)-I (17%) and *aac*(6')-Ib) (15%)]. None of the colistin resistant isolates were positive for *mcr*1-5 variants. Among 100 *A. baumannii* strains, 85% carried the *ade*B efflux pump gene. According to their biofilm formation ability, 29.7% were strong, 34.1% moderate biofilm formers; 16.5% were non-biofilm formers. 11 out of 14 colistin resistant isolates were resistant to all tested antibiotics and classified as pan-drug resistant. All pan-drug resistant strains were positive for *bla*OXA-51 and *bla*OXA-23. All isolates except one was carrying ISAba1. *arm*A was positive in 8 pandrug isolates, whereas only 1 isolate with *aac*(3)-I and 1 isolate for *aac*(6')-Ib were detected among pandrug isolates. 8 of them were positive efflux pump multidrug transporter protein *ade*B.

In metabolomics analysis, results showed that 28 metabolites were altered significantly. These metabolites were evaluated in pathway analysis. Results revealed that amino acid and energy metabolism pathways significantly changed in resistant isolates.

The use of metabolomic methods in the clinical treatment of MDRAB is of utmost importance. It has the potential to provide new diagnostic tools and identify new drug targets. The integration of metabolomics into clinical practice will not only improve patient outcomes but also help to combat the emergence of MDR *A. baumannii*.

Keywords: Acinetobacter baumannii, Metabolomics, GC-MS, PCR, Multi-drug resistant

YS-023

Sophisticated Mass Spectrometry-Based Techniques for the Identification of Novel Mycosporine-Like Amino Acids in Marine Organisms

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Marine organisms like algae, cyanobacteria, and even fish often contain secondary metabolites with the ability to filter harmful UV-A and -B radiation due to their exceptionally high

molar extinction coefficients at wavelengths between 310 and 360 nm. These natural UV filters include mycosporine-like amino acids (MAAs), which also have promising effects on immunostimulation, skin ageing and inflammation, indicating a potential use in cosmetic formulations [1]. However, their commercial success is hampered by their low abundance in biomaterials, their labor-intensive isolation, and a lack of creative ways to identify new MAAs.

This lecture will present an application of the novel mass spectrometry-based technique "Ion Identity Molecular Networking" (IIMN) [2], combining efficient chromatographic separation (UHPLC), high-resolution tandem mass spectrometry ($HRMS^2$) and advanced bioinformatics, for the analysis of MAAs. After a general introduction to the basic principles of IIMN, its appeal as an accessible tool for the visualization and multifaceted exploration of large datasets (e.g., related to chemo-/taxonomy, UV response, bioactivity, etc.) will be highlighted. For this purpose, the results of a proof-of-concept study are addressed in which crude algal and fish eye extracts were analyzed using a dedicated UHPLC-$HRMS^2$ IIMN workflow. By leveraging the fact that MAAs consist of a limited number of building blocks, namely a cyclohexenone or cyclohexenimine scaffold substituted with different amino acids, a combinatorial database of theoretical structures was created and used for combinatorial molecular network annotation propagation [3]. Spectral matching of the obtained data with open-source and combinatorial libraries afforded the straightforward dereplication of known MAAs as well as the putative annotation of undiscovered ones. SIRIUS [4], a java-based software framework for the analysis of LC-MS^2 data, was employed to further increase the credibility of spectral hits. Based on the overall output of this contemporary approach, the genus *Bostrychia* was identified as a highly promising source for the isolation of novel MAAs.

The combination of state-of-the-art separation techniques, mass spectrometry, and user-friendly bioinformatics tools provides an intriguing approach for the comprehensive and comprehensible assessment of the chemical space covered by natural extracts. Its application in phytochemical research could pave the way for rapid progress, even in areas struggling with difficult-to-handle compounds such as MAAs.

Keywords: mycosporine-like amino acids, marine organisms, molecular networking, network annotation propagation, UHPLC-HRMS/MS

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YS-024

Fast and Sensitive Determination of Orotic Acid in Urine Samples with Capillary Electrophoresis Coupled with Contactless Conductivity Detection

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Orotic acid (1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic acid), a small organic molecule in carboxylic acid structure considering as key biomarker for diagnosis of several metabolic disorders [1]. Especially for newborns assessment of the amounts of orotic acid in urine is vitally important. Capillary electrophoresis as both analysis and separation technique, depends on the migration of the analytes under high voltage in a capillary column serves many advantages; namely small amount of sample consumption, high sensitivity and shorter analysis time [2]. In this novel work, simple, rapid and highly sensitive diagnostic method was developed with employing capillary electrophoresis coupled with contactless conductivity detection towards orotic acid. The sensitivity of the method further increased with field amplified sample stacking procedure which depends on altering the conductivities of the separation buffer and the sample solution after a large volume of sample injection. The optimized separation conditions were selected as 20 mM MES, 10 mM Histidine and 0.1 mM CTAB at pH of 6.5. The separation was performed at 25 kV electric field at 25°C, sample injection at cathodic side electrokinetically. The developed method was validated and the reproducibility of the method was tested and satisfactory results were found. Applicability of the proposed method to the urine samples were also tested and the orotic acid amounts of were quantified without any time-consuming sample preparation procedures. The satisfactory recoveries were found in between 80.02- 92.25 %.

Keywords: Orotic acid, capillary electrophoresis, contactless

conductivity detection

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YS-025

Enhancing Anticancer Potential: Optimization of Niclosamide-Loaded Lipid Nanocapsules for Improved Delivery and Quantification

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Abstract

Niclosamide, a highly hydrophobic molecule, has shown promise as an anthelmintic drug and potential anticancer agent in recent studies. With the rise of nano-sized drug delivery systems in cancer treatment, lipid nanocapsules (LNCs) have emerged as a promising approach to enhance the bioavailability of drugs with low water solubility due to their advantageous properties. This study focuses on the preparation of niclosamide loaded LNC formulations (NIC-LNCs) using the phase inversion method, which were subsequently optimized utilizing the Box-Behnken experimental design approach. Additionally, the development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of niclosamide in the LNC formulations was presented.

To achieve optimal chromatographic separation, an Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 µm) was employed with a mobile phase consisting of acetonitrile and 0.1% H_3PO_4 phosphate buffer in a 50:50 v/v ratio, flowing at a rate of 1.2 mL/min. The detection wavelength was set at 335 nm, and the analysis was performed at a temperature of 35°C. Validation of the developed method encompassed assessments of linearity, precision, accuracy, limit of detection, limit of quantitation, specificity and stability.

Furthermore, the optimization of NIC-LNC formulation was conducted using the Box-Behnken design. The optimized formulation, designated as LNC 5, comprised 4% NIC, 20% lipid and 20% surfactant. The successful development and validation of the RP-HPLC method facilitated the accurate determination of niclosamide content in the LNC formulations, while the optimized NIC-LNC formulation demonstrated improved properties based on the Box-Behnken experimental design. These findings contribute to the advancement of LNC as a potential therapeutic option in cancer treatment, particularly for cancers with low water solubility.

Keywords: Niclosamide, HPLC, analitic validadion, optimization, lipid nanocapsules, Box–Behnken design

I. Introduction

Niclosamide (NIC) is a FDA-approved oral anthelmintic agent used in various tapeworm treatments in humans. In recent years, it has been found to show anticancer activity, especially by inhibiting the STAT3 signaling pathway. The low water solubility of niclosamide is a major problem when a systemic effect is desired. Lipid-based nanocarriers are gaining increasing attention as drug delivery systems for drug with with poor oral bioavailability. LNCs are new generation nanocarriers systems consisting of an oily liquid core surrounded by a hydrophilic and lipophilic rigid surfactant shell[2]. High Performance Liquid Chromatography (HPLC) is the most widely used technique to identify, quantify and separation of the components. Analytical method validation is a crucial part of formulation development. In this study, NIC-LNC formulations were prepared by the phase inversion method that does not contain any organic solvents. The one of the main aim of this research is the method development and validation for the quantitation of NIC from LNCs. In addition, it is also aimed to determine the optimum formulation by examining the effects of various formulation parameters on the physicochemical properties of NIC-LNC with experimental design.

II. Material and method

Analytical grade phosphoric acid (85%), sodium hydroxide, sodium chloride (NaCl) HPLC grade acetonitrile (ACN), methanol and NIC were purchased from Sigma-Aldrich. Labrafac® WL1349, Lipoid® S75-3 and Kolliphor HS15 were gifts from Gattefossé, Lipoid GmbH and BASF, respectively. The Agilent 1100 series LC system was used for the method development, forced degradation and validation studies. The method was validated according to International Council on Harmonisation (ICH) guidelines. NIC-LNCs were prepared according to the phase inversion technique described by [2] . NIC were dissolved

in the Labrafac® WL1349 prior to all preparation steps by ultrasonication for 15 min. Lipoid® S75-3, Solutol® HS15, NaCl and purified water were added to this oil phase and all components were homogenized under magnetic stirring. Three cycles of progressive heating and cooling in between 60°C and 85°C were carried out, followed by an irreversible shock induced by dilution with cold purified water added to the mixture at 72°C. Finally, the nanosuspension was stirred under slow magnetic stirring for 5 min. Box Behnken experimental design was used for the optimization of NIC-LNCs (Table 1). Encapsulation efficiency (EE) was selected as a dependent parameter and determined by the indirect method. The design data was analysed using the design Expert 6.0.8 statistical package program (USA). Particle size, polydispersity index (PDI) and zeta potential analyses were performed by Malvern Zetasizer Nano ZS.

Table 1. Variables used in Box–Behnken experimental design

Levels	Independent Variables		
	X1	X2	X3
-1	2	10	10
0	4	20	20
+1	8	30	40

X1: Niclosamide %; X2: Oil %; X3: Hydrophilic surfactant %

III. Results and discussion

During the method development process, the most suitable analytical conditions were tried to be found by changing the wavelength, mobile phase ratio, temperature, pH, flow rate, organic solvent type and stationary phase, respectively (Fig. 1)

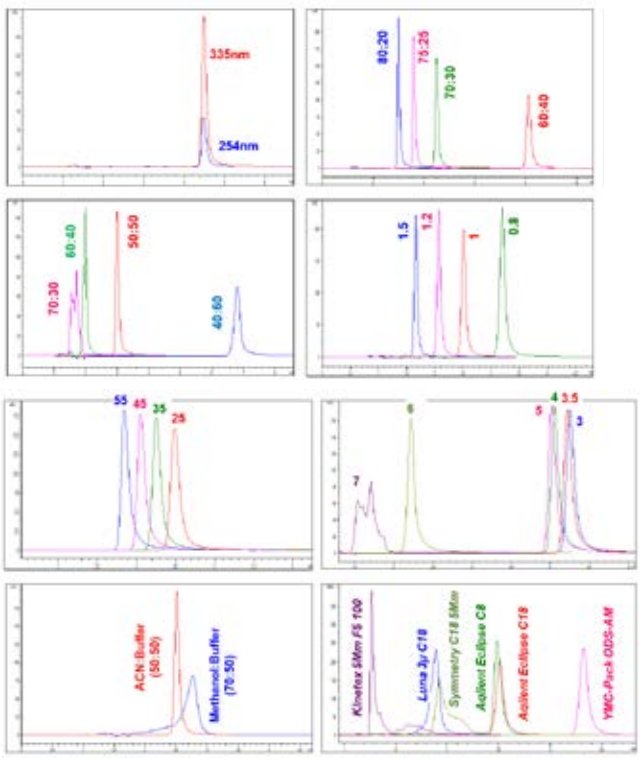


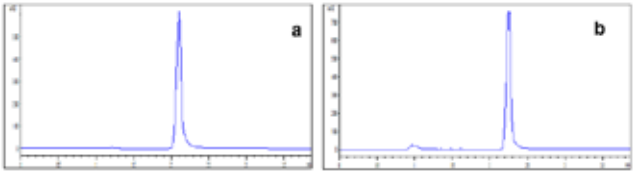
Fig. 1. a) Determination of wavelength b) Optimization of mobile phase [Agilent Eclipse XDB-C18 (5µm, 150x4.6mm) column, Buffer pH:3, 25°C temperature, Flow rate: 1mL/min)] c) Optimization of temperature [Agilent Eclipse XDB-C18 (5µm, 150x4.6mm) column, ACN:Buffer: 70:30 (v/v), Buffer pH:3, Flow rate: 1mL/min)], d) Optimization of pH, [Agilent Eclipse XDB-C18 (5µm, 150x4.6mm) column, ACN:Buffer: 70:30 (v/v), 35°C temperature, Flow rate: 1mL/min)], e) Optimization of the mobile phase at pH 7, [Agilent Eclipse XDB-C18 (5µm, 150x4.6mm) column, 35°C temperature, Flow rate: 1mL/min)], f) Optimization of flow rate [Agilent Eclipse XDB-C18 (5µm, 150x4.6mm) column, 35°C temperature, ACN:Buffer: 50:50 (v/v), Buffer pH:7], g) Optimization of organic solvent [Agilent Eclipse XDB-C18 (5µm, 150x4.6mm) column, 35°C temperature, Buffer pH:7], h) Optimization of stationary phase [35°C temperature, ACN:Buffer: 50:50 (v/v), Buffer pH:7, Flow rate: 1.2 mL/min]

As a result, the most suitable experimental conditions obtained through optimization studies are reported in Table 2.

Table 2. Conditions for optimum in vitro assay of NIC by HPLC

Column	Agilent Eclipse XDB-C18 (150x4.6mm, 5 µm)
Mobile Phase	ACN: 0.1% H_3PO_4 phosphate buffer (50:50 v/v)
Buffer pH	7
Flow Rate	1.2 mL/min
Temperature	35°C
Wavelength	335 nm
Injection Volume	20 µL

The optimized method was validated according to the ICH guidelines. Linear regression data showed good linearity at nine concentration levels between 0.1-200 µg/mL and the coefficient of determination for the calibration curve was found to be 1.00. The results for intraday precision and interday precision presented in terms of RSD% are less than 2%. The specificity was approved by forced degradation studies. To evaluate the ability of the optimized chromatographic method to separate possible degradation products of NIC, studies were carried out under mild and forced stress conditions over acidic and basic degradation, oxidation, photodegradation and thermal degradation (Fig. 2 and Fig. 3). With the separation of the observed degradation products and NIC peaks, the developed method proved to be highly specific. LOD and LOQ values were founded 0.0285 and 0.0863 µg/mL, respectively. The accuracy of the method was proven with the results close to 100% (98.68%-100.97%) and the % RSD values were less than 2%.



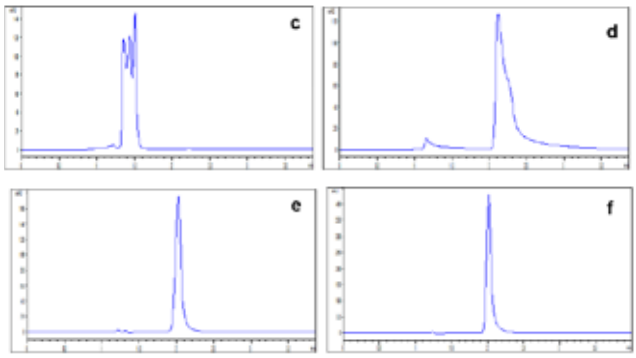


Fig. 2. Chromatograms presented mild conditions (a) Standard chromatogram, (b) 0.1M HCl, (c) 0.1M NaOH, (d) 3% H_2O_2 (e) 24h dry heat in oven at 60°C, (f) 12h UV light at 254 nm

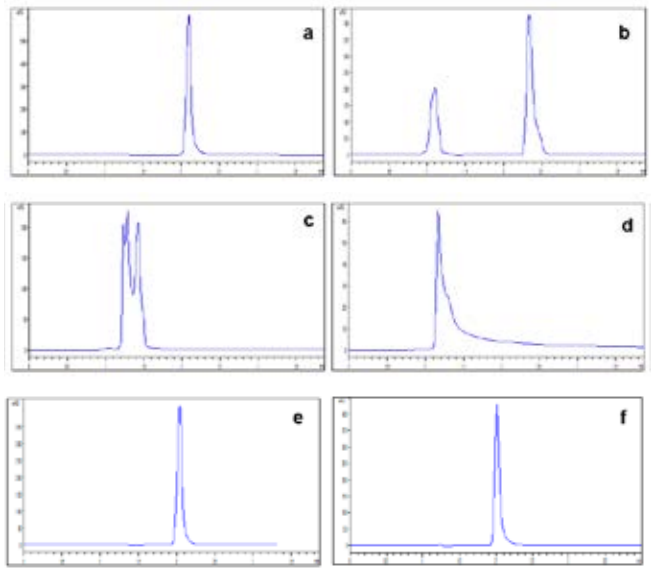


Fig. 3. Chromatograms presented drastic conditions (a) Standard chromatogram, (b) 1M HCl, (c) 1M NaOH, (d) 30% H_2O_2 (e) 48h dry heat in oven at 60°C, (f) 24h UV light at 254 nm

EE values of the NIC-LNCs were determined between 98.18-100% (Table 3). According to the Box-Behnken experimental design results, it was found that dependent variable X1 (p=0.6293) did not have a significant effect on the EE values of the nanocapsule formulation of NIC, whereas, both input variables X2 (p=0.0753) and X3 (p=0.0867) had a statistically significant effect on EE data (p<0.05). It has been possible to achieve high EE at nanocapsule formulations of NIC due to the low water solubility of the active molecule and the lipophilic nature of the nanocarrier. In all formulations, NIC-LNCs had a monomodal particle size distribution with a narrow distribution and their PDI ranged between 0.020 and 0.141. LNC formulations showed negative zeta potential (ZP) values ranging from -6.31 to -18.2 mV. The particle size of the NIC-LNCs ranged from 19.27 nm to 72.00 nm. Among thirteen NIC-LNC nanoparticles formulations, LNC5-coded formulation which was produced by using 4% NIC concentration, 20% oil and 20% Solutol contents was found as the optimum formulation by the Box-Behnken experimental design.

Table 3. Formulation content and the characterization results of NIC-LNC formulations.

Formulation	NIC (%)	Oil (%)	Solutol (%)	PS (nm)	PDI	ZP (mV)	EE (%)
LNC1	2	10	20	26.91 ± 0.22	0.084±0.015	-11.7±0.95	99.72
LNC2	8	10	20	25.59 ± 0.17	0.041±0.004	-6.31±0.59	99.97
LNC3	2	30	20	62.53 ± 0.64	0.028±0.013	-14.4±0.42	99.99
LNC4	8	30	20	64.38 ± 0.97	0.064±0.005	-15.1±0.79	99.96
LNC5*CP	4	20	20	43.29±0.32	0.064±0.006	-12.7±1.92	99.96
LNC6	2	20	10	72.00 ± 0.07	0.022±0.004	-13.6±0.90	100.00
LNC7	8	20	40	27.16 ± 0.28	0.038±0.014	-9.77±1.19	99.82
LNC8	2	20	40	28.43 ± 0.21	0.078±0.014	-5.35±0.74	98.95
LNC9	8	20	10	70.93 ± 0.13	0.036±0.019	-11.4±0.78	99.65
LNC10	4	10	10	36.42 ± 0.29	0.020±0.015	-18.2±1.48	98.18
LNC11	4	30	10	61.42±0.62	0.046±0.011	-11.5±1.04	99.32
LNC12	4	10	40	19.27±0.21	0.095±0.009	-7.63±0.30	99.96
LNC13	4	30	40	51.11±0.45	0.141±0.012	-9.54±0.39	95.50
LNC14*	4	20	20	43.17±0.53	0.048±0.002	-14.0±1.07	99.65
LNC15*	4	20	20	42.69±0.43	0.053±0.001	-12.3±0.950	99.97
LNC16*	4	20	20	42.67±0.61	0.058±0.017	-12.5±0.61	99.73
LNC17*	4	20	20	43.25±0.44	0.028±0.008	-13.3±3.32	99.99

IV. Conclusion

In this study, a simple, rapid, specific, linear, precise and accurate RP-LC method has been developed and optimized. NIC-LNCs were successfully prepared by solvent-free phase inversion technique. Box-Behnken experimental design was utilized to optimize the formulation variables. The optimized NIC-LNC formulation was obtained with high EE, small particle size, narrow size distribution and negative zeta potential values. Findings of the present study showed that the optimized LNC formulation could be an effective vehicle for NIC delivery.

Acknowledgements: This research was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK) with 123S083 project number. The authors would like to TUBITAK for their financial support and also Gattefossé, Lipoid GmbH and BASF for providing Labrafac, Lipoid and Kolliphor, respectively.

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YS-026

Solid Lipid Nanoparticles Loaded with Vemurafenib: A Promising Approach for the Treatment of Metastatic Melanoma

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Metastatic melanoma is an aggressive form of cancer, and it represents 80% of skin cancer deaths except for 1% of skin tumors [1]. In 2011, FDA approved Vemurafenib (VEM) for the treatment of being unable to remove with surgery or metastatic melanoma [2]. However, the steady-state concentration of VEM in plasma is quite variable between individuals, and low plasma concentrations of VEM are associated with tumor progression and melanoma resistance to VEM [3]. Furthermore, treatment with VEM has pharmacological limitations because of the adverse side effect [4].

To overcome treatment limitations, this study aimed to develop an analytical method for quantification VEM concentration by using HPLC-UV and investigate the anti-cancer effects of solid lipid nanoparticles (SLNs) loaded with VEM on metastatic melanoma by using the hot homogenization technique. A novel HPLC-UV methodology was developed using a Kinetex C18 analytical column (150 mm × 4.6 mm i.d., 5 µm) with a 1.5 mL/min flow rate of acetonitrile: grade water containing 0.1% orthophosphoric acid (60:40, v/v), the column oven is set to 45°C and detection wavelength was adjusted to 249 nm. The method was validated according to International Council on Harmonisation (ICH) guidelines. The method's limits of detection (LOD) and quantification (LOQ) have been determined and reported as 0.05 and 0.1 µg/mL, respectively. The loading efficiency of VEM into solid lipid nanoparticles was determined to be in the range of 14.57% - 89.06% with a size of between 220 and 360 nm. Dissolution rate studies showed that VEM was released from the SLN formulations at rates ranging from 53.80% to 90.88% on the fourth day. The results of the study showed that the developed chromatographic analysis method could be successfully applied for the analysis of VEM loaded into solid lipid nanoparticles and VEM-loaded solid lipid nanoparticles significantly reduced the proliferation of A2058 cells in a concentration-dependent manner ($p<0.01$). Specifically, SLNs loaded with 10 µM VEM caused membrane blebbing and nuclear damage in the cells, indicating a potent anti-cancer effect.

In conclusion, the study suggests that VEM-loaded SLNs have the potential as a promising effective therapeutic approach for the treatment of metastatic melanoma.

Keywords: HPLC, Solid lipid nanoparticles, Therapeutic drug monitoring, Vemurafenib,

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YS-027

A new Nano-Composite Material from Recycled PLA for Magnetic Dispersive Solid-Phase Extraction

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In recent years, a lot of new materials have been realized and tested as sorbents within the field of sample preparation. Materials used for solid phase extraction (SPE) are, usually, very expensive and no attention is paid to their environmental sustainability. In this study, nanocomposite sorbents were prepared through the recycling of polylactic acid (PLA) from waste materials such as PLA sheets, recovered from tobacco products for the non-burning technology. A washing procedure was optimized to regenerate PLA. Among a wide group of solvents tested, tetrahydrofuran was chosen to dissolve the regenerated PLA so to obtain a saturated solution. Magnetic nanoparticles, made up of Fe_4O_3, were prepared from aqueous solution 0.5 M in HCl of $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot nH_2O$ (2:1 molar ratio). This solution was added drop by drop to an aqueous solution 1.25 M in NaOH. A black precipitate of magnetic Fe_3O_4 particles was formed after the addition. The nanoparticles were added to the PLA organic solution together with an active carbon-based material, the last one to assist the extraction of analytes from the matrix. Carbon nanotubes (CNTs) and graphene oxide (GO) were selected as coadjutants for the extraction. With the technique of the emulsion precipitation, the three-component organic phase was combined with a water solution, saturated with sodium chloride. As a result of the salting out effect, the organic and aqueous phases were immiscible, and a cloudy solution was formed under magnetic stirring. The drop-by-drop addition of water to the emulsion broke the emulsion and promoted the precipitation of PLA in the form of nanospheres. A complete characterization study was performed via Scanning Electron Microscopes (SEM) and Transmission electron microscopy (TEM) analysis, as well as Fourier-transform infrared spectroscopy (FTIR) for the qualitative confirmation of the obtained material composition. PLA@CNTs and PLA@GO were tested as sorbents for the magnetic-SPE of a wide group of pollutants (pesticides, drugs, hormones, etc.) of different polarity and polarizability, from different chemical classes.

Ideal peculiarities of such composites are the sustainability (recyclability of the PLA and its complete biodegradability) and the simplicity of their realization. The perfect compatibility of PLA with different secondary components enables to control the adsorption capabilities of the material depending on specific requirements. For CNTs and GO, partially overlapping performances were registered. In both cases, it was possible to obtain recoveries greater than 80% for a fair number of analytes. The new PLA-based materials were tested for the extraction of the same contaminants from urine. The ease of the application and the ruggedness of the technique guarantees high level of recovery and reproducibility. The matrix effect for the considered samples is lower than 20%, as requested by the FDA guidelines for bioanalytical methods.

Keywords: Polylactic acid, Nanocomposite, Magnetic dispersive solid-phase extraction, Sample preparation, Contaminants

PLA@CNTs magnetic nano-particles

Magnetic properties and shape of PLA@CNTs nano-particles

YS-028

Two-step Microwave Extraction with NADES and Biological Activity Comparison of Origanum Michrantum and Origanum Minutiflorum Plants

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The use of deep eutectic solutions as alternative solvents, which has attracted a great deal of attention in recent years and is one of the subjects of interest in green chemistry, has come to the fore in research. In this study, synthesized natural deep eutectic

solvent (NADES) (p-coumaric acid- tert-butyl ammonium bromide) were used for pretreatment. Pretreatment was carried out in a microwave oven. Essential oil extraction was performed with the hydrodistillation system combined with a microwave oven (MHD). While the yield of essential oil analysis without pretreatment was 19% and 21%, respectively, the results were obtained as 23% and 22% in pretreated ones. While the number of essential oil components without pretreatment was 20 and 27, the number of compounds obtained with pretreatment was determined as 52 and 57. In the analysis without pretreatment, the major compounds γ-Terpinen (10.69%) and Terpinen-4-ol (31.32%) were found for Origanum micranthum, Linalool (13.85%) and α-Terpineol (24.61%) for Origanum minutiflorum. In the pre-treated analysis, the major compounds Terpinen (13.02%) and Terpinen-4-ol (14.23%) were found for Origanum micranthum, Linalool (19.96%) and α-Terpinyl acetate (22.71%) for Origanum minutiflorum. Antioxidant activities of all essential oils obtained from two plants were analyzed by DPPH, FRAP and CUPRAC tests. It was observed that the oils obtained from pretreated plants had higher antioxidant activity compared to FRAP and CUPRAC analyzes, but DPPH analyzes showed lower activity. Considering all, NADES had a significant impact on the extraction of essential oil with higher yield, and more quantity, especially when it was combined with microwave technology. Moreover, the NADES-MHD as an economical and eco-friendly technique had the potential to be applied to other plant materials.

Keywords: NADES, Essential oils, Antioxidant Activity, Microwave Assisted Hydrodistillation

YS-029

Determination of the Dissolution of Hypericin from Polymeric Nanoparticles by HPLC Analysis

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Abstract

Analysing of active ingredients from herbal sources is challenging due to difficulty in effectively analysing a small amount of an active component among multiple components in an extract. Active ingredient of *Hypericum perforatum* (*H.perforatum*), hypericin, faces issues like low solubility, photodegradation, and aggregation, resulting in a loss of biological activity. Our study aimed to develop a precise and rapid method for the analysis of hypericin amount loaded in polymeric nanoparticles and their dissolution properties. The extract was prepared using ethanol in a soxhlet apparatus and lyophilized. A validated high-performance liquid chromatographic (HPLC) method was developed to determine the hypericin content in different dissolution media. It is concluded that developed HPLC method was effective for the determination of hypericin from polymeric carrier. Without interference with the excipients and polymers of the nanoparticle. Hypericin loaded polycaprolactone nanoparticles were prepared using nanoprecipitation method, and nanoencapsulation efficiency of hypericin was calculated, and *in vitro* dissolution studies were conducted in phosphate buffer with cosolvents. The dissolution of hypericin was found to be enhanced by combining with other components in the extract, but hypericin was unstable in aqueous media. Encapsulation of the extract into a nanoparticle decreased the dissolution rate of hypericin, also the instability of hypericin was prevented. Although the analysis of hypericin from plant extract is complex, the results of the determination of hypericin from nanoparticles and the determination of release of hypericin in different media in this study proved that these developed HPLC methods can be used reliably for further formulation studies.

Keywords: *Hypericum perforatum*, hypericin, nanoparticles, *in vitro* dissolution, polycaprolactone, solubility

I. Introduction

There is a significant interest in the use of medicinal plant extracts as part of basic healthcare needs. However, there are many issues associated with their use, including low aqueous solubility and stability, which are the most common problems encountered with their use. The use of a carrier system for the extracts is one of the most commonly used methods to overcome these problems [1][2]. *H. perforatum*, contains hypericin, one of the active ingredient that is sensitive to acidic conditions, light exposure, and aggregation in aqueous environments, leading to a loss of biological activity [3]. Nanoparticles are drug carrier systems that have been extensively studied due to their ability to protect sensitive active ingredients from the external environment, enhance the solubility and biocompatibility of lipophilic active ingredients, and provide stability. Analysis of active ingredients from herbal sources is a challenging problem because it is difficult to effectively analyse a small amount of a component that is believed to be active among multiple components in an extract. Additionally, when herbal extract is loaded in a polymeric carrier system, the amount of loaded main compound can be less than limit of detection. Therefore, in our study, we aimed to develop a precise and rapid method for the analysis of hypericin from polymeric nanoparticles without interference of excipients used in formulation and analysis of hypericin released in different

dissolution media.

II. Material and method

Materials: Standard Hypericin (Cayman, Germany), polycaprolactone (PCL) (Mw: 80,000 Da) (Sigma Aldrich, UK), dichloromethane (DCM) (Lab-Scan, Ireland), Polyvinyl alcohol (PVA) (Mowiol 4-88 Mw: 31,000 Da) (Aldrich, Germany) were used. All other chemicals were of HPLC grades. Voucher specimens of *H. perforatum* (AEF 30944) were stored in the Herbarium of the Faculty of Pharmacy of Ankara University.

Preparation of extracts: 20 g of dried and ground plant samples were extracted with 200 mL of 96% ethanol in a soxhlet apparatus, evaporated under vacuum using a Rotavapor (Buchi RII, Switzerland) at a temperature not exceeding 40°C, and lyophilized for 24 hours (TRST 4/4 Teknosem, Turkey) to obtain the dried extract.

Chromatographic conditions: A reversed-phase HPLC (Agilent 1100 series, USA) method adapted from European Pharmacopoeia (EP10) was used to determine hypericin. Separation was achieved using an ACE5 C18 column (150x4.6mm, 5µm). Mobile phase composition, modified from the EP10, is a mixture of methanol/ monosodium phosphate buffer at 15.6 g/L in ethyl acetate (66:8:26, v/v/v). The injection volume was 20 µL, flow rate was 1 mL/min and, the detection wavelength was 590 nm. The column temperature was 25°C (for a medium containing 25% v/v methanol), 40°C (for a medium containing 25% v/v ethanol), and 20°C (for a 0.3 w/v% PVA solution as supernatant). 0,1 mg/mL stock solution of Hypericin was prepared in DMSO. A calibration curve was created by analysing the medium at 8 different concentrations. Analytical validation of HPLC results was conducted in the medium according to (ICH) guideline Q2R1 (2005) for linearity and range, accuracy, repeatability, limit of detection (LOD), and limit of quantification (LOQ).

Dissolution of lyophilized plant extract: It was conducted in different media containing surfactant and cosolvents. The media used were phosphate-buffered saline (PBS) (pH 7.4), PBS containing 0.2% w/w Tween 80, PBS containing 25% v/v methanol, and PBS containing 25% v/v ethanol. Plant extract equivalent to 25 µg and 50 µg hypericin were separately mixed with 5 mL of each medium and shaken at 100 rpm in a temperature-controlled shaker incubator (Thermo Scientific, MaxQ Mini 4450 Shaker, USA) at 37°C for different time intervals (6, 12, 24, 48, 72, and 96 hours). Samples were withdrawn at each time interval by filtering 1 mL of each mixture and were analysed using the HPLC method described above. To avoid loss of the undissolved extract from the medium, the same volume of fresh solvent at the same temperature was added to the medium after filtering.

Preparation of the nanoparticles: *H. perforatum* loaded PCL nanoparticles were prepared by a modified nanoprecipitation method (Javaid et al., 2021) and lyophilized overnight.

Determination of encapsulation efficiency: The amount of encapsulated hypericin (EE%) in the supernatant (0,3% w/v PVA solution) was determined by HPLC (*n=3*). EE%= [(initial added amount drug amount in supernatant)/ initial added amount] x100.

In vitro dissolution studies: Extract and nanoparticles (equivalent to 4,80 µg hypericin) placed in 1 mL of PBS including 25% v/v methanol or ethanol as cosolvent separately in eppendorf tubes which then agitated by vortex for 1 min and shaken in a 37°C horizontal shaker at 75 rpm. At the predetermined time interval (6, 12, 24, 48, 72, and 96 hours), the tubes were centrifuged at 10,000 rpm for 10 min. The supernatant was withdrawn and filtered, and the same volume of fresh medium at same temperature was added to eppendorf. The samples were analysed by obtained HPLC method for each medium *(n=3)*.

III. Results and discussion

For the *in vitro* dissolution medium, a PBS solution containing 25% v/v methanol and a PBS solution containing 25% v/v ethanol were used, and a method for quantitative analysis using HPLC was developed for these media. For the analysis of encapsulation efficiency, a water phase medium for the nanoparticles (0.3% w/v PVA solution) was utilized, and an HPLC method was developed for the quantitative analysis. Hypericin calibration couldn't be performed in PBS because the dissolved amount of hypericin was below the limit of detection due to its low solubility. The resulting calibration curves, which plotted peak areas against hypericin sample concentrations, were found to be linear within the ranges of 2-8 µg/mL (for PBS containing 25% v/v methanol), 0.5-8 µg/mL (for PBS containing 25% v/v ethanol), and 1-8 µg/mL (for 0.3% w/v PVA solution), respectively. All correlation coefficients were greater than 0.99. Calibration data was given in Table 1. These HPLC methods are enable for the effective analysis of a small amount of a hypericin among multiple components in an extract. The analytical validation data was presented in Table 2. Standard deviations were smaller than 2% for all injections. Thus, the methods are considered reliable and reproducible.

Table 1. Calibration data of standard hypericin in different medium

Medium	Slope (m)	Intercept (n)	Correlation coefficient	Retention time (min)	LOD (µg/mL)	LOQ (µg/mL)
PBS-Methanol (25% v/v)	109.87	-115.48	0.9991	3.4	0.128	0.388
PBS-Ethanol (25% v/v)	90.800	27.582	0.9993	2.6	0.111	0.335
PVA solution (0,3% w/v)	87.687	30.017	0.9998	3.6	0.129	0.391

Table 2. Analytical validation data of standard hypericin in different medium *(n=6)*

Medium	Theoretical Hypericin Concentration (µg/mL)	Concentration (µg/mL) ± SD	RSD Intraday (%)	RSD Interday (%)	Mean Recovery (%) ± SD
PBS-Methanol (25% v/v)	2	2.05 ± 0.01	0.31	1.83	102.3 ± 0.31
	5	4.68 ± 0.01	0.13	1.63	96.94 ± 0.10
	8	7.97 ± 0.01	0.22	1.20	95.37 ± 0.07
PBS-Ethanol (25% v/v)	3	3.03 ± 0.02	0.53	0.70	101.3 ± 0.66
	5	5.04 ± 0.01	0.26	0.33	100.7 ± 0.32
	7	7.01 ± 0.01	0.10	0.40	100.8 ± 0.61
PVA solution (0.3% w/v)	3	2.92 ± 0.01	0.27	0.77	97.84 ± 0.55
	5	5.01 ± 0.01	0.15	0.87	100.6 ± 0.80
	7	6.96 ± 0.02	0.31	0.93	99.85 ± 0.43

To implement the specificity, different medium, standard hypericin solution and the solution of hypericin loaded nanoparticle with (1:1) drug:polymer ratio in medium were injected separately. The known amount of the standard hypericin solutions were added to pre-analysed hypericin loaded formulation, and the mixtures were analysed by the proposed method. High percentage of recovery shows that the method is free from the interferences of the excipients and polymers used in the formulation of the nanoparticle for given Fig. 1.

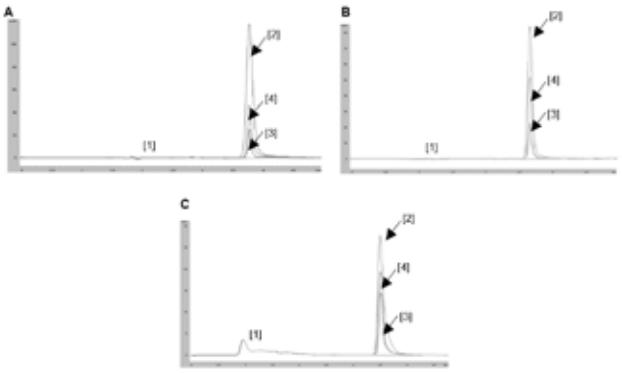

Fig. 1. HPLC chromatograms of hypericin for different mediums: 25% v/v methanol-containing PBS (A), 25% v/v ethanol-containing PBS (B) and 0.3% w/v PVA (C) (dissolution medium without hypericin [1], hypericin [2], hypericin loaded nanoparticle [3], mixture of hypericin loaded nanoparticle and known amount of Hypericin solution [4]).

When the dissolution data of plant extract equivalent to 25 µg and 50 µg hypericin concentrations were examined, an increase in the amount of dissolved hypericin from the extract was higher with 50 µg hypericin concentration (Fig. 2). This indicates that the combination of hypericin with other components in the extract enhances the solubility of hypericin. According to the United State Pharmacopeia (USP 44), saturation (equilibrium) has been reached when multiple samples, assayed after different equilibration time periods, yield equivalent results (e.g., change by less than 5% over 24 h, or less than 0.2%/h). For plant extract equivalent to 50 µg hypericin, the equilibrium solubility results in different media were 18.47 ± 1.05 µg/mL (for PBS in 48 hours), 14.59 ± 0.98 µg/mL (for PBS containing 0.2% v/v Tween 80 in 72 hours), 24.49 ± 1.52 µg/mL (for PBS containing 25% v/v methanol in 12 hours), and 23.34 ± 1.39 µg/mL (for PBS containing 25% v/v ethanol in 24 hours), respectively. It has been found that the results obtained are close to the data in previously published studies (Disch et al., 2017). Since pure active ingredient was not used, analysis of extract does not fully represent the solubility of hypericin, so the dissolution term was preferred in case of solubility. However, since hypericin is present in the extract form in the formulation, the data obtained can provide preliminary information for the selection of the solvent system.

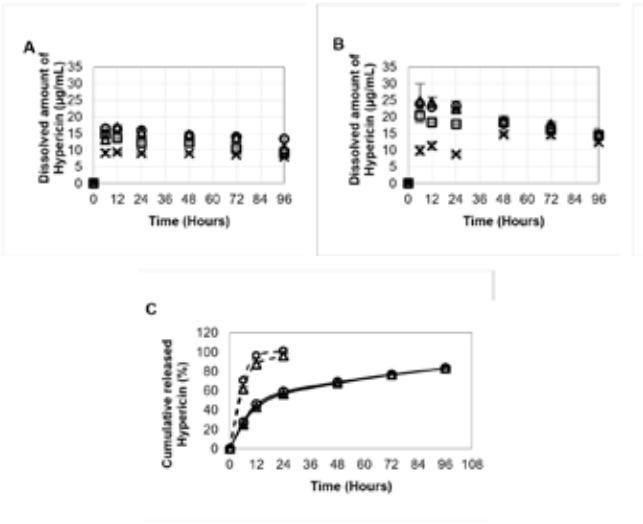

Fig. 2. Dissolution of hypericin data in various solvent systems (n=3): plant extract equivalent to 25 µg hypericin (A) and plant extract equivalent to 50 µg hypericin (B), in vitro release of Hypericin from extract and nanoparticle (C) (▬: PBS,▬:PBS containing 0.2% w/v Tween 80,▬: PBS containing 25% v/v methanol,▬: PBS containing 25% v/v ethanol, - - : extract,—: nanoparticle).

In the dissolution study conducted with plant extract, a decrease in the amount of hypericin over time is observed in all media studied. This decrease can be explained by the instability of hypericin in the aqueous media. Therefore, it is decided that sampling time from *in vitro* dissolution media should not be more than a day. When the release profiles of hypericin at the PBS medium and PBS medium with Tween 80 were compared, the least dissolution was obtained by using surfactant in the medium (Fig. 2A and B). Hypericin tends to aggregate in physiological pH and all aqueous environments. It has been found that Tween 80 facilitates the transformation of less soluble aggregates of hypericin and has a negative effect on the dissolution. The solubility of hypericin in the medium containing methanol or ethanol was more than PBS-Tween 80 medium. As can be seen from Fig. 1. there is

absence of interference of the used excipients and polymers in the formulation of the nanoparticle. The encapsulation efficiency of the prepared polymeric nanoparticles was found to be 63.84 ± 0.36%, indicating that the formulation was able to load the extract. The amount of encapsulated hypericin could be calculated with the calibration equation developed. It was determined that hypericin had a faster release in the medium containing 25% v/v ethanol compared to the medium containing 25% v/v methanol (Fig.2C). Hypericin was released by over 90% in approximately 24 hours in both media. When the release profile of hypericin from the nanoparticle was examined, it was determined that hypericin had a faster release in the buffer containing 25% v/v ethanol, similar to the release profile of hypericin from the extract. The hypericin was released from extract in 24 hours, achieving about 100% release rate (Fig.2C). In contrast, the nanoparticle only exhibits about 60% hypericin release rate, indicating that the polymeric nanoparticle can effectively modulate hypericin dissolution. By encapsulating the extract into the nanoparticle, the release rate decreases, thus increased aqueous stability could be obtained in the dissolution medium.

IV. Conclusion

A precise and fast HPLC method modified from EP10 was developed for analysing hypericin from different dissolution media with appropriate LOD and LOQ. Hypericin loaded PCL nanoparticle could be prepared with high encapsulation efficiency. Loaded hypericin amounts could be analysed without interference with excipients used in nanoparticle preparation. These developed HPLC methods could be used effectively to determine the release of hypericin from nanoparticle at different dissolution media. Encapsulation of the extract into a nanoparticle decreased the release rate and increased the stability of the hypericin in aqueous medium. Although the analysis of hypericin from plant extract is complex, the results of the determination of hypericin from nanoparticles and the determination of release of hypericin from different media in this study proved that these developed HPLC methods can be used reliably for further formulation studies.

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YS-030

Drug Release Profles of Atenolol and Benidipine from pH Responsive Polymeric Hydrogel Matrix

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Due to the limitations such as short biological half-life and extensive hepatic frst-pass metabolism causing fuctuations in drug concentration in plasma, prolonged time dosing frequency following a single dose, unwanted side efects, age-dependent personal risks (i.e., forgetfulness to use drugs regularly on time, or the having to use more than one drug), and consequent poor patient compliance, the design of novel drug carrier systems is of vital importance. In this sense, herein, the potential utilization of a crosslinked free-radical polymerized acrylic acid-based hydrogel system for delivering of two model antihypertensive drugs belonging to Beta Blocker [(BB), Atenolol (ATE)] and Calcium Channel Blockers [(CBB), Benidipine (BEN)] was demonstrated. The in vitro release behavior of ATE and BEN from the poly(acrylic acid) (PAA)-based hydrogel system was examined in response to three diferent conditions mimicking the gastric (SGF, pH of 1.2), intestinal (SIF, pH of 7.4) and physiological body fuids (SPF, pH of 7.4) at 37 °C. Degree of swelling of the respective hydrogels and release kinetics were also compared. As a result, the highest swelling for all PAA-based hydrogel matrixes was observed in SIF (pH 7.4) and a Fickian difusion mechanism was predominant. The distribution of the loaded drug in the hydrogel matrix was found to be quite uniform, and the PAA-based hydrogels prepared in this work have the potential to act as a suitable drug carrier for the delivery of BB and CCB.

Keywords: Atenolol, Benidipine, Hydrogel, Drug release

YS-031

Development of Colorimetric Aptasensor for Detection of 17β-Estradiol in Milk Based on Newly Designed Aptamers and Gold Nanoparticles

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The contamination of 17β-estradiol (E2) in food is a wide concern due to its potential negative effects on human health, leading to a research interest in the development of a simple detection system to monitor its safety level in food. In this work, a new colorimetric E2 detection is developed, and its principle is based on the newly designed aptamers and gold nanoparticles (AuNPs). AuNPs were synthesized by a reduction method and their average size was 12.7 as analyzed by transmission electron microscope (TEM). Their crystalline structure was analyzed by selected area electron diffraction (SAED) and X-ray diffraction (XRD). The new aptamers were designed to contain 2-5 repetitive crucial loops in their structures, referred to as L2-L5. Their E2-binding capabilities were compared with the original aptamer (L1). The results showed that L3-L5 aptamers demonstrated the highest E2-binding efficiency. However, only L3-aptasensor effectively detected E2 in a linear range of 0.05–0.80 nM with the limit of detection at 13.1 pM. The developed L3-aptasensor selectively detected E2 but did not significantly detect other tested chemicals with similar structures including progesterone, genistein, diethylstilbestrol, bisphenol A, and chloramphenicol. Also, the aptasensor showed the correct detection of E2 values in E2-spiked milk samples; the recovery rates of 100.1–113.0 % and relative standard deviations of 5.2–11.1 %. These results suggest that this developed colorimetric aptasensor is potentially used as a simple and rapid tool for monitoring E2 in milk and food samples for food safety applications.

Keywords: Biosensor, Estrogen, Food, Nanomaterials, Optical sensor

YS-032

Development of Electrochemical Sensor Based on Glassy Carbon Electrode Modified with CeO_2/PNMA/fMWCNTs Composite for Simultaneous Detection of Uric Acid and 5-Fluorouracil

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Uric acid (UA) is a biomolecule produced as a degradation product during human purine metabolism. It also acts as a scavenger for radicals in the biological system and helps maintain blood pressure stability and antioxidant stress. Excess of UA in the body can cause hyperuricemia, kidney failure, chronic inflammation of the joints, gout, and some cardiovascular or prolonged low UA level can result in some genetic diseases. Therefore, developing a rapid and accurate UA detection method is very important [1,2].

5-Fluorouracil (5-fluoro-1H-pyrimidine-2,4-dione, 5-FU) is one of the most important chemotherapeutic agents consisting of a fluorine atom in the fifth position of uracil and is frequently used in the treatment of stomach, breast, pancreas, cervix, and colorectal cancer. It is also an antineoplastic agent that blocks cell division in the body by interfering with RNA, DNA, and protein molecules in cells[3]. However, increased concentration may cause serious side effects such as neurotoxicity, cardiotoxicity, and mortality [2]. Controlling the amount of 5-FU in a given formulation and the dose given to patients is necessary for pharmaceutical control and clinical diagnosis [4].

As mentioned above, quantifying UA and 5-FU concentrations in biological fluid is essential for diagnosing and treating many diseases. 5-FU is one of the most critical UA derivatives reported worldwide as a potential cancer drug for cancer treatment. Both molecules, UA and 5-FU, have a similar structure[2]. Therefore, it is very important to develop high-sensitivity and selective electrochemical sensor systems for the simultaneous detection of two molecules.

This study aimed to fabricate a highly efficient electrochemical sensor to determine UA and 5-FU simultaneously. For this purpose, a cerium oxide/poly(*N*-methyl aniline)/functionalized MWCNTs (CeO_2/P*N*MA/*f*MWCNTs) composite was fabricated. A glassy carbon electrode (GCE) electrode modified

with CeO_2/PNMA/*f*MWCNTs composite was utilized for the electrochemical sensing of UA and 5-FU. The electroactive performance of the composite-modified GCE was evaluated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. CV and DPV results showed that the CeO_2/PNMA/*f*MWCNTs composite has high electroactivity and is suitable for the simultaneous determination of UA and 5-FU. The GCE/CeO_2/PNMA/*f*MWCNTs sensor has excellent stability, reproducibility, anti-interference ability, and reproducibility. Furthermore, the applicability of the sensor was tested with a synthetic human serum sample. It was determined that the prepared composite is a promising platform for simple, rapid, and simultaneous analysis of UA and 5-FU.

Keywords: Composite, Electrochemical sensor, Simultaneous detection, Uric acid, 5-Fluorouracil

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YS-033

A Novel Electrochemical Tyrosinase Biosensor for the Monitoring Inhibition of Rosmaniric Acid from Plants

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Abstract

Tyrosinase (Tyr, EC 1.14.18.1), which catalyzes the biosynthesis of melanin and other pigments through oxidation from tyrosine, is a copper-containing metalloenzyme found in the plant bacterial, mammalian, and fungal tissues. In this study, Tyr inhibition was followed with Rosmaniric acid with the selected Rosmaniric acid-containing plants. Firstly, an electrochemical biosensor was developed by immobilizing Tyrosinase in a novel three component organoselenium-based random conjugated polymer matric with NH_2 functionalized quantum dots. The response of Tyrosinase towards its well-known substrate, catechol, was optimized in terms of NH_2 functionalized quantum dots amount, conducting polymer amount, tyrosinase amount, and glutaraldehyde percent. Following then, Tyrosinase inhibition was observed using Rosmaniric acid-containing plants, *Rosmarinus* and *Eryngium campestre*.

Keywords: Tyrosinase, Catechol, Inhibition, Rosmaniric acid, Electrochemistry, Biosensor

I. Introduction

Tyr causes hyperpigmentation and hypopigmentation in mammals, and it is vital to control enzyme activity with Tyr inhibitors. Tyrosinase reacts with many different substances, and it is the most used enzyme to monitor phenolic compounds, such as catechol. In addition, Tyr is involved in melanin biosynthesis, in which excess amounts cause dermatological problems. Several natural products, peptides, drugs, etc., could inhibit melanin biosynthesis (Seo, Sharma, & Sharma, 2003). Rosmarinic acid is a hydroxylated compound frequently found in herbal plants and is primarily responsible for the anti-inflammatory and antioxidative activity. There are 317 species, subspecies, and variations in the *Eryngium genus* (Apiaceae). The majority of Europe, northern Africa, and North America are home to the species *E. campestre* L. There are 23 species of *Eryngium* in the Turkish and East Aegean Islands flora, and *E. campestre* is referred to as "Bogadikeni" in Turkish traditional medicine (Kartal et al., 2005). Rosemary

(*Rosmarinus officinalis* L.), a perennial shrub in the Lamiaceae family with a pleasant scent, can be found growing in a variety of climates around the globe. In Turkish, it is referred to as "biberiye hasalban, kus dili." Turkey is a Mediterranean nation abundant in fragrant and medicinal plants (Özcan & Chalchat, 2008).

II. Material and method

Prior to the usage, all graphite rod electrodes were polished with emery paper, rinsed with ultra-pure water and dried in air. Conducting polymer of [*a*-2-thienyl-*ω*-2-thienyl-poly[4,8-bis((2-ethylhexyl)oxy)benzo[1,2-*b*:4,5-*b*′]dithiophene-*alt*-(5,6-dimethoxybenzo[*c*][1,2,5]selenadiazole;5-(2-ethylhexyl)-4*H*-thieno[3,4-*c*]pyrrole-4,6(5*H*)dione)] (PSe), (1mg/1 mL $CHCl_3$) was prepared. The nanobiosensor was fabricated by casting 3 µL PSe, and allowed to dry. Tyrosinase was prepared as 1mg in 50 µL of PBS, and adding 5 µL of Tyr mixed 2 µL NH_2QDots then crosslinked with 5 µL of 2.5% glutaraldehyde solution. Nanobiosensor were left to dry for 2 hours before biosensing experiments at room temperature. The chronoamperometric technique was used to follow catechol biosensing at room temperature under stirring conditions by applying a -200 mV constant potential. The designed nanobiosensor was used as a working electrode, Ag/AgCl (BASi; 3 M NaCl) was used as a reference electrode and a platinum wire used as an auxiliary electrode. Three electrode system was immersed in a reaction medium containing 10 mL 50mM, pH 7 phosphate buffer. After reaching a steady-state, 50 µM catechol biosensing.followed. The dried aerial parts of plants were crushed into a fine powder (20g), extracted by maceration with 70% EtOH (200 ml) for 24 hours at room temperature, followed by an ultrasonic bath for 1 hour, and then filtered. Five times this extraction process was carried out, with each addition of a new solvent to the residue. After filtering, EtOH was evaporated until dry and then kept at 4°C until use.

III. Results and discussion

In this study, Tyr inhibition was followed with Rosmarinic acid with the selected Rosmarinic acid-containing plants. Firstly, an electrochemical biosensor was developed by immobilizing Tyrosinase in a novel three component organoselenium-based random conjugated polymer, matric of [*a*-2-thienyl-*ω*-2-thienyl-poly[4,8-bis((2-ethylhexyl)oxy)benzo[1,2-*b*:4,5-*b*′]dithiophene-*alt*-(5,6-dimethoxybenzo[*c*][1,2,5] selenadiazole;5-(2-ethylhexyl)-4*H*-thieno[3,4-*c*]pyrrole-4,6(5*H*)dione)] (PSe) with NH_2 functionalized quantum dots. The response of Tyrosinase towards its well-known substrate, catechol, was optimized in terms of NH_2 functionalized quantum dots amount, conducting polymer amount, tyrosinase amount, and glutaraldehyde percent. The optimum conditions were obtained when 3 µL PSe is utilized to immobilize 5 µL Tyrosinase together with 2 µL NH_2QDots. Gluteraldehyde % optimization studies also performed and optimum responses were obtained with 2.5% glutaraldehyde solution. Under optimized conditions, catechol was determined in the range of 0.1-88 µM with a limit of the detection value of 0.023 µM and a limit of quantification value of 0.07 µM.

Following then, Tyrosinase inhibition was observed using Rosmaniric acid-containing plants, *Rosmarinus* and *Eryngium campestre*. The HPLC analysis of *R. officinalis* and *E. campestra* plants showed excess rosmarinic acid levels. Following then,their potential inhibitor effect was researched using the novel designed electrochemical biosensor. Inhibition % is calculated from the I_o and I values that are the current corresponding to the enzyme activity of the nanobiosensor towards catechol and when the inhibitor is not present and present, respectively. Firstly, the incubation time was optimized since inhibition needs time. After optimization of the inhibition conditions, inhibitory strength, which is expressed as the I_{50} value, which is the half-maximal inhibitory concentration where 50 % inhibition is followed at a specific substrate concentration, was calculated. Hence, I_{50} was reported as 17 µM and 21 µM for *Rosmarinus* and *Eryngium campestre*, respectively.

IV. Conclusion

Rosmarinic acid has recently gained bright prospects in cosmetic and medical utility on the whitening effect as it inhibits Tyrosinase activity. In this study, Tyrosinase inhibition was followed with Rosmaniric acid with the selected Rosmaniric acid-containing plants. For this aim, an enzyme based electrochemical biosensor was designed using three moieties of benzoselenadiazole, thienopyrroledione, and benzodithiophene containing selenium-bearing conjugated polymer and Tyrosinase wired with NH_2- functionalized quantum dots. This random conjugated polymer, enhanced the catechol response, together with the Tyrosinase wired NH_2-QDots matric. Moreover, parameters affecting biosensing were optimized, such as NH_2-functionalized quantum dots amount, pPSe amount, tyrosinase amount, and glutaraldehyde percent. Under optimized conditions, catechol biosensing and rosmarinic acid inhibitory effect was followed. The developed electrochemical nanobiosensor would provide excellent potential for different enzyme immobilization and enzyme-based inhibition studies for other types of drugs.

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YS-034

The First Electrochemical Sensor for the Determination of Ibrutinib in Human Serum in the Presence of Anionic Surfactant

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Abstract

Ibrutinib is a Bruton's tyrosine kinase inhibitor, which is a selective, highly potent, and irreversible small molecule used for treating chronic lymphatic leukemia. In this study, ibrutinib was detected at the nanomolar level using adsorptive stripping square wave voltammetry in the presence of an anionic surfactant medium. A glassy carbon electrode was used to examine the effects of supporting electrolyte, pH, surfactant concentration, and scan rate on the voltammetric peak responses of ibrutinib. After optimizing the conditions, a concentration range of 4.00 x 10^{-9} M to 2.00 x 10^{-7} M and a detection limit of 2.73 x 10^{-10} M were obtained in the presence of 2.00 x 10^{-3} M surfactant in 0.1 M sulfuric acid. This method was applied for the determination of ibrutinib in biological samples and was also tested with potential interference substances.

Keywords: Ibrutinib, square wave voltammetry, biological samples, anionic surfactant, glassy carbon electrode.

I. Introduction

Ibrutinib (IBR) is an orally administered Bruton's kinase (Btk) inhibitor. IBR is reversibly bound to human plasma protein and eliminated primarily through feaces (approximately 80%), mainly in metabolite form (Veeraraghavan et al., 2015). Surfactants, such as sodium lauryl sulfate (SLS), have both hydrophilic and hydrophobic characteristics (Aksoz & Topal, 2021). Sodium lauryl sulphate (SLS) is an anionic surfactant having an anionic organosulphate (Sener et al., 2020). Various analytical methods are used for the quantification of IBR, such as high performance liquid chromatography (HPLC) (Yasu et al., 2019), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Rood et al., 2016), and ultra performance LC-MS/MS (UHPLC-MS/MS) (Beauvais et al., 2018). In this study, sensitive, rapid and reliable adsorptive stripping square wave (SWAdS) voltammetic method was developed to quantification of IBR using a glassy carbon electrode (GCE) in the presence of SLS. This method was applied to human serum sample.

II. Material and methods

Electrochemical studies were conducted using PSTrace 5.6 software and a PalmSens instrument. A triple electrode system was used, consisting of a glassy carbon electrode (GCE) as the working electrode, Ag/AgCl as the reference electrode, and a platinum electrode as the counter electrode. IBR was purchased from DEVA, and a stock solution was prepared using dimethyl sulfoxide (DMSO). Supporting electrolytes such as 0.1 M sulfuric acid solution, acetate buffers, phosphate buffers, and Britton-Robinson buffers were used.

III. Results and discussion

The anodic peak response of IBR was examined using cyclic voltammetry (CV), differential pulse (DP), and square wave (SW) voltammetry on GCE in the presence of 2.00 x 10^{-3} M SLS in all pH ranges. The scan rate studies reveal that the electrochemical reactions are realized under mixed diffusion-adsorption controlled process in 0.1 M sulfuric acid. The optimum stripping conditions were found as deposition potential of 0V and deposition time of 60 s in 0.1 M sulfuric acid by SWAdSV. SWV parameters were also optimized as step potential of 16 mV, amplitude of 25 mV, and frequency of 100 Hz for 2.00 x 10^{-7} M IBR in the presence of 2.00 x 10^{-3} M SLS in 0.1 M sulfuric acid. The proposed method was applied to human serum. Under optimum conditions, the linearity ranges of 4.00 x 10^{-9} M to 8.00 x 10^{-8} M with a detection limit of 7.32 x 10^{-10} M were obtained for human serum. The interferences studies such as KCl, NaCl, $NaNO_3$, dopamine, paracetamol, uric acid, ascorbic acid, and glucose were examined for IBR. Recovery values for human serum was found to be 100.99%, and the % relative error in interference solutions was found to be ±10 in the proportion of 1:1, 1:10, and 1:500 (IBR:interfering agent).

IV. Conclusion

A simple, sensitive, rapid, and environmentally friendly method was developed in 0.1 M sulphuric acid for the quantification of IBR in the biological samples with no need for preprocessing steps including sample pretreatment, time-consuming extraction, and evaporation. It is concluded that the developed method can be used for the determination of IBR in the biological complex matrix. The developed method may offer an alternative to LC techniques in therapeutic drug monitoring with the desired accuracy and precision.

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YS-035

Detection of Mutations on ctDNA Samples with All-MEMS Photoelectrochemical Sensor

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Regardless of the type, cancer is one of the leading causes of death, according to the World Health Organization (WHO). Diagnosing the disease in its early stages could help save millions of lives. Circulating tumour DNA (ctDNA), one of the major liquid biopsy samples, is a type of DNA that originates from tumour or cells with cancerous background circulating in the body and can be used in lab-on-a-chip (LoC) systems as a non-invasive detection tool. Recent advances in sensing technology and Biomedical Micro-Electro-Mechanical-Systems (BioMEMS) have led to the development of various biosensors to detect ctDNAs. MEMS-fabricated biosensor platforms for developing LoC systems together with well-established surface modification strategies upgrade sensors' sensitivity. Significant improvements can be achieved by integrating electrochemical (EC) sensors that allow working with small volumes of samples and offer low limit of detection values. Thin film technology based on sputtering allows fabrication of homogeneous, pollutant-free metal electrodes that are crucial for the formation and integration of mechanical and electronic components on-chip, resulting in fully integrated biomedical analysis platforms. Compared to conventional EC setups, MEMS-based EC sensors integrate sensing and detection electrodes directly into the microchip, providing improved output signal levels. Within the scope of this study, all-MEMS EC chips with 3 electrodes: gold working electrode (Au WE), platinum counter electrode (Pt CE), and silver reference electrode (Ag RE) were fabricated. In the proposed biosensor platform, the Au WE was modified with single-stranded DNA (ssDNA) probes for electrochemical ctDNA detection experiments. Chips were initially tested with complementary and non-complementary target DNA with various number of mismatched bases based on Electrochemical Impedance Spectroscopy (EIS). Next, the system was built where the target DNAs were labelled with nanoparticles. Upon excitation with a laser selected at the appropriate wavelength, the system generated a photocurrent. Photocurrent responses were compared with those of commercially available screen-printed electrodes and found to be more sensitive by detecting changes in nano-ampere (nA) level.

Keywords: MEMS, Biosensor, Electrochemistry, Liquid Biospy, ctDNA

YS-036

Determination of Liposomal Vitamin C Using Polarography

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Humans cannot synthesize Vitamin C, it must be consumed enough in their diets.Vitamin C has diverse functions in the body from antioxidation function to cancer treatment (1). Vitamins are delicate chemicals; they should be protected from damaging factors like heat and oxidants. Like other vitamins, Vitamin C can degrade with oxygen, light, temperature, and alkaline or neutral aqueous media (2). Liposomes are a type of microencapsulation consisting of lipid bilayers and through this procedure, liposomes can protect and control their release at controlled rates under

desired conditions (3). Thus, Vitamin C is protected from degradation. Several chromatographic techniques were developed for the qualitative and quantitative analysis of liposomal Vitamin C or other liposomal pharmaceuticals. However, before applying these techniques, the liposomal coat must remove by extraction process (4). Also, common chromatographic techniques are time-consuming, expensive, and due to solvent requirements, not suitable for green chemistry.

This study aims to develop a rapid, highly sensitive, and low-cost electroanalytical methods for the determination of Vitamin C in liposomal forms. The first reason for choosing differential pulse polarography (DPP) is, such solutions or suspensions allow for direct determination in the combination without the need for prior separations (5). Therefore, DPP is a delicate method to analyze vitamins even in multivitamin forms (6). Surfactants such as gelatine or Triton-X have been tried to degrade liposomes and the free form of Vitamin C has been determined quantitatively. With the help of DPP, a validated electroanalytical method developed for the direct determination of Vitamin C.

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Keywords: Polarography, Dropping mercury electrode, Liposomal vitamin c, Quantitative analysis

YS-037

Spectroscopic and Electrochemical Studies on the Interactional of Purine Nucleoside Antimetabolite Clofarabine with Double Helix Deoxyribonucleic Acid

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Clofarabine (Fig) is a purine analog, cytotoxic antineoplastic agent used in the treatment of acute lymphoblastic leukemia [1,2]. Clofarabine inhibits DNA synthesis and repair by terminating DNA chain elongation. It stops the growth/proliferation of cancer cells. In addition, clofarabine 5'-triphosphate disrupts the integrity of the mitochondrial membrane, leading to the release of an apoptosis-inducing factor that causes programmed cell death [3].

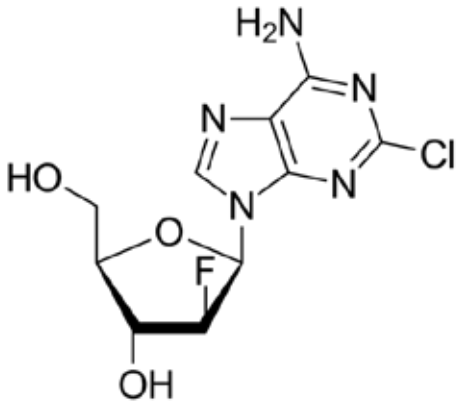

Fig. Chemical structure of Clofarabine

DNA has been known to be the cellular target for many cytotoxic anticancer agents for several decades. Understanding how drug molecules interact with DNA has become an active research area at the interface between chemistry, molecular biology and medicine. The interaction of drugs with DNA is a significant feature in pharmacology and plays a vital role in the determination of the mechanisms of drug action and the designing of more efficient and specifically targeted drugs with lesser side effects. Several techniques are used to study such interactions [4]. In this study, the molecular interactions between Clofarabine and fish sperm double strain DNA have been studied using UV–Vis, fluorescence spectrophotometry and voltammetric methods. The DNA binding constant of Clofarabine was calculated for each method and the binding mode was determined.

Keywords: Clofarabine, DNA binding, spectroscopy, voltammetry

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YS-038

Evaluation of Thermal and Structural Stability of Pediocin PA-1 Mutants with MD Simulations

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Pediocins and pediocin-like bacteriocins are classified as Class IIa bacteriocins. These peptides have become increasingly important in the food industry as they exhibit antimicrobial and protective effects, particularly in dairy products, and possess high heat resistance, up to 121°C. Pediocins have a typical length of 37-48 amino acids and contain two disulfide bonds that play a critical role in their structural and thermal stability and affect their antimicrobial properties. In this study, we used molecular dynamics (MD) simulations to investigate the thermal stability of Pediocin PA-1 and its mutants. MD simulations are popular in the pharmaceutical industry due to their cost-effectiveness and time-saving advantages. We introduced various amino acid replacements, such as Ser13-Cys13, Gln39-Cys39, His38-Cys38, Gly40-Cys40, Asp17-Cys17, Lys20-Cys20, Thr35-Cys35, Gly37-Cys37, Asn28-Cys28, Gly6-Cys6, Val16-Cys16, and His42-Cys42, in the peptide sequence of Pediocin PA-1, generating five different mutant peptides. We compared these mutants with the reference peptide for their thermal and structural stability using MD simulations. The cysteine-containing mutant peptides, such as Thr35-Cys35, Gly37-Cys37, Val16-Cys16, and Lys20-Cys20 replacements, showed higher stability than the reference pediocin structure, particularly in terms of the helical structure, which plays an essential role in antimicrobial activity. Therefore, this *in silico* study proposes the use of new pediocin mutant peptides that warrant evaluation in an industrial setting.

Keywords: Pediocin PA-1, Thermal Stability, MD Simulations, Food Industry, Peptit Engineering, Cys residues

YS-039

Elucidation of Binding Interactions and Mechanism of Tyrosine Kinase Inhibitor Axitinib with dsDNA via Multi-Spectroscopic and Voltammetric Studies

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Abstract: Axitinib, a second-generation tyrosine kinase inhibitor (TKIs) group of oral medication is used as a first-line treatment of metastatic renal cell carcinoma (RCC). It can induce DNA damage which results in cell cycle arrest and senescence, and mitotic catastrophe in RCC cells. This drug also has been found to bind with deoxyribonucleic acid (DNA) to exert its function. Therefore, studying this binding interaction is not only important to understand the pharmacodynamic profile of axitinib, but also for developing and designing better DNA- binding drugs. In our literature search, we did not find any study related to the DNA binding mode of Axitinib. So, this study is aiming to explore the interaction between DNA and axitinib by using different analytical methods like UV-spectroscopy, thermal denaturation, electrochemical and fluorescence spectroscopy. The DNA binding constant Kb for Axitinib was determined by spectroscopic and voltammetric techniques. Intraday and interday repeatability and these values are given with their standard deviations. As a result, it was seen that there were no significant differences between them.

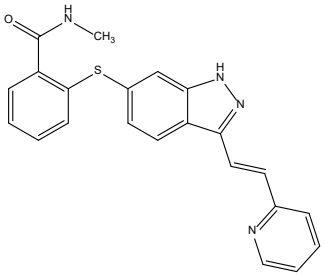

Figure 1. Chemical structure of Axitinib

Keywords: Axitinib, Double strain deoxyribonucleic acid (dsDNA), Spectroscopy, Voltammetry, Thermal denaturation

YS-040

A Validated Electrochemical Method for Quantification of Molnupiravir in Capsule Dosage Forms

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Molnupiravir (MOL) is an antiviral drug that inhibits the replication of certain RNA viruses and is used to treat COVID-19 SARS-CoV-2 infected individuals. Clinical trials have shown that treatment with MOL reduces deaths and hospitalizations in COVID-19 infections. MOL is a pro-drug of the synthetic nucleoside derivative N4-hydroxycytidine and exerts its antiviral effect by introducing copying errors during viral RNA replication (1). Electrochemical methods have gained considerable importance in drug analysis in pharmaceutical formulations, and biological, and food samples (2, 3). This study aimed to develop a sensitive, inexpensive, simple and rapid square wave voltammetry (SWV) method for determining MOL in pharmaceutical preparations. The developed method requires a one-step procedure for the electrochemical determination of MOL. A direct, simple, and sensitive method based on electrochemical deposition of reduced graphene oxide (rGO) on a glassy carbon electrode (GCE) using cyclic voltammetry (CV) was developed for MOL quantitative analysis. Here, the electrochemical oxidation of MOL was examined using SWV method and a well-defined peak at 0.2 V was measured against the Ag/AgCl reference electrode with the rGO sensor in Britton-Robinson pH 9 buffer. Various operational parameters for MOL analysis such as pH of supporting electrolyte, instrumental parameters, electropolymerization cycles, and GO concentration were optimized. The sensor exhibited a linear range from 0.09 to 4.57 µM with a detection limit of 0.03 µM. The electrochemical response of the rGO sensor was characterized by electrochemical impedance spectroscopy and CV. The character of current and reversibility of the electrode reaction, the number of electrons and protons transferred were studied by the CV method. The developed sensor was validated according to the International Council for Harmonisation (ICH) guidelines (4) and found stable, linear, accurate, precise, robust and rugged. The developed rGO sensor was successfully applied for the quantification of MOL in capsule dosage form and can be reliably applied for the routine analysis of MOL.

Keywords: COVID-19, Molnupiravir, Electrochemical sensor, Reduced graphene oxide, validation

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YS-041

Photodegradation of Favipiravir and Oseltamivir in the Presence of Microplastics

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The consumption of antiviral drugs, which are used in the treatment of Influenza A and B has increased during COVID-19 pandemic, leading to high concentrations in raw wastewater. Their fate in wastewater treatment plants and in the environment may change with several reactions such as hydrolysis, photodegradation, adsorption and accumulation in biosolids. Intentional of unintentional water reuse for irrigation purposes or the use of biosolids as soil conditioners may bring these pharmaceuticals into contact with soil organisms. In addition, the use and improper disposal of plastic personel safety equipment (*e.g.,* masks and gloves) during the pandemic is expected to have an effect in the environment when these plastic compounds degrade and turn into secondary microplastics (MP). It has been already shown that the fate and toxicity of some pollutants might change depending on the presence or absence of microplastics in the matrix. Therefore, this study aims to investigate two commonly used antiviral drugs, Favipiravir (FAV) and Oseltamivir (OSE), in terms of their photodegradation in the presence of microplastics. Synthetic samples with 50 µg/L concentration for both pharmaceuticals were prepared with distilled water and exposed to natural sunlight

irradiation for 72 hours. The effect of the presence of microplastics was examined by addition of polyethylene MP at a concentration of 0.1 mg/L. The change in concentrations of FAV and OS were determined by ultra performance liquid chromatography tandem mass spectrometry with the detection limit of 5.2 and 1.5 µg/l, respectively. The toxicity of FAV and OSE was determined with a soil invertebrate, *E.crypticus*. After 24-hour daylight exposure in the absence and presence of MP, the concentration of FAV in the sample decreased below the detection limit. The possible adsorption of FAV on MP was investigated with control studies carried out in dark conditions, and the 72-hour FAV concentration showed that adsorption does not occur even if the concentration of MP was increased to 5 mg/L. The results demonstrated that the effect observed during daylight exposure is only photodegradation by UV light. On the other hand, the results showed that OSE does not undergo photodegradation even in quartz containers and does not get adsorbed on MP. Based on these results, the presence of FAV in treated wastewater effluent is not expected to lead to toxicity to soil organisms due to probable photodegradation in surface waters. However, OSE in treated effluent or on biosolids might pose a threat to the environment (NOEC< 12.5 mg/kg) and therefore additional treatment methods may be needed to reduce its concentration in wastewaters.

This study was funded by the Scientific and Technological Research Council of Turkey (TUBITAK), Project No: 121Y383. LC-MS/MS analyses were performed in SUMER Laboratory, GTU.

Keywords: Adsorption, antivirals, NOEC, photolysis, ecotoxicity

YS-042

The Effect of Vitamins on Oxidation in Experimentally Diabetes Conditioned Rats

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Diabetes Mellitus (DM) is a metabolic disorder that is very common in our society, is caused by a combination of hereditary and environmental factors, and results in an increase in blood glucose levels. Diabetes mellitus develops as a result of increased blood glucose level and impaired insulin secretion. Antioxidant enzymes cannot be synthesized enough in patients with diabetes mellitus and free radicals damage tissues.

In this study, the effects of vitamin C and vitamin E on lipid peroxidation and antioxidant enzyme activities in kidney tissue of streptozotocin-induced diabetic rats were investigated. Male wistar rats, were grouped into five each consisting 8 rats as (non-diabetic control (K), diabetic (D), diabetic Vitamin E (E), diabetic Vitamin C (C), and diabetic Vitamin E and C (EC). Diabetes mellitus was induced in rats by intraperitoneal injection of 55mg/kg STZ. After the injection, i.p. 268mg/kg. vitamin E, 250 mg/kg vitamin C by gavage were administrated for four weeks. As an indicator of lipid peroxidation malondialdehyde (MDA) levels, antioxidant enzymes as superoxide dismutase (SOD), catalase (CAT) and and glutothione peroxides (GSH-PX) activities were measured in kidney tissue homogenates.

In the study, MDA level of group D was significantly higher than groups K, E, C and EC. Statistically significant difference was not observed in CAT levels among the groups. SOD enzyme levels in group D was higher compared to group K, and lower in group EC compared to groups C, and D. GSH-Px enzyme levels in group D and group C were significantly higher compared to group K. Group D GSH-Px levels were significantly lower whwn compared to group E, C and EC, GSH-Px levels of EC group was lower compared to group C.

As a result, in this study it was demonstrated that vitamin C and vitamin E have positive effect on lipid peoxidation and as a result decreased the high levels of MDA, SOD, GSH-Px levels of diabetic rat kidney tissues.

Keywords: Diabetes Mellitus, Streptozotocin, Antioxidant, Radicals Damage, Kidney

YS-043

Electrochemical Biosensors for Healthcare Applications

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Analytical devices that produce semi-measurable or measurable information about a biological process with a transducer are called biosensors. Electrochemical biosensors are a type of biosensor that detect biological events by converting information into electrical signals such as current and impedance. Electrochemical biosensors provide rapid, cost-effective, simple, and sensitive analysis (Low et al., 2021). In recent years, there has been an increasing trend towards integrating nanomaterials,

molecularly imprinted polymers (MIPs), and aptamers within biosensor systems. These materials offer improved sensitivity, selectivity, and stability, making them attractive for a range of applications in fields such as environmental monitoring, medical diagnostics, and food safety.

MIPs are synthetic substances that mimic the antigen-antibody interaction and selectively bind to the target molecule using a key-lock mechanism. Recently, MIP-based biosensors have gained an important place in the sensitive and selective detection of biological molecules (Hasseb et al., 2022). Nanomaterials are often utilized in biosensors to enhance sensitivity and selectivity due to their larger surface-to-volume ratio, electrical conductivity, and durability. As a result, they are commonly incorporated into biosensor designs for the detection of various analytes such as DNA, proteins, and pathogens (Sharifi et al., 2019).

In this talk, we will present the development of a biosensor based on a bacteria-imprinted polymer synthesized by electropolymerization in order to detect the causative bacteria of urinary tract infections. After the formation of the MIP on the electrode surface, the MIP-coated electrodes were incubated with the target bacteria, and detection was performed via electrochemical impedance spectroscopy. The biosensor based on bacteria imprinted polymer synthesized by electropolymerization was successfully developed and utilized for the detection target bacteria, in both artificial and real urine samples. The ability to detect target bacteria in urine samples is significant as it allows for rapid and accurate diagnosis of urinary tract infections, potentially leading to more timely and effective treatment. We will also discuss the electrochemical detection of the interaction between metal nanomaterials and DNA/RNA in this talk. The oxidation signals of both the metal nanomaterial and guanine in nucleic acids were measured using differential pulse voltammetry before and after the interaction. By analyzing the signal changes, we observed that the metal nanomaterial affects single and double stranded DNA at varying rates.

Acknowledgement: This study was supported by the grant of Izmir Katip Celebi University Scientific Research Projects Coordination Unit, Project No: 2022-GAP-ECZF-0009.

Keywords: biosensors, molecularly imprinted polymers, nanomaterials

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YS-044

Development of a Prototype Biosensor to Determine the Severity of Demyelination due to Diabetic Neuropathy in Serum

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Neuropathy is a nerve damage or dysfunction development due to different etiological grounds. Physical symptoms most of the time appear in the form of tingling, numbness, motor weakness seen in the area affected by the damaged nerve. Diabetic neuropathy is the occurrence of neuropathic processes in the peripheral nervous system on diabetes-related hyperglycemia and the pathways it triggers. Diabetic neuropathy is a common complication in diabetic patients, it frequently causes diabetic foot ulcers, high morbidity and mortality. The early stages of neuropathic changes are asymptomatic in most patients. Complaints and findings such as numbness, tingling and loss of sensitivity can usually be detected already in advanced stages of nerve damage.

In current practice, the diagnosis of diabetic neuropathy is made by physical examinations, questionnaires and nerve conduction studies. All of these methods are based on findings that can only be detected at late stages of the disease. Diagnostic methods that would enable diagnosis in the early stages of the disease are urgently needed in order to start the treatment at the early stages. Thus, it would be possible to treat patients without the need for amputation in advanced stages.

Aptamers are short single-chain oligonucleotides that are able to bind to specific target molecules with high affinity. In addition

to having high binding affinity and specificity, aptamers can be easily produced in vitro with required modifications. Advantages of aptamers also include rapid tissue penetration, ability be stored and used stably in a very long time and to be used as a bioreceptor in biosensor applications. Thus, aptamer-based biosensors (aptasensors) have been designed and applied for development of effective diagnostic methods of various diseases.

As a new approach within the scope of our project, an electrochemical aptasensor-based prototype diagnostic method will be developed for the rapid, sensitive, specific, low-cost Point-of-Care (POC) analysis of Myelin Basic Protein (MBP) biomarker, which increases in serum as a result of demyelination due to diabetic neuropathy. In the aptasensor designed, disposable graphite pencil electrodes (PGE) are used as the sensor surface and nanomodification is carried out by coating the surfaces with graphene oxide (GO-PGE) in order to increase sensitivity. For this purpose, MBP-specific aptamer LJM-5708, which was revealed by the team members in their previous studies but has not yet been tested at the clinical stage, is used. LJM-5708 aptamer is covalently attached to GO-PGE surfaces and subsequently analysis of patients' serum samples is performed. Binding of aptamers and subsequently MBP molecules from serum samples onto the sensor surface is visualized by Electrochemical Impedance Spectrometry (EIS). The place of the aptasensor in clinical use is investigated by comparative analyzes to be made with samples taken from healthy, diabetes and diabetic neuropathy cases. The groups investigated include healthy individuals, individuals with a diagnosis of diabetes without neuropathy findings, and diabetic patients with neuropathy and below-knee amputation indication.

This is still going on study and it is an original research dedicated to the aptasensor-based method development that would allow measuring the severity of demyelination in diabetic neuropathy via MBP.

Keywords: Myelin Basic Protein, Diabetic Neuropathy, Aptasensor

YS-045

Development of N-doped Holey Graphene-based Aqueous Inks to Construct Flexible Electrochemical Sensors

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Inkjet printing of graphene and graphene-related materials has taken a great deal of interest in the development of flexible electrodes and (bio)sensors in the last couple of years. While graphene displays unique physicochemical properties including large physical surface area, high electronic conductivity, and tunable surface properties; due to having hydrophobic nature and insufficient electrochemical activity in the absence of structural defects, the dispersibility and the chemical activity of graphene-based two-dimensional (2D) materials should be boosted. In the present work, we enhanced the dispersibility and chemical activity of graphene by exploiting defect engineering strategies, which are the introduction of nitrogen (N) heteroatoms and the creation of nanoholes in the basal plane. It should be highlighted that these approaches have not been implemented in the inkjet printing of graphene in the literature. Furthermore, the presence of holes in the basal plane addressed the challenges related to the restacking problem peculiar to all 2D materials. In the first part of the study, we developed highly concentrated N-doped holey graphene-based aqueous inks and evaluated the properties of the inks in terms of stability, printability, and homogeneity. Then, the inks were printed on flexible substrates to fabricate flexible electrochemical sensors. The results indicated that the defect engineering approaches enabled us to fabricate highly conductive and homogeneous working electrodes. In the last part of the study, the electrochemical performance of the flexible sensors was evaluated against hydrogen peroxide (H_2O_2) and glucose.

This work was supperted by TUBITAK with the project number of 118F433.

Keywords: Inkjet printing, holey graphene, defect engineering, flexible sensor

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ORAL ABSTRACTS & PROCEEDINGS

OP-001

Photochemical Immobilization of Cellulose tris(3-chloro-5-methylphenylcarbamate) on Silica and Its Use for Separation of Enantiomers in High-Performance Liquid Chromatography

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Analytical and preparative separation of enantiomers is considered as the most difficult problem in separation science for many years due to identical properties of enantiomers in achiral environment. However, in chiral environment their properties are different. Only since 1980s chiral columns for high-performance liquid chromatographic (HPLC) separation of enantiomers became commercially available. Among all chiral stationary phases (CSP) available today for HPLC separation of enantiomers polysaccharide-based chiral columns are most widely used. Polysaccharide-based CSPs are commonly prepared by physical coating of polysaccharide derivative onto the surface of silica. This is a fast method that does not require pre-activation of silica or polysaccharide derivative. As for covalently immobilized CSPs, the main mechanism of immobilization is crosslinking of polysaccharide chains. The advantage of this method is that immobilized chiral stationary phases are stable and compatible with various mobile phases.

The major research goal of the present study was photochemical immobilization of cellulose tris(3-chloro-5-methylphenylcarbamate) on silica and its comparison with CSP prepared by coating cellulose tris(3-chloro-5-methylphenylcarbamate) on silica for separation of enantiomers in HPLC. We also compared separation of enantiomers in different mobile phases, such as methanol, acetonitrile and mixture of n-hexane and propan-2-ol with various ratios. In the frame of the present work we also studied the thermodynamics of enantioseparations on these newly prepared materials.

Keywords: Cellulose tris(3-chloro-5-methylphenylcarbamate), chiral selector, immobilization, enantioseparation, mobile phase, thermodynamic parameters

OP-002

Analytical Methodologies and Challenges in the Analysis of Synthetic Phosphodiesterase Type 5 Inhibitors (PDE-5i) Found as Adulterants in Health Supplements

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As of today, there are already 111 unapproved PDE-5i found as adulterants in illegal health supplements. Seventy-five of these unapproved PDE-5i are analogues of sildenafil (68%), followed by 26 analogues of tadalafil (23%), 8 analogues of vardenafil (7%) and 2 other compounds (2%). Common screening techniques for such analogues include high performance or ultra-high performance liquid chromatography tandem with ultra-violet detector (HPLC-UV or UHPLC-UV) (57, 50%) and thin-layer chromatography tandem ultra-violet detector (TLC-UV) (8, 7%). Screening by mass spectrometry is relatively less common with the use of single-, triple-quadrupole and time-of-flight (TOF) mass spectrometers (10, 9%). Meanwhile, the combined detection by UV-MS has been recorded at 10%. Recently, screening by proton nuclear magnetic resonance spectroscopy (NMR) (13, 11%) has also been applied. For compound structural elucidation, NMR spectroscopy has been preferred (101 out of 111 compounds), followed by high-resolution mass spectrometry (HRMS) (73 out of 111 compounds) and Fourier transform infra-red spectroscopy (FTIR) (44 out of 111 compounds). Over the past decade, analytical technology has been evolving with enhanced sensitivity and resolution. Despite this, structural elucidation of the new emerging analogues in illegal health supplements remain a challenge, especially when the analogues involve complex structural modification. Common techniques as abovementioned may not be adequate to characterize the analogues. Additional works involving chiroptical methods, 2D NMR experiments and X-ray crystallography are likely required.

Keywords: Phosphodiesterase type 5 inhibitors (PDE-5i), Health Supplements, Nuclear Magnetic Resonance (NMR), High-resolution Mass Spectrometry (HRMS)

Reference:

[1.] C. L. Kee, X. Ge, M. Y. Low, V. Gilard, M. Malet-Martino, Unpublished data

OP-003

The Enantiseparation of Chiral Compounds by Chiral Stationary Phases

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Chiral stationary phase (CSP) method is the preferred method for enantioseparation of chiral drugs because it not only can be used in analyzing but also in preparation of chrial compounds. We prepared and evaluated four kinds of chiral stationary phases with macrocyclic antibiotics, natural product saponin, 1,1'-bi-2-naphthol(BINOL), aniline, respectively. Two macrocyclic antibiotics, named vancomycin and teicoplanin was used as material and different isocyanate, such as p-chlorophenyl isocyanate, m-methylphenyl isocyanate as derivatives respectively, then 10 CSPs were prepared and enantioseparated forty chiral solutes under normal phase, polar phase and reversed phase mobile phases. The interactions, including hydrogen bonding, π-π stacking, dipolar-dipolar, hydrophobic, hydrophilicity, electrostatic interaction, etc, between solutes and CSPs have influenced the reservation and enantioseparation. Two natural products saponin, named ginsenoside Rg1 and diosgenin was used material to prepared HPLC stationary phase, respectively. They have typical reversed characteristics and good at separating structure alike compounds, so they were used to established the fingerprint of Traditional Chinese Medicine including total saponins of Panax notoginseng and Gongxuening capsule. These two saponin stationary phases were derived by different isocyanate, such as phenyl isocyanate, 3,5-dimythyl phenyl isocyanate and saponin-based CSPs were fulfilled. Under reversed phase mobile phase, more than ten amino acids got different certain enantioseparation using these saponin-based CSPs. We prepared a series of CSPs from R-/S-BINOL. The results showed that the same solute got reversed elution order by R-/S-BINOL CSP and R-BINOL CSP always got better chiral ability than S-BINOL CSP. They can be used for the separation of enantiomer of N free chiral compounds and the hydroxyl group of CSP was not essential, which are different from literature reports. Furthermore, the non-conjugate structure or acyl group, is benefit for the enantioseparation, indicating that the π-π interactions and spatial stereointeractions between solutes and chiral stationary phases have a significant impact on chiral recognition. Using aniline as raw material, single chiral camphor sulfonic acid as inducing acid and dopant, we synthesized chiral polyaniline (PANI) in water, organic solvent and water organic solvent by interfacial polymerization, self-assembly, oligomer assisted method, secondary doping method, chemical oxidation method, etc. Three "sandwich" CSPs were prepared using silica gel as the core, PANI as the intermediate layer, and polysaccharide derivatives such as cellulose and starch as the shell. The baseline separation of chiral compounds such as trans-oxypyrene and 2,2,2,2-trifluoro-1- (9-anthracyl) ethanol was achieved using these PANI-based "sandwich" CSPs.

Keywords: Chiral stationary phase, macrocyclic antibiotics, natural product saponin, 1,1'-bi-2-naphthol, aniline

OP-004

Quantitative Analysis of Estradiol and Estradiol Metabolites by UPLC-MS/MS Method

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Human cytochrome P450 enzymes (CYPs) play a role in human diseases and various biological processes (Kwon, Shin, & Chun, 2021). Oxidation, reduction or hydrolysis of drugs in phase I metabolism is done by Cytochrome P450 (CYP) enzymes. CYPs not only catalyze the phase I metabolic reactions of endogenous (eg, sex hormones and arachidonic acid) or exogenous (eg drugs, poisons and procarcinogens) substances, but are also associated with drug resistance, disease occurrence, and tumor susceptibility. CYPs play a role in the metabolism of carcinogens (Luo, Yan, Yan, & Yuan, 2021). Estradiol is hydroxylated from the C2 and C4 positions by NADPH-dependent CYP enzymes and converted into 2-OH estradiol and 4-OH estradiol molecules known as catechol estradiol (Santen, Yue, & Wang, 2015). Catechol estradiol quinones, which are produced from 4-OH estradiol, react with DNA and lead to the formation of DNA adducts, that may be the start of a mutation and without proper DNA repair it can lead to carcinogenesis, the initiator of cancer. Tumor formation may result from the formation of metabolites due to the metabolism of the endogenous hormone estradiol. (Markushin et al., 2003). As the population ages, the risk of developing the disease increases, but especially in the postmenopausal stage, the increased adipose tissue, which participates in the production of estrogen - contributes to the increase of metabolites, therefore obesity in society. As the elderly population continues to grow, there is a rising demand for medical drugs and functional foods. Propolis, one of the most popular functional food in the world, has been shown to have an

inhibitory effect on some isoforms of CYP enzymes (Ryu et al.).

An analytical method was developed for in vitro drug metabolism of estradiol using liver microsome (Knights, Stresser, Miners, & Crespi, 2016). In this study, an in-house UPLC-MS/MS method was developed and validated for the quantitative analysis of Estradiol, 2-OH Estradiol and 4-OH Estradiol. The method has been validated according to the European Medicines Agency (EMA) Bioanalytical method validation guidelines (Agency, 2011).

In the bioanalytical method we developed for the quantitative analysis of estradiol and its metabolites, the lower limits of quantitation for estradiol, 2-OH Estradiol and 4-OH Estradiol were determined as 0.25 µg/mL, 50 ng/mL and 10 ng/mL, respectively. The calibration working ranges of the method for Estradiol, 2-OH Estradiol and 4-OH Estradiol molecules were selected as 0.25 – 25.00 µg/mL, 50 – 5000 ng/mL and 10 – 1000 ng/mL, respectively. The correlation coefficient of the calibration working intervals determined for all molecules is > 0.99. The developed method was used to measure the inhibitory effect of propolis on estradiol metabolites 2-OH Estradiol and 4-OH Estradiol. Propolis inhibited the rate of formation of both metabolites.

Keywords: UPLC-MS/MS, ostradiol, 2-OH ostradiol, 4-OH ostradiol, propolis

OP-005

Mechanistic Studies on Enantioselective Noncovalent Selector-Selectand Interactions Using Capillary Electrophoresis, Nuclear Magnetic Resonance Spectroscopy and Isothermal Titration Microcalorimetry

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The project aimed to explore the potential of capillary electrophoresis (CE) for studying enantioselective intermolecular interactions. It focused on utilizing cyclodextrins and their derivatives as chiral selectors and a range of chiral compounds, particularly chiral drugs, as selectands. Cyclodextrins offer advantages such as well-characterized structures, transparency in the UV range for compatibility with CE instruments, and well-defined NMR spectra for elucidating complex structures.

Cyclodextrins are widely studied complex formation agents with applications in various fields such as analytical chemistry, physical chemistry, organic chemistry, supramolecular chemistry, materials sciences, and the pharmaceutical industry. They are commonly used to improve water solubility, mask taste and odor, and address formulation challenges of hydrophobic drugs.

The study employed CE, NMR spectroscopy, and isothermal titration calorimetry (ITC) to elucidate the mechanisms of enantioselective complex formation between cyclodextrins and chiral guest molecules. CE proved to be highly sensitive for detecting weak enantioselective effects, while NMR spectroscopy provided insights into complex structures and association constants. ITC was used to measure small energetic effects in guest-cyclodextrin interactions.

The project advanced knowledge in enantioselective intermolecular interactions by combining CE, NMR spectroscopy, and ITC. These techniques provided comprehensive insights into the structural, thermodynamic, and enantioselective aspects of cyclodextrin complexes with chiral analytes. The findings contribute to further investigations and potential applications in various fields.

Keywords: Chirality, ITC, CE

OP-006

Novel Solutions in Drug Discovery from Complex Natural Matrices for the Prevention and Treatment of Neurodegenerative Diseases

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Neurodegenerative diseases (NDs) and other age-associated chronic disorders have emerged as one of the greatest medical challenges of the 21st century. Despite numerous years of research very few effective treatment options are available for Alzheimer's or Parkinson's disease. Complex natural matrices are rich in phar-

macologically active compounds and have served as a great source of drug leads for NDs. Additionally, epidemiological data suggest consumption of a diet rich in fruits and vegetables confers protection against chronic diseases, including NDs. Key challenges in the identification and extraction of pharmacologically active compounds from complex natural mixtures, including dietary sources is a lack of suitable assays and the need for laborious isolation and characterization steps to identify drug hits within an active mixture. Here I will present our recent work focused on the development of bioaffinity chromatography columns and cell membrane coated nanoparticles for the identification of new potential ligands targeting transmembrane receptors. The focus will be on tropomyosin receptor kinase B (TrkB) that has been proposed as a potential novel target in the development of drugs for numerous neurological disorders. Additionally, I will present our efforts focused on unraveling the neuroprotective mechanism of action of dietary phytochemicals. We have proposed that certain lipophilic dietary soft electrophiles nonenzymatically post-translationally modify regulatory proteins, such as transcription factors, which ultimately results in neuroprotection. I will provide an overview of bioanalytical methods used in our laboratory, based on in vivo animal model studies and MALDI-MS analysis, aimed at the identification of these post-translationally modified proteins. Overall, this presentation aims to depict new approaches that hopefully will reinvigorate drug discovery from natural matrices for neurological disorders.

Keywords: Neurodegenerative diseases, drug discovery, bioaffinity chromatography, nanoparticles, tropomyosin receptor kinase B, MALDI-MS

OP-007

Development of Capillary Electrophoresis-Mass Spectrometry Method for the Simultaneous Analysis of Boswellic Acids and Non-Steroidal Anti-Inflammatory Drugs

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The separation of boswellic acids (BAs), natural compounds with anti-inflammatory activity, has only been achieved by capillary electrochromatography [1], while the use of other capillary electrophoresis (CE) methods in their analysis has not been described. Non-steroidal anti-inflammatory drugs (NSAIDs) could be potential adulterants of dietary supplements based on boswellic acids. Thus, the development of CE method which allows the simultaneous analysis of both classes of these compounds is of high importance. Such a method can play an important role in the detection of the potential adulteration of dietary supplements containing BAs.

During the preliminary experiments, capillary zone electrophoresis (CZE), non-aqueous capillary electrophoresis, and CZE with a high content of organic modifier were tested. The last approach based on 40 mmol/L ammonium acetate (pH 8.5), MeOH and ACN (5:1:4, v/v/v) background electrolyte was the most successful in terms of simultaneous analysis of both BAs and NSAIDs. The coupling of CE with mass spectrometric detection increased the selectivity of the method compared to UV detection. However, this approach also presents many challenges, including the high fragility of separation capillaries under certain experimental conditions [2]. This problem could be successfully solved by (i) avoiding high ammonia content in the sheath liquid, which is based on 75% aqueous isopropanol solution, and (ii) setting low temperatures and flow rates of both the sheath and drying gases of the Agilent Jet Stream ion source. Although the optimization of ion source parameters is usually a routine task, we had to use design of experiments to understand the effect of ion source settings on the method performance in more comprehensive way. In the first stage, screening based on a two-level full factorial design allowed rapid selection of variables that significantly affected ion abundances in 19 runs. The most significant factors, i.e., nozzle voltage (500-2000 V), needle voltage (1000-4500 V), iFunnel high pressure RF voltage (80-120 V), and iFunnel low pressure RF voltage (10-210 V) were further optimized using a central composite design with 27 experiments. The optimal method run was carried out in the selected reaction monitoring mode under the negative polarity of the ESI voltage and allowed the separation of BAs in addition to 13 NSAIDs. Using our method, the oleane and ursane types of boswellic acids cannot be distinguished due to the same electrophoretic behavior as well as the same transitions. Therefore, further research is needed to find suitable separation conditions that allow the separation of all 6 major BAs. The optimal method will be validated and applied to the analysis of dietary supplements containing *Boswellia serrata* extract.

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Keywords: Adulteration, boswellic acids, capillary electrophoresis, frankincense, mass spectrometry, non-steroidal anti-inflammatory drugs

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OP-008

Determination of Tamoxifen in Biological Fluids Using Liquid-Phase Microextraction and Liquid Chromatographic Analysis

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Breast cancer is one of the most common types of cancer in women and affects approximately one in ten women worldwide. Tamoxifen is the treatment of choice for patients with all stages of estrogen receptor positive breast cancer. In study a new, rapid enrichment and determination method for Tamoxifen in biological samples has been developed based on liquid–liquid microextraction and HPLC-DAD analysis. In proposed method; n-octanol was used for extraction solvent the presence of pH 5.0 buffer and extraction phase was diluated with ethanol prior to chromatographic determinations The samples were transferred to HPLC microvials by filtration with a 0.45 µm porous PTFE filter before HPLC analysis. Experimental variables such as pH, volume of n-octanol, ionic strength, extraction time were studied and optimized in detail. By extraction using optimal conditions, analytical parameters such as linear ranges, detection limits, pre-concentration factor for tamoxifen were calculated by using experimental data. In the developed method; tamoxifen in biological samples was analyzed on isocratic elution of acetonitrile:pH 3.0 buffer solution (50 mM) (30:70 v/v) with DAD detector at 240 nm wavelength. Low Limit of Quantification (LLOQ) values for tamoxifen in plasma and urine calculated as 5.0 and 2.0 ng mL-1, respectively. Relative standard deviations (RSD) were below 6.0 % for determinations of model biological sample solutions. Finally, the developed method has been applied to biological samples and quantitative results have been obtained in recovery experiments. The developed liquid phase microextraction method is faster than classical sample preparation procedures such as solid phase extraction or liquid-liquid extraction, with a minimum sample handling, less solvent consumption, and is a promising screening procedure for analysis of tamoxifen.

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Keywords: Tamoxifen, liquid phase microextraction, biological samples, HPLC

OP-009

Separation and Determination of Enantiomers of 2-Hydroxyglutaric Acid in Urine by Capillary Electrophoresis with Contactless Conductivity Detection

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2-hydroxyglutaric acid (2-HG) is a chiral metabolite whose enantiomers accumulate in urine in D-2-hydroxyglutaric aciduria (D-2-HGA) and L-2-hydroxyglutaric aciduria (L-2-HGA). These acidurias are autosomal recessive neurometabolic disorders. The identification of the enantiomers of 2-HG plays an important role in the diagnosis of these diseases. Capillary electrophoresis is an effective technique for the enantiomeric separation of chiral species because of its high separation efficiency, superior resolution, rapid analysis time, and small consumption of the sample and electrolyte. In this study, an easy and fast capillary electrophoresis system combined with a contactless conductivity detection method was developed for the separation and determination of 2-HG enantiomers in urine. The optimal separation conditions for enantiomers were achieved by the use of a buffer containing 50 mM 4-(N-morpholino) butane sulfonic acid solution (pH 6.5), an electroosmotic flow modifier (0.001% (w/v) polybrene), and 30 mM vancomycin as chiral selector. The analysis time was 7 min under optimal conditions. The linearity of the method was determined to be in the range of 2–100 mg/L for D- and L-2-hydroxyglutaric acid in urine. The precision (relative standard deviation%) was obtained as < 7%. The limits of detection for D- and L-2-hydroxyglutaric acid were 0.566 and 0.497 mg/L, respectively. The optimized and validated method was successfully implemented for quantifying L-2-HGA in patients' urine, without any pretreatment step. The quantity of L-2-HG in patient urine samples was determined to be in the range of 108.0–4654 mg/L. The method could be potentially used for routine clinical analysis of D- and L-2-HG in patient urine samples with D- and L-2-HGA.

Keywords: Chiral separation, metabolic disorders, clinical analysis, urine

OP-010

A Deep Eutectic Solvent-Based Ferrofluid for Vortex-Assisted Liquid-Liquid Microextraction of Nonsteroidal Anti-Inflammatory Drugs from Environmental Waters

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the main classes of environmental contaminants due to their widespread application, poor degradability, and incomplete removal in wastewater treatment plants [1]. Consequently, simple, reliable, and sensitive analytical methods are needed to determine environmental NSAID levels.

The development of smart materials has significantly influenced sample preparation methods. Among them, ferrofluids are of particular interest because of their liquidity and magnetic controllability, which facilitates their recovery after extraction [2]. In this work, a ferrofluid, prepared from magnetic nanoparticles and a deep eutectic solvent (DES), was applied in vortex-assisted liquid-liquid microextraction (VALLME) of NSAIDs (nimesulide, naproxen, and flurbiprofen) from environmental waters prior to their analysis by high-performance liquid chromatography with photodiode array detection (HPLC-DAD). Initial studies were conducted to select the optimum DES composition using thymol, menthol, acetic acid, pentanoic acid, and azelaic acid as precursors. The best results were obtained with pentanoic acid and menthol as the hydrogen bond donor and the hydrogen bond acceptor, respectively. The ferrofluid was prepared by mixing the DES and oleic acid-coated Fe_3O_4 nanoparticles, followed by ultrasonication for 30 min [3]. For VALLME, 200 µL of ferrofluid was vortex-mixed with the 16 mL sample for 45 s. No centrifugation step was needed for phase separation, but the ferrofluid was simply separated from the aqueous phase by placing a neodymium magnet outside the vial. Following the removal of the sample matrix, 200 µL methanol was added to the extract in order to destroy the ferrofluid structure. Finally, the magnet was placed at the bottom of the vial to separate magnetic nanoparticles, and 10 µL of the clear supernatant was injected into HPLC. Experimental parameters affecting the extraction efficiency were optimized, including the weight percentage of magnetic nanoparticles, ferrofluid volume, pH, salt concentration, and vortex time. Under optimum conditions, calibration curves were linear in the range of 5-100 µg/L. Relative standard deviations of intra- and inter-day experiments at 50 µg/L level were in the ranges of 2.0-4.1% and 3.6-5.3%, respectively. The limit of detection values ranged from 1.6 to 2.0 µg/L, and enrichment factors were between 38.9 and 50.6. The applicability of the developed method was demonstrated by intra- and inter-day accuracy values ranging from 90.3% to 108.0% for tap, river, and lake water samples spiked at 20 and 80 µg/L levels. The results show that the use of ferrofluid in liquid-phase microextraction methodologies can be considered a desirable alternative for the analysis of environmental contaminants.

Keywords: Deep eutectic solvents, ferrofluids, environmental analysis, liquid-phase microextraction

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OP-011

Selective Determination of D-Amino Acids in Human Plasma Using a Two-Dimensional LC-MS/MS System Toward Early-Diagnosis of Chronic Kidney Disease

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Several D-amino acids, the minor enantiomers of L-amino acids, have been discovered in living organisms with the recent advances in analytical techniques. Especially in the medical/health science areas, the relationships between intrinsic D-amino acids and diseases, for example kidney diseases, have been gathering attention because non-negligible amounts of several D-forms were found in the blood of patients with chronic kidney disease (CKD). Accordingly, D-amino acids are expected as new diagnostic biomarkers for the estimation of renal function. Various analytical methods discriminating D- and L-enantiomers

have been reported, however, the precise determination of intrinsic chiral amino acids is frequently disturbed by the uncountable co-existing biomolecules even using an LC-MS (MS/MS). Due to the insufficient selectivity of the methods, the scientific insights about the usefulness of various D-amino acids for the assessment of kidney function were limited, therefore, the development and utilization of highly selective analytical methods are continuously required. In the present study, the two-dimensional (2D) LC separation and the detection of specific ion pairs by an MS/MS were integrated for the selective/comprehensive analysis of chiral amino acids in clinical samples. Using the system, D-amino acids in the plasma of CKD patients were determined to find potential biomarkers for early/precise diagnosis.

Alanine, aspartate, glutamate, leucine, lysine, methionine, phenylalanine, proline, serine and valine were selected as the targets considering the presence of their D-forms in mammals, and were subjected to the HPLC analysis after derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). The 2D LC-MS/MS method was designed/developed as a fully-automated system combining a reversed-phase C18 column (Singularity RP18, 1.0 x 500 mm, 3 µm, 45°C), a Pirkle-type enantioselective column (Singularity CSP-001S, 1.5 x 150 mm, 5 µm, 25°C) and an MS/MS (Triple Quad 5500). In the first dimension, the NBD-amino acids were separated by the gradient elution using aqueous 10–30% acetonitrile solutions containing 0.05% trifluoroacetic acid. Only the fractions containing the target analytes were transferred to the next dimension in order by switching the valves automatically. The enantiomers of individual amino acids were, then, separated by the Singularity CSP-001S column (Rs≥1.38) with isocratic elution using mixed solutions of acetonitrile/methanol (50/50 or 75/25, v/v) containing formic acid (0.05–0.2%). The developed system was validated by checking the linearity (r^2≥0.9952), precision (RSD≤6.67%) and accuracy (94.2–114.1%) for all analytes, and applied to human plasma. As a result, no interfering peaks were observed and all target chiral amino acids were successfully determined by one injection to the HPLC system. The amounts of D-enantiomers (%D, D/(D+L)x100) were regulated in low ranges in the plasma of healthy volunteers, while those of several D-forms significantly elevated with the progress of kidney diseases ($p<0.05$). To confirm the results, target amino acids in tested plasma samples were further determined using different mobile phase conditions for the enantiomer separation, and the obtained %D values were consistent with those using original mobile phase conditions. These D-amino acids are the potential biomarkers for the early/precise diagnosis of kidney diseases, and further evaluation using a large number of CKD samples are expected.

Keywords: D-Amino acids, Chiral LC-MS/MS, Chronic kidney disease

OP-012

Development of a Method for Quantification of Some Metallic Content of *Humulus lupulus* L. Plant with ICP-OES

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Abstract

Metallic content of herbs is very important research area in pharmacognosy especially for food supplements, herbal teas etc. An efficient extraction procedure should be developed for each specific plant which will be used further. Additionally, rapid and highly sensitive analytical technique should be selected. In this study, quantification of some important metallic content of *Humulus lupulus* L. will be done with ICP-OES analytical technique. The appropriate harvesting time can be determined by using results obtained from this study. Additionally, the developed method can be used as a quality control analysis for the commercial products produced from this plant.

Keywords: Metallic content, Humulus lupulus L., ICP-OES

I. Introduction

Humulus lupulus L. plant belongs to the family Cannabaceae of the Urticales order from a taxonomic point of view and hops are the female inflorescences of this plant [1]. Hops are mainly used in the brewing industry due to not only increase the bitterness and aroma but also provide microbial stability and strengthen the stability of the foam [2]. Additionally, hops have been also used in traditional medicine mainly to treat anxiety, restlessness, insomnia and others [3]. Many biological activities of hops have been discovered in recent studies in the literature such as treatment of menopausal hot flashes and osteoporosis, the ability to whet one's appetite and to improve digestion, treatment of tension headache, relief for toothaches, earaches and neuralgia, anti-atherogenic properties, anti-inflammatory properties, strong antioxidant properties, and anticancer properties [4].

Analysis of metallic content of hops are very important because some metals can be derived from hops products which are constantly maintaned over time in organisms. Different analytical techniques have been used for the quantification of metallic content in hops such as AAS, ICP-MS and ICP-OES [5]. In this study, ICP-OES technique is used to quantify some metallic content of hops and herbal tea samples.

II. Material and method

0.2500 g of each hops sample was placed into a PTFE digestion vessel in triplicate, followed by addition of 4.0 mL of nitric acid (65%) and 2.0 mL of hydrogen peroxide (35%), and 1.0 mL

of perchloric acid (70%). Firstly, the temperature was kept at 60 °C for 1 h for absolute digestion of total organic matter. Next, the temperature was kept at 180 °C for 10 min to obtain clear and transparent solution. Blanks were prepared in each lot of samples. All experiments were performed in triplicate.

The plant was dried under the shade and grinded by grinder. The grinded plant was extracted with absolute ethanol three times. The extract was filtered and evaporated at 40°C until dryness under the low pressure by rotary evaporator.

The plant was extracted with water three times. The extract was filtered. The filtered extract was freezed at -20°C and lyophilized until dryness.

III. Results and discussion

External calibration technique was followed for the quantitative analysis of samples. Standard solutions were prepared in 1% (w/w) nitric acid by diluting a multi-element standard solution containing nine analytes: Al, Ca, Fe, Mn, Mg, K, Na, Ba, and Ti. The measured wavelength of each element is shown in Table 1. Under optimized measurement conditions, five different concentrations of standard solutions were measured and calibration curves were plotted from the limits of detection of the corresponding elements. Calibration curves of the standard solutions were linear having the square of correlation coefficient ($R2$) exceeded 0.9990.

The standard solutions having lowest concentrations were used for estimating LOD and LOQ. The LODs were calculated as triple standard deviation (σ) of the signal obtained from a set of independently prepared standard solutions (n=10), and the LOQ as 10σ. LOD and LOD values are also shown in Table 1.

Tab. 1: Measured wavelength of metals, LOD and LOQ values

Elements (emission λ)	LOD (ppb)	LOQ(ppb)
Al (396.153)	10,23	33,8
Ca (317.933)	12,36	40,8
Fe (238.204)	9,86	32,5
Mn (257.610)	12,65	41,7
Mg (285.213)	2,56	8,5
K (766.490)	2,36	7,8
Na (589.592)	2,56	8,5
Ba (233.527)	1,54	5,1
Ti (334.940)	2,36	7,8

The concentration values of the nine metals for hops and tea samples are listed in the Table 2 below.

Tab. 2: Concentration values of the metals in hops and tea samples

Sample	Al (ppm)	Ca (ppm)	Fe (ppm)	Mn (ppm)	Mg (ppm)	K (ppm)	Na (ppm)	Ba (ppm)	Ti (ppm)
Hops	16,306	9975,355	99,603	42,856	2465,698	17972,839	459,357	21,731	0,313
W. E.	14,553	7778,805	82,367	16,887	2174,651	9143,662	114,333	10,497	0,018
E. E.	0,000	0,870	0,005	0,011	0,788	5,893	0,130	0,000	0,001
T1	13,246	7080,653	74,975	15,372	1979,474	8323,013	104,072	9,555	0,016
T2	95,100	12878,196	1034,472	52,281	3957,832	12241,494	513,109	13,849	4,134
T3	18,266	13431,979	123,442	19,833	3859,259	15528,054	280,653	15,167	0,093
T4	15,243	13974,087	97,198	27,336	3751,746	10041,968	224,864	21,438	0,079
T5	33,537	17046,173	146,441	29,950	3280,826	8492,368	414,058	17,086	0,043
T6	77,136	11807,504	627,161	42,708	4220,217	11653,788	395,839	12,143	2,097
T7	20,681	12189,051	126,279	27,840	3896,312	14618,297	656,551	14,931	0,117
T8	1,477	14147,986	1136,471	57,436	4348,074	13448,505	563,702	15,215	4,542
T9	47,614	17079,992	605,039	46,626	4516,487	12825,663	296,875	9,324	1,103
T10	0,681	119,616	3,049	0,129	52,605	107,745	0,000	0,238	0,000

IV. Conclusion

The developed method is reliable, sensitive and economical by considering the discussed results and data. The LOD and LOQ values are quite good with the other studies in the literature. This study demonstrated a method for quantification of some metals in hops and tea samples. This study contributes the general features of the metallic content of hops in Bilecik city, which can be used to preliminarily prediction of different varieties and origins of hops. In vivo relationship between functional ingredients and certain metal elements should be further investigated. In conclusion, the developed method can be used as an alternative method for quality control of hops and tea products in the market.

Acknowledgements: I thank to Assoc. Prof. Dr. Mert İlhan and Prof. Dr. Ufuk Koca Çalışkan.

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OP-013

Synthesis of Chiral Sulfoxides and Study of Structure-Retention and Structure-Enantioselectivity Relationships

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Chiral sulfoxides are of great interest due to their biological and pharmaceutical activities and diverse applications in organic synthesis. The sulfinyl group has been shown to be an efficient chiral auxiliary in carbon-carbon and carbon-oxygen bond forming reactions, in cycloaddition reactions, radical addition reactions and in asymmetric catalysis. There is a high interest to sulfoxides in medicinal and pharmacological chemistry due to their variety of biological activities, including anti-cancer, anti-viral, anti-bacterial properties, for treatment of schizophrenia and others.

The main goal of the present study was to investigate structure-retention and structure enantioselectivity relationships in the series of structurally related chiral sulfoxides. In order to reach this goal some novel chiral sulfoxides were synthesized. Enanioeparations of these analytes were studied on different commercial and non-commercial polysaccharide-based chiral stationary phases using various mobile phases.

Based on obtained separation results interesting conclusions regarding the effect of structural characteristics (substituents nature and position) of the chiral sulfoxides and chiral selectors can be drawn that shall allow further optimization of chiral chromatographic separation systems.

Keywords: Chirality, Chiral sulfoxides, Enantiomers, High Performance liquid chromatography, enantioseparations

OP-014

A Molecularly Imprinted Polymer-Based Electrochemical Sensor for the Determination of Emtricitabine

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Abstract

This study explains the fabrication of the highly sensitive electrochemical sensor based on a molecularly imprinted polymer (MIP) for the determination of Emtricitabine (EMT). In this work, the MIP-based electrochemical sensor was designed by photopolymerization on the glassy carbon electrode (GCE) surface. The characterization of the electrode performance was evaluated with electrochemical analyses (cyclic voltammetry and electrochemical impedance spectroscopy). It was successfully applied for EMT determination in standard solution, and serum sample with high sensitivity and accuracy using differential pulse voltammetry method meanwhile utilizing the 5 mM solution of $[Fe(CN)_6]^{3-/4-}$ as the redox probe. Additionally, interference studies and imprinting factor calculations were performed with common interfering agents and using similar compounds to demonstrate the selectivity of the sensor. Finally, the non-imprinted polymer (NIP)-based sensor confirmed the performance of the MIP sensor.

Keywords: Emtricitabine, Molecularly imprinted polymer, glassy carbon electrode

I. Introduction

EMT is used along with other medications to treat human immunodeficiency virus (HIV) infection. EMT is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs) (Al-Majed vd., 2020). Electrochemical sensors have continued to be the most popular technology due to having distinctive features such as fast determination, simplicity, low-cost, and easy-to-use. However, electrochemical methods displays poor selectivity. Therefore, many efforts have recently been made to design and fabricate electrodes to improve the performance of the electrochemical sensors, especially significant improvements in molecularly imprinted polymer (MIP) technology make them superior candidates to possess high affinity and selectivity, better detection performance, reproducibility, long-term stability, and easy preparation. MIP is considered an artificial receptor, produced by the copolymerization of functional monomer with cross-linkers in the presence of a template molecule, which has a three-dimensional network structure with complementary to the template in

shape, size, and chemical functionalities of the template molecule (Crapnell vd., 2020; Lu vd., 2014).

This study, therefore, aims at developing an interface imprinting method to be used in the electrochemical sensor for EMT detection in serum samples. Herein, differential pulse voltammetry was employed as a detection method, while the molecularly imprinted polymer (MIP) films were formed with EMT as a template molecule. MIP films were developed on glassy carbon electrodes by copolymerization of 4-amino benzoic acid (4-ABA) and hydroxyethyl methacrylate (HEMA) with the crosslinker ethylene glycol dimethacrylate (EGDMA) in the presence of EMT, and the sensor was subjected to UV radiation at room temperature for 5 min.

II. Material and method

Potassium ferricyanide ($K_4[Fe(CN)_6]$, ≥99.0%) and potassium ferrocyanide ($K_3[Fe(CN)_6]$, ≥98.5%) were provided by Merck. Methanol (99.8%), ACR, hydroxyethyl M-methacrylate (HEMA; ≥ 99%), ethylene glycol dimethacrylate (EGDMA; > 98.0%), 2-hydroxy-2 methyl propisphenone, , sodium hydroxide (>97%), sodium acetate trihydrate (> 99%), acetic acid (99%), sodium phosphate monobasic (≥99.0%), paracetamol, ascorbic acid, potassium nitrate (KNO_3), sodium sulfate (Na_2SO_4), magnesium chloride ($MgCI_2$), dopamine, and sodium dihydrogen phosphate dihydrate were purchased from Sigma-Aldrich.

III. Results and discussion

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) are mostly used electrochemical techniques for the surface characterization and comparison of bare and modified electrodes. CV and EIS techniques are also preferred for sensor's characterization. In this way, the surface properties were characterized at different stages during the preparation of the sensor, before polymerization, after polymerization, after removal, and after rebinding. A polymeric film covering the electrode surface was obtained thanks to the photopolymerization process.

Analytical validation and performance evaluation of the MIP/GCE sensor was performed by determining EMT in the standard solution using the DPV method. The selectivity study was performed using DPV methods. According to the results, it was found that the interference substances did not affect the analytical ability of the MIP/GCE sensor. Serum sample application studies are important in applicability and accuracy. Therefore, the developed MIP/GCE sensor was applied to commercial human serum samples using DPV method.

IV. Conclusion

This study describes the first MIP-based electrochemical sensor application for the determination of EMT in standard solution and serum samples. Additionally, the applicability of electrochemical methods, DPV, was confirmed with analytical performance evaluation studies. Very low LOD and LOQ values were acquired. The MIP film obtained via photopolymerization showed very selectivity towards EMT compared to other similarly structured molecules. Serum sample studies yielded very good recovery% and RSD% results demonstrating the accuracy and applicability of the sensor with DPV. In conclusion, this newly developed MIP-based electrochemical sensor enables a selective, sensitive, short analysis time, stable, and low-cost approach for EMT determination.

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OP-015

Mycosporine Like Amino Acids – Isolation and Analysis of Promising Marine Sunscreens

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Among the compounds found in marine organisms, especially red algae, mycosporine like amino acids (MAAs) are unique due to their extremely high UV extinction coefficients, rendering them interesting candidates as sunscreens for human use. MAAs are small secondary metabolites with a cyclohexenone or cyclohexenimine scaffold, substituted with different amino acids like glycine, valine or alanine. However, because of a very polar nature and low abundance, their analysis as well as isolation is challenging.

For nearly ten years we intensively have been working on this interesting group of natural compounds and our respective achievements are summarized in this presentation. After a short general introduction to MAAs, their isolation by Fast Centrifugal Partition Chromatography is presented using an uncommon aqueous two-phase system (ATPS). It permitted the isolation of two common representatives, shinorine and porphyra-334, in good yield and purity from *Porphyra* sp. (Nori) in a single run of

90 min [1]; this is not possible by conventional means like column chromatography. The quantitative determination of MAAs in diverse algae has been achieved by several analytical techniques, ranging from (Ultra) High Performance Chromatography [2] over Hydrophilic Interaction Liquid Chromatography [3] to Capillary Electrophoresis [4]. The methods are compared and individual pros and cons of each approach highlighted. For example, for fully resolving eleven most common MAAs by HPLC 35 min were required, whereas with UHPLC this could be achieved in only 8 min. In terms of economic operation CE is definitely unmatched, as sample and buffer consumption are neglectable; however, analysis time was longer (27 min for 5 MAAs) and reproducibility problematic. All methods were validated and respective results are opposed, additionally supplemented by quantitative data of real samples, e.g. the determination of bostrychines, novel MAAs occurring in the red algal genus *Bostrychia*.

Despite of their simple structure, MAAs are challenging from an analytical point of view. Previous concerns regarding insufficient retention and selectivity of the available assays as well as questionable peak assignments have been solved with the here presented methods. Therefore, an excellent starting point for future research on these highly interesting natural products is provided.

Keywords: Mycosporine like amino acids, MAA, analysis, isolation

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OP-016

Inkjet-Printing of MXene/ Holey CNT-based Inks for the Construction of Flexible Nicotine Sensors

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Inkjet printing of two-dimensional (2D) materials has gained a great deal of interest in the development of flexible electrodes, sensors, and biosensors in the last couple of years. MXenes belong to the 2D material family and exhibit unique physicochemical properties, including large physical surface area, high electronic conductivity, and abundant surface functional groups. The tailorable surface properties of MXenes make them excellent functional ink materials owing to their high dispersibility in water-based solutions. On the other hand, the MXene-based inks should be modified with more conductive carbon-based materials to achieve high electrode conductivity, which is essential to develop electrochemical sensors with high sensitivity and a low limit of detection. In this regard, in the present work, we developed MXene/holey CNT-based inks with high stability and dispersibility in water-based dispersants for the inkjet printing process. The inks were then printed on flexible polymer-based substrates to construct electrochemical nicotine sensors. The performance of the sensors against nicotine was evaluated and real sample analyses were conducted to validate the applicability of the sensors to detect nicotine in complex media.

Keywords: Inkjet printing, flexible sensors, MXene, holey CNT, electrochemical sensor

OP-017

Biopolymers as Antibacterial Wound Dressing and Controlled Drug Release Agents

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Biopolymers are natural polymers and are obtained from animals, plants, or algae. They are biocompatible and biodegradable and have wide pharmaceutical and biomedical applications. The use of biopolymers as wound dressing and controlled drug release agents are the research topics that have appeared intensively in recent years. This presentation provides a review of our laboratory's research on developing composite biopolymers as wound dressing and controlled release agents.

Recently, the wound-healing properties of numerous metal nanoparticles have been demonstrated. Cerium oxide nanoparticles (CeO_2 NPs) have recently attracted attention in biomedical applications due to the unique reversible transition between Ce_3^+ and Ce_4^+ states, which is the basis of these particles' biological mimetic, antioxidant, anti-inflammatory, and anti-bacterial activity properties. We prepared antibacterial CeO_2 NPs /chitosan/cellulose acetate [1] and CeO_2 NPs / chitosan/hydroxyethyl cellulose/polyethylene glycol composite films [2], respectively as po-

tential wound dressing materials. Films were characterized with physical, mechanical, and antibacterial tests and were proposed as suitable potential wound dressing materials. Alginate films were crosslinked with cerium (III) solution and chitosan-added cerium (III) solution [3]. Both cerium ions crosslinked, and cerium ion-chitosan crosslinked alginate films gained antibacterial activity against Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacteria. Results show that cerium alginate-chitosan films can be flexible, ultraviolet-protecting, and antibacterial wound dressings. Barium ion cross-linked alginate-carboxymethyl cellulose composites were prepared for controlled release of the anticancer drug methotrexate (MTX) [4]. The results showed that almost the total amount of MTX release (98.1%) was completed in 5 h. $ZnFe_2O_4$-Chitosan- NPs were prepared and loaded with imatinib, a tyrosine kinase inhibitor known as "Glivec". At the end of 5 hours, 91.6% of the total amount of imatinib was released from nanoparticles [5]. The SDS-modified alginate beads was proposed as suitable carriers for the passage of orally taken protein-type drugs into the colon medium by preventing their degradation in acidic gastric fluid [6]. Novel pH-sensitive alginate–protein–clay composite beads were investigated for the in vitro oral delivery of the model protein, bovine serum albumin (BSA) [7]. No BSA release was detected until 60–90 min after the first contact time of beads with a gastric solution. The presence of clay in alginate beads prevented burst release in higher pH of the intestine by slowing the release rate of BSA to 45–55% within around 9 h, resulting in a potential matrix for intestinal release of protein drugs.

Keywords: Biopolymers, Wound Dressing, Controlled Drug Release, Nanoparticles

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OP-018

An Immunosensor Using A Specific Aptamer as A Bioreceptor for Quantification of Sarcosine

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Abstract

Sarcosine has been found as a urine-detectable differential metabolite that is significantly elevated with the progression of prostate cancer (PCa) to metastasis. It has also been demonstrated that adding sarcosine to cultures of benign prostate cells increases their invasiveness and motility. It has been reported that sarcosine, when combined with other markers and diagnostic techniques, has the potential to enhance the future accuracy of PCa detection. In this context, a gold quartz tuning fork (GQTF)-based aptasensor was developed to assess the correlation between sarcosine levels and PCa. SH-modified sarcosine-specific single-stranded DNA aptamers were covalently linked to the surface of GQTFs for sensitive detection in a complex environment mimicking urine conditions. Following the optimization studies conducted to construct an effective bioactive layer, extensive characterization processes were carried out to characterize the surface properties and assess the performance of the generated biosensor. The designed aptasensor showed a low detection limit of 5.19 fg/mL. The electrochemical aptasensor's promising sensing performance demonstrates its potential as a superior alternative to existing sensing technologies.

Keywords: Aptasensor, quartz tuning fork, sarcosine, prostate cancer, electrochemical impedance spectroscopy

I. Introduction

Electrochemical impedance spectroscopy (EIS) is an efficient technique for biosensor characterisation and construction. EIS can be used successfully to measure target molecules and charge transfer resistance (Rct). The Randles-Ershler electrical equivalent circuit model is the chosen electrical circuit model for a simple electrochemical reaction. This equivalent circuit model consists of an ohmic resistance (Rs) in relation to the electrolyte solution resistance, Rct between the working electrode and the reference electrode, the double layer capacitance associated with

the capacitance of the complex bioactive layer, and the normal diffusion across the complex layer. It addresses the Warburg impediment (Zw) (Dhillon & Kant, 2017)fuel cells, sensors etc. It is also used in understanding response of the fundamental systems with coupled processes like charge transfer, diffusion, electric double layer charging and uncompensated solution resistance. We generalize phenomenological theory for the Randles-Ershler admittance at the electrode with double layer capacitance and charge transfer heterogeneity, viz., non-uniform double layer capacitance and charge transfer resistance (cd and RCT. Ideally, electrochemical biosensors would be able to overcome the constraints of these detection methods due to their simple detection procedure, on-site quantitative analysis, rapid detection time, high sensitivity, and mobility. These advantageous characteristics make electrochemical biosensors particularly desirable for biomarker detection in situ and in real-time in test samples. Due to their superior features and simple production, aptamer-based electrochemical sensing systems, also known as electrochemical aptasensors, are widely utilized in most diagnostic applications (Keefe et al., 2010; Zambry et al., 2022)accurate, and efficient detection methods for Salmonella can significantly control the outbreak of salmonellosis that threatens global public health. Despite the high sensitivity and specificity of the microbiological, nucleic-acid, and immunological-based methods, they are impractical for detecting samples outside of the laboratory due to the requirement for skilled individuals and sophisticated bench-top equipment. Ideally, an electrochemical biosensor could overcome the limitations of these detection methods since it offers simplicity for the detection process, on-site quantitative analysis, rapid detection time, high sensitivity, and portability. The present scoping review aims to assess the current trends in electrochemical aptasensors to detect and quantify Salmonella. This review was conducted according to the latest Preferred Reporting Items for Systematic review and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR.

II. Material and method

Electrochemical studies were conducted with a Gamry Reference 600 Potentiometer/Galvanometer and a 5mM $[Fe(CN)_6]^{3-/4-}$ 50 mM phosphate buffer (PB solution) (pH 7) containing 0.01M KCL as the redox probe. In the triple electrode system, GQTF was employed as the working electrode, 3M Ag/AgCl as the reference electrode, and platinum as the counter electrode. Shoulder Crystal Corporation (China) supplied the GQTF electrodes, while Sigma-Aldrich Company supplied all the other substances utilized in this work (Germany). Aptamers were selected using the systemic evolution of ligands by exponential enrichment (SELEX) method with the aid of graphene oxide (GO), as described in previous research (Özyurt et al., 2019). Figure 1 illustrates the schematic of the immobilization operation.

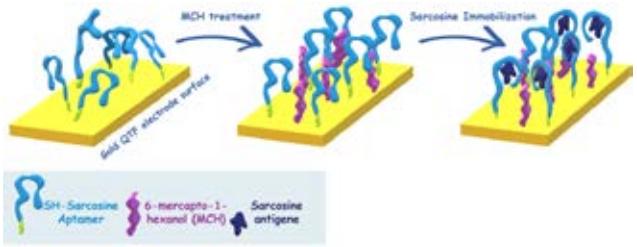

Figure 1. A strategy for immobilizing the sarcosine aptasensor

III. Results and discussion

In this research, the very sensitive EIS technology was used with aptamers as a recognition element to detect sarcosine. To design a functional aptasensor, it is required to determine the optimal parameters. Establishing certain conditions permits the establishment of parameters under which appropriately aligned recognition components are sufficient to build a homogeneous layer. After the construction of an effective bioactive layer was optimized, detailed characterization operations were done to characterize the surface characteristics and evaluate the performance of the created biosensor. The reproducibility of the aptasensor was evaluated using modified GQTF electrodes manufactured at various periods and under optimal circumstances. Since reproducibility is crucial for biosensors, it was evaluated using 10 separate calibration plots of biosensors calibrated under the same conditions. Identical aptasensors displayed a comparable linearity level between 5 and 1000 fg/mL. The relative standard deviation was 3.01% based on the slopes' overlapping line plots. These results demonstrate that the proposed aptasensors for detecting sarcosine have excellent reproducibility.

The created aptasensor allowed for the successful and practically feasible detection of sarcosine without the need for a crosslinker or marker. The aptasensor is notable because of its extraordinarily low limit of detection (LOD), which is just 5.19 fg/ mL. In addition to an increase in accuracy, the biosensor that has been developed has good levels of reproducibility, repeatability, and storage stability. It is a highly pratical approach with a preparation phase that takes two and a half hours and a marker measurement that takes a few minutes. Because of these characteristics, it is possible to conclude that the suggested biosensor has great potential for clinical applications that include sarcosine analysis.

IV. Conclusion

This study describes the development of a very sensitive electrochemical aptasensor for detecting sarcosine. The aptasensor that has been presented has a low LOD of 5.19 fg/mL and a linear detection range of 5 to 1000 fg/ml. The developed aptasensor system will, in the course of further research, make it feasible to develop an electrochemical immunosensor that has a greater detection range for a variety of biomarkers.

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OP-019

Acoustic Particle Manipulation in 3-D Printed Acoustophoretic Microfluidic Chips

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Acoustophoretic micro/bio-particle separation is becoming an increasingly popular method in microfluidics due to its low chip cost and relatively high throughput. A bulk acoustic wave microfluidic device generally consists of five main components: piezoelectric actuator(s), chip material, fluid inside the microchannel, a glass lid and tubing. The purpose of the piezoelectric actuator(s) is to generate waves in the chip. The function of the chip material is to transfer the elastic waves into the microchannel etched inside the chip material. The purpose of fluid flowing in the channel is to sustain the acoustic pressure and force field and the glass lid is used to form a fluid flow conduit by covering the etched surface on the chip material. The piezoelectric actuator, the glass lid and the tubing are generally glued on the chip material which can be an unreliable connection type.

The interface and orientation of the piezoelectric actuator(s) are quite important. The orientation and poling arrangement of the piezoelectric actuator(s) determines the concentration location of the micro/bioparticles. The most commonly used actuation orientation is asymmetric placement to the top of the chip material. Symmetric but partitioned actuation is also used in the literature along with the studies of the authors of this work. A rarely used actuation strategy is actuation with dual piezoelectric actuators from the sides of an acoustophoretic chip. In this study, we demonstrate that this actuation strategy results in the efficient manipulation of micro/bioparticles. Also, we demonstrated with experiments that this configuration results in the concentration of micro/bio particles, where efficient manipulation was not successful at high flow rates when top actuation configuration is used for polymer chip materials. Also, important geometrical parameters related to successful actuation were investigated using finite element methodology.

A flexible manufacturing method that enables the implementation of geometrical features is important for the efficient actuation of an acoustophoretic chip. In that sense, we fabricated polymer-based acoustophoretic chips by 3D printing. The flexibility and versatility in manufacturing due to 3-D printing method enabled us to implement several features which improve both the performance and the use of the manufactured acoustophoretic such as buried microchannels, placement of alignment features, and introducing luer connections to the chip design. We utilized stereolithography (SLA) (Form3+, Formlabs, USA) for 3D printing of the microfluidic chips by using acrylic resin. Obtaining embedded channels in SLA printing of microfluidic chips is typically challenging as uncured resin may block the channels during printing. To prevent blocking of the microchannels, the acoustophoretic chip was printed such that the main channel axis was perpendicular to the building plate and the layer thickness was set to 0.25 µm. Another challenge is that SLA printed parts become typically translucent after postprocessing, which includes washing of uncured resin and flush exposure to UV. To enhance transparency, we applied a thin layer of resin on the surface of the chip before postprocessing.

Keywords: Acoustophoresis, Microfluidics, 3D printing

OP-020

Unveiling the Metabolic Signature of Preeclampsia: A Promising Analytical Tool for Early Detection

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Abstract

Preeclampsia, known as pregnancy poisoning, is stated but not limited as a high blood pressure (hypertension) disorder that can occur during pregnancy. Metabolomics is the comprehensive is the comprehensive and systematic determination of the amount and changes of metabolites that occur in tissues, cells and physiological fluids with high-throughput technologies. Thanks to metabolomic analysis, the metabolic pathways of diseases can be determined, and individual treatment can be planned by early diagnosis. Although the etiology of preeclampsia has been investigated for years, it has not been fully determined until today. The metabolomic data of this preliminary study using Q-TOF LC/MS combined with the latest data processing strategies were evaluated together with the data collected from literature. 21 pregnant women with preeclampsia (H), 19 healthy pregnant women (G) and 23 healthy women (C) aged between 18-36 were followed up within the scope of the study. Plasma samples were collected and a protein precipitation using methanol was performed to prepare the metabolite fractions. A Zorbax C18 (1.8 µM, 100 x 2.1 mm) column was used as the chromatography column. Water and acetonitrile, both containing 0.1% formic acid, were used as mobile phase. The MS device was operated in positive ion mode. The raw chromatograms were processed using XCMS and MS-Dial. Totally 118 metabolites, statistically different ($p<0.05$ and fold change >1.5) between group P and G were identified and 26 of them were identical with the ones reported in literature. The identified metabolites were involved in metabolism of lipids, metabolism of steroids, primary bile acid biosynthesis and arachidonic acid metabolism.

Keywords: Q-TOF LC/MS, metabolomics, preeclampsia, XCMS, data analysis

I. Introduction

Although pregnancy is a natural process for women, because of the development of pregnancy complications, many pregnant women and babies diebabies die, or experience permanent damage due to the inability due to the inability to perform early diagnosis and appropriate intervention. Preeclampsia is a multisystem disease of unknown cause, characterized by increased systemic vascular resistance, increased platelet aggregation, activation of the coagulation system, and abnormal vascular response associated with endothelial dysfunction. Clinical findings of preeclampsia may manifest manifest in the mother or the the fetus. Preeclampsia, which is specific to human pregnancy, ranks among the top three causes of maternal death worldwide. However, maternal mortality rates can be reduced by providing appropriate management of the disease. In this sense, early diagnosis is of great importance. Conditions characterized by hypertension, edema and proteinuria occur after the 24th week of pregnancy in a pregnant woman in a pregnant woman who is usually normal until the 24th week of pregnancy. These three symptoms do not always coexist in the same patient. The most common symptom is hypertension. In severe cases, subjective symptoms such headaches, visual disturbances, and acute epigastric pain were added to the three cardinal symptoms, and objective symptoms such as increased reflexes, motoric restlessness, and blurred consciousness (Walker 2000). Such a complex picture also causes difficulties for the physician in the decision-making process. This demonstrates how important it is to identify disease-specific metabolites as biomarkers at the metabolome level for such a common disease with complex symptoms. The complexity of this disease and the fact that and the fact that it is a well-studied disease in the literature encouraged us to plan a Q-TOF LC/MS-based study using the most up-to-date metabolomics approaches. We combined the data in the literature with the ones that we obtained in the present study to find novel biomarkers.

II. Material and method

21 pregnant women with preeclampsia (H), 19 healthy pregnant women (G) and 23 healthy women (C) aged between 18-36 were followed up within the scope of the study. The pregnant women were between 34th and 37th week of their pregnancy. Pool H was formed by taking 100 µL of the plasma of pregnant individuals with preeclampsia into a clean centrifuge tube. Pool G was formed by taking 100 µL of plasma from healthy pregnant individuals into a clean centrifuge tube. Pool C was formed by taking 100 µL of plasma from healthy female individuals into a clean centrifuge tube. Centrifuge tubes for Pool H, Pool G, and Pool C were vortexed, and 500 µL of and 500 µL of plasma mixtures were taken from the tubes into the QC eppendorf. Individual samples, pooled sampes and QC samples were precipitated via methanol and vacuum centrifugation was performed. The final residues were dissolved using acetonitrile:water (50:50 v/v) mixture before injected. Plasma samples of preeclampsia patients, healthy pregnant women and healthy women; Pool H, Pool G, Pool C, and QC samples were injected into Agilent 6530 LC/MS Q-TOF. A Zorbax C18 (1.8 µM, 100 x 2.1 mm) column was used as the chromatography column. Water and acetonitrile, both containing 0.1% formic acid, were used as mobile phases. The analysis (0.20 mL min^{-1}) started with 10% acetonitrile and increased linearly to 90% acetonitrile within 10 min. The acetonitrile ratio was then reduced linearly to 10% until the 17th minute and the ratio was kept constant till the 25th minute. The scanning range for the MS device is set to 100-1700 m/z. The column temperature was set to 35 °C, the drying gas temperature to 350 °C and the capillary voltage to 4000V. The MS device was operated in positive ion mode. The raw chromatograms were processed using XCMS and MS-Dial. The identification was performed using MetaboAnalyst 5.0.

III. Results and discussion

The overlapped chromatograms obtained as a result of the

injection of QC samples (first six injections) are given in Figure 1. Metabolite profiling studies were performed in C18 column and positive MS mode. Raw data were evaluated with optimized XCMS parameters and MS-Dial. When the raw data was processed, a total of 5470 peaks were detected. A new table was created for the normalized peaks using the total peak areas, and these values were compared with the 't statistics test' and it was determined that 2636 peaks differed between the H and G groups at the $p<0.05$ confidence interval. The obtained results were loaded into MetaboAnalyst 5.0 software and PLS-DA (Partial Least Squares Discriminant Analysis) graphics were created (Fig. 2.). When matching was achieved for peaks with a coefficient of variation (DK) of more than 1.5 times, 118 peaks were matched with at least one metabolite.

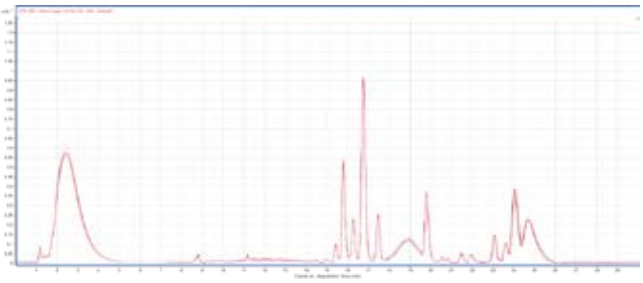

Fig. 1. BPC chromatograms for the overlapped QC samples

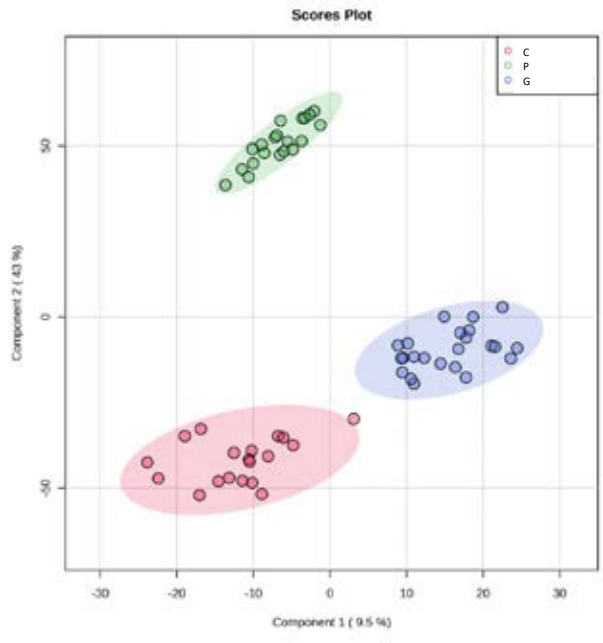

Fig. 2. PLS-DA graphs to show the differences of the groups in metabolome level (C:red, P:green, G:blue)

Regarding the complexity and heterogeneity of preeclampsia, it is unlikely to find a single biomarker that can predict all types of preeclampsia disorders (Nobakht M. Gh 2018). The results of this study were compared with the metabolites recommended as biomarkers in the studies found as a result of a literature review. Metabolites found in common with the metabolites found in previous studies can be listed as follow: Glucose, L-Isoleucine, L-Phenylalanine, L-Leucine, Choline, Octanoylcarnitine, glycerophosphocholine, Myo-Inositol, N6,N6,N6-Trimethyllysine, Linoleic acid, Docosahexanoic acid, Pregnenolone, Progesterone, Cholesterol, Cortisol, Cortisone, Taurodeoxycholic acid, Leukotriene B4, LysoPC(18:0), N-Acetylputresin, Oleic acid, Sphingosine (Kenny, Broadhurst et al. 2010, Moon, Moon et al. 2014, Nobakht M. Gh 2018, Kivelä, Sormunen-Harju et al. 2021). When a metabolic pathway analysis performed using IMPaLA (http://impala.molgen.mpg.de), the mainly involved pathways with preeclampsia were found as metabolism of lipids, metabolism of steroids, primary bile acid biosynthesis and arachidonic acid metabolism.

IV. Conclusion

The results show that the analytical method and data processing we have used are enabling us to match with the 26 metabolites that have been reported for preeclampsia in the literature so far. This provides partial validation of the other data we obtain. As a result of the metabolic pathway analysis, the metabolic pathways associated with or affected by preeclampsia were deciphered. Although some of these pathways have been associated with preeclampsia in the literature, this preliminary study provided useful data to indicate some potential biomarkers involved in the relevant metabolic pathways.

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OP-021

A New Competitive Lateral Flow Immunoassay Test for Rapid Detection of Total Antibody in Vaccinated Puppies Serum Against Canine Parvovirus-2

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Canine Parvovirus-2 is a viral disease of dogs causing acute hemorrhagic gastroenteritis and myocarditis with high morbidity and mortality rates. The infection is widespread all over the world. CPV-2 is now only available in vaccine formulations and not circulating in the field. Vaccines developed against the infection have great importance in preventing it. However, it is difficult to recommend a practical vaccination program without knowing the antibody level of a pup. Despite widespread vaccination, difficulties in detecting the maternal antibodies in puppies, remain the main cause of vaccination failure. The Hemagglutination Inhibition (HAI) test is the gold standard to determine the immune status of dogs for Canine Parvovirus 2, but HAI test has several disadvantages such as the need for fresh porcine blood, well equipped laboratory, long incubation periods.

In this study, first time we developed a colloidal gold based competitive lateral flow assay (CLFA) system for rapid detection of total antibody in canine serum. The working principle of a CLFA is based on the competition between capture molecule and the target analyte in a biological sample for binding to a labelled molecule on a test strip. Unlike LFAs, the absence of a colored band on the test strip indicates the presence of target analyte, while the presence of a colored band indicates the absence of analyte in sample. For this purpose, recombinantly expressed capsid protein of CPV-2 in prokaryotic expression system was used as labeled molecule in CLFA.

We carried out studies on our CLFA system using the clinic samples obtained from vaccinated pup serum. We compared the results of the LFAs with the HAI test. Competitive lateral flow assay results showed good correlation with the gold standard method, HAI test. In the developed platform, we determined cut off level of antibodies as below 1:80 HAI titer. Our reported system represents a strong alternative to be used in CPV-2 antibody-based detection applications.

Acknowledgement: This research is supported by the Scientific Research Projects (BAP) Commission of Ankara University with Project Number

Keywords: lateral flow immunoassay, competitive immunoassay, canine parvovirus-2, viral disease, gold nanoparticle

OP-022

An Overview of Current Approaches in Quantum Dot-Based Electrochemical DNA Biosensors and Their Potential for Use in the Diagnostic Kit Field

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Since the first commercial biosensor instrument for the detection of blood glucose levels was found in the 1970s, numerous different types of biosensors have been designed, and this area of research still remains popular around the world. In the biosensor research reports prepared in line with the literature and industry statistics published in recent years, it is predicted that biosensor design technologies, including handheld or wearable devices, will be preferred in many areas in the near future. However, new generation biosensor technologies using nanoparticles still maintain their very important place in the scientific world and are frequently preferred in innovative research projects.

Among the nanoparticles, semiconductor quantum dots (QDs) with an average particle size between 1 and 20 nm are considered to be one of the most valuable nanomaterials, especially in the research field of biosensors. In this context, recent and prominent developments in electrochemical DNA biosensor technologies using QDs-based nanomaterials and their potential for use in the diagnostic kit area are briefly summarized.

Keywords: Electrochemical DNA biosensors, quantum dots, diagnostic kit

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OP-023

Drug Screening Based on Proteomics

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Target identification is critically important for understanding the mechanism of action of drugs. Here we reported a new strategy for deconvolution of drug targets (or off-targets) with photoaffinity labeling chemoproteomics in combination of untargeted metabolomics by using doxorubicin (DOX) as a model. The DOX derived photoaffinity probes were prepared and applied to capture the DOX interacting proteins in living cells. The captured DOX interacting proteins were then identified by label-free quantitative (LFQ) proteomics. Totally 151 significant proteins were identified with high confidence (fold change > 4, p-value < 0.005). The GO (gene ontology) enrichment analysis suggested that the proteins mainly involved in carbon metabolism, citrate cycle, and fatty acid metabolism and metabolic pathways. Therefore, the untargeted metabolomics was applied to quantify the significantly altered metabolites in cells upon drug treatment. The pathway enrichment analysis suggested that DOX mainly interrupted with the processes of pyrimidine and purine metabolism, carbon metabolism, methionine metabolism, and phosphatidylcholine biosynthesis. Integrative analysis of chemoproteomics and metabolomics indicated that adenosylhomocysteinase (AHCY) is a new target (off-target) of DOX leading to the accumulation of S-adenosyl homocysteine (SAH). This deduced DOX target was confirmed by the cellular thermal shift assay, affinity competitive pull-down assay, biochemical assay, and siRNA knock down experiments. Our result suggested that AHCY is the uncovered off-target of DOX.

Keywords: Chemoproteomics, Doxorubicin, Photoaffinity labeling, Target identification, Untargeted metabolomics

OP-024

Simultaneous Spectrophotometric Analysis of Emtricitabine and Tenofovir Disoproxil Fumarate in Pharmaceutical Formulation

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A simple, accurate and sensitive spectrophotometric method for quantitative analysis of Emtricitabine (ETB) and Tenofovir Disoproxil Fumarate (TDF) in pharmaceutical dosage forms has been developed. Ratio spectra first derivative spectrophotometry was used for this purpose. In the method; first derivative spectra of the ratio spectra of the solutions of ETB and TDF in distilled water were recorded between 200 and 350 nm. 10 µg/mL solution of ETB and 10 µg/mL solution of ETB were used as divisors. The first derivative spectra were recorded as Δλ= 4 nm. Derivative absorbance values were read at 222 and 242 nm in their derivative spectra. The mean recovery and relative standard deviation of the method were found as % 99.2 – 101.2 and % 0,8 - 1,1 respectively in the method. The working range of the method was found as 2-15 µg/mL for both ETB and TDF with an excellent linearity (r^2 = 0,999). The developed method was successfully applied to tablet formulation containing ETB/TDF and commercially available in Turkiye. According to the results, we have found that this method can be applied for the routine simultaneous analysis of ETB and TDF in pharmaceutical formulations.

Keywords: Tenofovir disoproxil fumarate, emtricitabine, spectrophotometric, ratio, first derivative

OP-025

The role of Analytical Pharmacotoxicology in Adressing the Main Functions of the National Early Warning System on NPS

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AIM: In compliance with the European Council Decision 2005/387/JHA, the Italian AntiDrug Policies Department des-

ignated the National Center for Addiction and Doping of the National Institute of Health (ISS) to organize and manage the National Early Warning System (NEWS) on New Psychoactive Substances (NPS) to promote a rapid exchange of information on NPS within and between Italy and the EU. The analytical pharmacotoxicology Unit of the Center supports NEWS with screening and confirmation of NPS presence in conventional and non conventional biological matrices from acute and fatal intoxication cases. Futhermore, yearly, pure standards of new NPS are tested and provided to Laboratories from collaborative centres and proficiency testing on NPS in oral fluid and hair are organized.

METHOD: Different analytical technologies, including GC-MS, GC-MS/MS, LC-MS/MS, LC-HRMS/MS were applied for a screening and confirmation analysis of classic drugs of abuse and NPS and their metabolites in conventional and unconventional biological matrices from intoxication cases and clinical trials. Sample preparation involves both liquid-liquid or solid phase extraction.

RESULTS: During 2021 and the first months of 2022, around 80 different psychoactive substances, including new synthetic opioids and/or metabolites were identified in the urine from 296 spanish patients with a history of opioid use disorder; 12 classic drugs and NPS were detected in hair from 300 mexican pregnant women; cocaine, THC, heroin, methadone, ketamine and mephedrone and metabolites were determined in urine from 76 italian prisoners; JWH-122 and JWH-210, UR-144 were quantified in oral fluid from consumers and finally some cases of intoxications with fentanyl, norfentanyl and analogs were also disclosed. The new benzimidazole opioid was detected in three fatalities that occured between July and September 2022. The ketamine comeback in the street market is suggested by the detection in hair, blood and urine samples from emergency department in Rome, along with synthetic cathinones, methamphetamine, MDMA and classic drugs of abuse. In the NEWS context, two exercises of Proficiency testing for NPS in oral fluid and hair were organized. Samples from both matrices were prepared in cooperation with Comedical (Trento, Italy). More Recently, the proficiency test for NPS in blood matrix was set up and managed in collaboration with Centro Regionale Qualità Laboratori of Sicily, an operative unit of NEWS. About 20 different pharmacotoxicological national laboratories participated in the exercised with very variable quali-quantitative results.

CONCLUSION: Although the NPS phenomenon is not uniform, it is well-known as a global problem. Due to its mutating nature, we believe that investments in toxicological and forensic analytical data sources are strongly needed. Currentl, more than 1000 NPS have been identified. Not all of them found the same diffusion in the illicit market, but many of them are temporary adulterants or substitute of controlled compounds or finally fake drugs. Indeed, the polydrug consumption imore frequently results intoxications. Drug consumption patterns are often characterized by polyconsumption of both classical drugs and NPS and laboratories supporting emergency rooms, forensic toxicologies and police forces must be enabled to identify the substance/s causing the acute or chronic intoxication or a fatality.

Keywords: New psychoactive substances, LC-HRMS/MS, national early warning system, toxicology, GC-MS/MS, biological matrices

OP-026

An Analytical Debate Regarding Nitrite Analysis in Raw Materials Associated with Nitrosamine Occurrence in Finished Products; The Importance of Selecting a Precise Testing Methodology

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Nitrosamine impurities were unexpectedly discovered in pharmaceuticals in 2018. Major contamination cases were reported in valsartan, ranitidine and metformin, among others. Interestingly, the mechanistic interpretations of the root cause of drug contamination were distinctive in each case, which necessitated the use of careful and accurate testing methodology. One of the repeatedly suggested theories is that the contamination is likely a result of interactions between a susceptible amino group in the active pharmaceutical ingredient (API) and a nitrite group in one of the inactive ingredients, at defined reaction conditions. Consequently, testing of nitrite species was initiated on different active and inactive ingredients. Two main techniques for testing nitrite were reported, namely: ion chromatography and spectrophotometry. In this regard, there was a scientific dialogue between two research groups concerning nitrite testing in one of the inactive ingredients used in metformin drug formulation. There was a dramatic difference between the generated results for nitrite testing using the two different techniques. Here, we critically analyze the tested methodologies while providing some insights into the interpretations of the major differences within generated results. Some recommendations for optimum nitrite testing are also discussed. We highlight the importance of implementing the golden criterion *fit for the purpose* for the proposed analytical method, and provide some critique on the used approach. Scientifically sound methodology for testing nitrite in either active or inactive pharmaceutical ingredients is deemed necessary in order to effi-

ciently mitigate nitrosamine formation.

Keywords: nitrosamine, nitrite, nitrate, NDMA, contamination, ion chromatography

OP-027

The Effect of Pharmacokinetic Genes Polymorphisms to the Plasma Clozapine and Norclozapine Levels in Patients with Schizophrenia

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Clozapine (CLZ) is an atypical antipsychotic with narrow therapeutic range used in the treatment of resistant. Polymorphisms in the encoding region of genes that related with CLZ metabolism have major importance and can alter the CLZ metabolism and CLZ plasma levels. Investigate the effects of *ABCB1* G1334A and *UGT1A4* L48V polymorphisms on CLZ and its main metabolite N-desmethlyclozapine (DCZL) plasma levels was aimed.

The 94 schizophrenia patients treated with CLZ were took part in the study, the high-performance liquid chromatography was used to quantified the plasma CLZ and DCLZ levels and PCR-RFLP method were used for the identified the genotypes of *ABCB1* G1334A and *UGT1A4* L48V polymorphisms.

In current study, the *ABCB1* C3435T and*UGT1A4* L48V variant allele frequencies are found 49% and 13%, respectively. The CLZ C/D level and DCLZ C/D levels were not statistically significant between *ABCB1* and *UGT1A4* genotypes. Nevertheless, CLZ C/D levels and DCLZ C/D levels were found 4.5% and 22.4% higher in the *UGT1A4 TT* genotype than in the *UGT1A4 TG+GG* genotypes. When two polymorphisms were combined, the current results showed that the cellular elimination and excretion of CLZ and its active metabolite DCLZ were slower in individuals with *ABCB1* TT + *UGT1A4* TT genotypes. Thus, the dose adjustment is recommended to do more carefully in these individuals.

*This research was funded by Ankara University BAP grant number 18L0217001.

Keywords: Clozapine, ABCB1, UGT1A4, Schizophrenia

OP-028

Benchtop-NMR for the Detection of Undeclared Drugs in E-Liquids and Aphrodisiac Honeys

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Over the past decade, a new generation of cryogen-free benchtop NMR (Nuclear Magnetic Resonance) spectrometers, based on permanent magnets, has enabled new applications. Although these new devices deliver a weaker magnetic field, resulting in losses of resolution and sensitivity compared to traditional spectrometers, the analytical information obtained can be of great interest, particularly in quality control or detection of adulteration. This study presents two cases of adulteration with undeclared drugs. Analyses were performed with a compact Oxford 60 MHz NMR spectrometer, conventional high-field NMR experiments being used as reference methods.

The first example presents the analysis of 13 samples of e-liquids in which five synthetic cannabinoids were detected. The high-field 1H NMR spectra allowed the detection of matrix signals, synthetic cannabinoids, and flavouring compounds. The ability of benchtop low-field 1H and 19F NMR spectroscopy was evaluated and then quantitative NMR experiments were performed to quantify synthetic cannabinoids.

The second example deals with aphrodisiac honeys; about 50 samples were analysed. The adulterants in these samples were sildenafil or tadalafil, two approved drugs used to treat erectile dysfunction and acting as phosphodiesterase-5 inhibitors. Low-field NMR analysis performed after extraction allowed quantification of the adulterants.

In the two cases presented, the matrices contain molecules that give rise to strong signals in the 1H NMR spectra, which

could be a hindrance to low-field NMR analysis and prevent the detection of adulterants. Despite this, we have proposed analytical procedures that allow their detection and quantification.

Keywords: NMR, Benchtop NMR, adulteration, synthetic cannabinoids, phosphodiesterase-5 inhibitors

OP-029

Targeting FLT3/ITD Mutation in Acute Myeloid Leukemia: Lessons from Natural Compounds

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Acute myeloid leukemia (AML) is a hematological malignancy involving the bone marrow and blood. It is clinically aggressive and spreads to many organs such as lymph node, spleen and liver with its migration and invasion capabilities. Since AML is not characterized by tumor complexes, it is subtyped by the diagnosis of point mutations, translocations, or fusions. The FMS-like tyrosine kinase 3 (FLT3) is a gene involved in the regulation of new blood cells, and its mutations mean poor outcomes and prognosis for AML patients. ITD or TKD mutations of FLT3 are present in 30% of AML cases and cause constitutive activation of oncogenic signaling cascades. The ITD mutation is more common and associated with an increased risk of recurrence and has become an attractive target in disease with such a complex molecular background. Tyrosine kinase inhibitors belonging to different generations are used according to the genotype of the disease in the clinic. Efforts are currently on the development of effective cures against FLT3 expression and especially ITD mutation with next generation inhibitors, immunochemotherapeutics and novel combinations. Natural compounds act as an active playmaker in this equation and offer a huge library of unique experimental therapeutics. The main aim of this presentation is to convey extensive laboratory experience in screening various natural compounds against FLT3 ITD-mutated leukemia and determining their mechanism of action. In this context, the MV4-11 cell model representing FLT3+ and ITD mutant biphenotypic B-myelomonocytic leukemia and was used. Endogenous expression levels of 43 different cell death, survival, and resistance targets specific to this cell line were determined by high-throughput protein profiling assays. Many novel oxindole derivatives as well as some essential oils were screened. Time and dose dependent cell death experiments were performed with active compounds and molecular targets affected by the treatment of the compounds were identified. The interaction mechanisms, binding abilities, and stability of active compounds with FLT3 were determined through molecular docking and molecular dynamics simulations. Compliance of the compounds with drug similarity rules was investigated by ADME modelling. In the light of these findings, cell growth inhibitors, which are specific to cells bearing FLT3/ITD mutation, were determined by their mechanism of action. The lead compounds found with these data are promising for new pharmaceutical formulations, combination studies and further mechanistic research.

Keywords: Acute myeloid leukemia, FLT3, Anticancer, Biological activity, Screening, Cell

OP-030

Electrochemical Development of Poly(L-alanine)-gold nanoparticles-modified Electrode for the Determination and Mechanism of Antipsychotic Drug Olanzapine

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Abstract

A novel electrochemical sensor for sensitive determination of antipsychotic drug or the sensitive determination of the antipsychotic drug olanzapine (OLA) was introduced. The fabricated sensor was based on pre-treated pencil graphite electrode (pre-PGE) modified with poly(L-alanine) and gold nanoparticles (AuNPs). The effect of monomer L-alanine and chloroauric acid concentrations, pH, and scan rate was tested with differential pulse voltammetry (DPV) and cyclic voltammetry (CV). The analytical method validation parameters were studied. The linear response of OLA was in the ranges of (0.271-1000.0) µM with the correlation coefficient 0.99. LOD and LOQ were found to be 0.081 µM, and 0.271 µM, respectively. The interference effect was investigated for fluoxetine (FLX), ascorbic acid (AA), uric acid (UA), acetaminophen (APAP), sodium (Na^+), potassium

(K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) cations. The utility of AuNPs-poly(L-alanine)/pre-PGE sensor was worked for the determination of OLA in its pharmaceutical dosage form, human serum, and human urine. Finally, the probable electrochemical oxidation mechanism of the OLA was suggested.

Keywords: Poly(L-alanine), gold nanoparticles, pencil graphite electrode, olanzapine, voltammetry, electrooxidation mechanism.

OP-031

Therapeutic Drug Monitoring of the Free and Total Valproic Acid in Human Plasma by LC-MS/MS

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Valproic acid (VPA) is a branched-chain carboxylic acid used to treat different types of epilepsies [1]. Its metabolism in vivo is affected by various factors, and it has a narrow therapeutic range (50–100 µg/mL). Thus, therapeutic drug monitoring (TDM) is usually required in the clinical use of VPA [2]. Its usage may be limited by potential hepatotoxicity and it has high plasma protein-binding (appr. 80–95%) [3] and the concentration of VPA detected by conventional methods in TDM is the total serum concentration [4].In addition to this, it is well known that only free drug concentration is pharmacologically active. The aim of this study developed an LC-MS/MS method for therapeutic monitoring of free and total concentrations of VPA in plasma.

Valproic acid was extracted from plasma by using protein precipitation. Free valproic acid was extracted from plasma by ultrafiltrate method. The test method has been validated according to the European Medicines Agency (EMA) Bioanalytical method validation guidelines [5].

In the study, we developed a method for therapeutic drug monitoring of the valproic acid in human plasma, the lower limit of quantification of valproic acid was determined as 5 µg/mL. The calibration curve of valproic acid for the method was validated between 5 and 150 µg/mL, showing correlation coefficients greater than 0.99. Moreover, the developed method was applied for therapeutic drug monitoring of valproic acid in real patient plasma samples.

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Keywords: LC-MS/MS; Valproic acid; Free valproic acid; Therapeutic drug monitoring; Human plasma

OP-032

LC-MS/MS-Based Quantitative Analysis of A Tricyclic Antidepressant and Its Major Metabolite

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Clomipramine, a tricyclic antidepressant, is widely employed in the treatment of depression. Due to its narrow therapeutic index, precise measurement of clomipramine and its major metabolite, desmethylclomipramine, in biological matrices is crucial to ensure both therapeutic effectiveness and patient safety. In this study, our aim was to develop a robust and accurate quantitative analysis method for clomipramine and desmethylclomipramine utilizing liquid chromatography-mass spectrometry (LC-MS/MS). LC-MS/MS offers exceptional sensitivity and precision, making it an ideal technique for the analysis of small molecules like pharmaceutical compounds.

To achieve the quantitation of clomipramine and desmethylclomipramine, we utilized an LC-MS/MS method, known for its ability to yield rapid and reproducible results at low detection limits. Chromatographic separation was successfully achieved using a Poroshell 120 column (3.0 mm × 100 mm, 2.7 µm) employing a gradient mobile phase system comprising a mixture of acetonitrile and buffer including formic acid at a flow rate of 0.6 mL/min while the injection volume was standardized to 2 µL.

To evaluate the applicability of the developed method, a set of real samples obtained from 24 patients diagnosed with major depression was analyzed for clomipramine and desmethylclomipramine concentrations. The results obtained demonstrated the suitability of the LC-MS/MS method for accurate and precise quantification of these compounds in biological matrices.

In conclusion, this study presents a novel LC-MS/MS-based quantitative analysis method for the determination of clomipramine and desmethylclomipramine. The developed method exhibited excellent performance characteristics, including sensitivity, reproducibility, and suitability for clinical applications. The proposed method holds great promise for facilitating therapeutic drug monitoring and optimizing patient care in the context of clomipramine therapy.

Keywords: Clomipramine, desmethylclomipramine, LC-MS/MS, plasma, forensic toxicology, therapeutic drug monitoring

OP-033

Investigation of New Thiazole Derivatives and their Biological Effects

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Abstract

This research focuses on the synthesis and analysis of twelve new thiazole compounds, which were evaluated for their potential biological activity against acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes. The *N*-[4-(4-acetamidophenyl)thiazol-2-yl]-2-heteroarylpropanamide compounds (**5a-5l**) were analyzed using spectroscopic techniques, including HRMS, NMR, and IR, to determine their chemical structure and properties. The compounds were then screened for determining AChE and BChE inhibitory activity, and the results showed that compound **5j,** namely *N*-[4-(4-acetamidophenyl)thiazol-2-yl)-2-((5-chlorobenzo[d]oxazol-2-yl)thio]propanamide, exhibited the most significant activity. Molecular docking and molecular dynamic simulation (MDS) studies were conducted. The docking analysis revealed that the three most active compounds (**5d, 5e** and **5j**) exhibited strong associations with the AChE binding site, indicating their potential as therapeutic agents for the treatment of neurological disorders. Overall, this study highlights the importance of utilizing various heterocyclic compounds to evaluate the potential of synthesized compounds and provides insights into the design of future studies aimed at developing novel AChE inhibitors.

Keywords: Thiazole, AChE inhibition, molecular docking, MDS.

I. Introduction

Neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, are significant causes of morbidity and mortality worldwide, affecting millions of people annually. Despite extensive research, there are currently no effective treatments that can cure or halt the progression of these diseases(Emmerzaal, Kiliaan, and Gustafson 2015). Therefore, the development of new therapeutic agents that can target the underlying mechanisms of neurodegeneration is of great importance(Leoni et al. 2019).

In recent years, the use of small molecules as therapeutic agents has gained increasing attention due to their potential to modulate specific targets and pathways, making them an attractive option for the development of novel drugs. Among these small molecules, thiazole-containing compounds have shown promise as scaffolds for developing drugs to treat neurodegenerative disorders(Yurttaş et al. 2014).

In this study, our aim was to synthesize and evaluate a series of twelve compounds containing a thiazole ring as potential therapeutic agents for neurodegenerative disorders. The synthesized compounds were analyzed using various techniques.

Overall, this study provides valuable insights into the potential of thiazole-containing compounds as scaffolds for the development of novel therapeutic agents for the treatment of neurodegenerative disorders.

II. Material and method

The compounds were acquired by a five-step synthesis procedure and their formulas are shown in **Figure 1. Method A** involves the synthesis of *N*-(4-acetylphenyl)acetamide using 5-aminoacetophenone, triethylamine, and acetyl chloride in tetrahydrofuran as a solvent. **Method B** utilizes *N*-(4-acetylphenyl)acetamide to synthesize *N*-[4-(2-bromoacetyl)phenyl]acetamide using bromine and acetic acid as a solvent. **In Method C**, *N*-[4-(2-bromoacetyl)phenyl]acetamide and thiourea are combined in etha-

nol to synthesize *N*-[4-(2-aminothiazol-4-yl)phenyl]acetamide. **Method D** involves the synthesis of *N*-[4-(4-acetamidophenyl) thiazol-2-yl]-2-chloropropanamide using *N*-(4-(2-aminothiazol-4-yl)phenyl)acetamide and 2-chloropropionyl chloride in tetrahydrofuran as a solvent. Finally, **Method E** involves the synthesis of *N*-[4-(4-acetamidophenyl)thiazol-2-yl]-2-heteroarylpropanamide by reacting *N*-[4-(4-acetamidophenyl)thiazol-2-yl]-2-chloropropanamide with various heterocyclic mercaptane derivatives and potassium carbonate in acetone as a solvent. The AChE and BChE enzyme inhibition activity of the compounds were tested according to modified Ellman method (Ellman, 1961).

III. Results and discussion:

The synthesized thiazole compounds **(5a-5l)**, which are potential candidates for treating neurodegenerative disorders, were obtained with yields ranging from 70-98%. To ensure the purity and chemical structure of all synthesized compounds, melting points were determined, and various chemical spectral analysis techniques such as IR, ^{1}H-NMR, ^{13}CNMR, and HRMS were employed. The results of these analyses confirmed the purity and chemical structure of all compounds, indicating that they were novel derivatives. The ^{1}NMR spectra showed that the synthesized compounds had the expected chemical shifts and multiplicity, indicating the presence of the thiazole ring and other functional groups. Additionally, the HRMS spectra exhibited intense signals corresponding to the molecular ion peaks, indicating the high purity of the synthesized compounds. Overall, the results of these analyses demonstrated the successful synthesis of novel thiazole derivatives with high yields and confirmed their purity and chemical structure. The biological activities of the compounds were determined as a percentage of the inhibitory effects on AChE (Sağlık et al. 2019) The results are presented in **Table 1**. Compounds **5d** (IC_{50}: 0.223 μM), **5e** (IC_{50}: 0.092 μM) and **5j** (IC_{50}: 0.054 μM) were determined to have the highest inhibitory effect against AChE.

IV. Conclusion:

Thiazole compounds have been explored as a potential drug scaffold for treating neurodegenerative disorders such as Alzheimer's disease. In this study, novel *N*-[4-(4-acetamidophenyl)thiazol-2-yl]-2-(heteroaryl)propanamide **(5a-5l)** derivatives were synthesized, starting from *N*-[4-(2-bromoacetyl)phenyl]acetamide. The synthesized compounds were found to be effective as AChE inhibitors, with three compounds (**5d**, **5e**, and **5j**) exhibiting high inhibition efficiency against AChE. Docking analysis revealed strong associations between these compounds and amino acids through various bonds. In future studies, it is considered to evaluate the anticholinesterase activities of the compounds by providing heterocyclic ring diversity, as well as to evaluate the monoamine oxidase inhibitory activities to enhance activity and ligand-protein binding.(Sahin et al. 2018)

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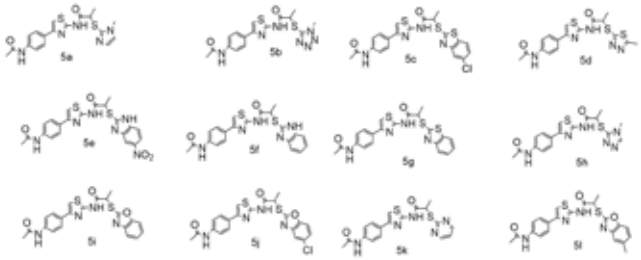

Figure 1: The chemical structures of the synthesized compounds **(5a-5l)**

Table 1: The AChE and BChE inhibitory activity of the compounds

Compound	AChE % Inhibition		AChE IC_{50} (μM)	BChE % Inhibition		MAO-B IC_{50} (μM)
	10^{-3} M	10^{-4} M		10^{-3} M	10^{-4} M	
5a	**52.323 1.122±**	42.597 1.454±	>100	26.462 0.834±	22.746 0.720±	>1000
5b	48.154 0.936±	41.357 1.010±	>1000	29.375 0.920±	26.128 0.967±	>1000
5c	42.759 0.806±	38.964 0.821±	>1000	30.613 1.258±	26.408 0.857±	>1000
5d	**89.646 1.958±**	**83.342 1.768±**	**0.223 0.010±**	31.045 1.041±	24.067 0.812±	>1000
5e	**92.155 1.874±**	**88.464 1.334±**	**0.092 0.003±**	24.246 0.865±	20.771 0.830±	>1000
5f	**72.849 2.485±**	48.328 0.889±	>100	32.749 1.059±	26.697 0.718±	>1000
5g	47.764 1.030±	31.146 1.302±	>1000	30.569 1.156±	20.203 0.801±	>1000
5h	43.233 0.955±	29.031 0.847±	>1000	27.322 0.948±	22.345 0.720±	>1000
5i	**57.954 1.537±**	30.897 0.867±	>100	28.048 0.864±	21.470 0.869±	>1000
5j	**93.576 2.846±**	**90.655 1.920±**	**0.054 0.002±**	26.468 0.854±	23.876 0.778±	>1000
5k	**74.290 2.110±**	47.231 0.886±	>100	29.567 0.975±	25.661 0.897±	>1000
5l	**69.067 1.854±**	38.468 0.736±	>100	28.357 0.923±	22.554 0.764±	>1000
Donepezil	99.156 1.302±	97.395 1.255±	0.0201 0.0010±	-	-	-
Tacrine	-	-	-	99.827 1.378±	98.675 1.450±	0.0064 0.0002±

OP-034

Screening of Trypsin Inhibitors in Cotinus Coggygria Scop. Extract Using At-Line Nanofractionation Coupled with Semi-Preparative Reverse-Phase Liquid Chromatography

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Trypsin inhibitors (TIs) have significant anti-inflammation, anti-tumor and anti-viral effects, thus being used in the treatment of diseases such as pancreatitis, hypertension, diabetes and thrombosis. In this study, an at-line nanofractionation (ANF) platform was successfully fabricated in parallel with mass spectrometry and trypsin inhibitory bioactivity assessment for rapid screening of trypsin inhibitors (TIs) from natural products for the first time. After systematic optimization, the ANF platform was applied to screen and identify TIs in the extract of a traditional Chinese herb, i.e., Cotinus coggygria Scop. Subsequently, semi-preparative reverse-phase liquid chromatography was used to further simplify and enrich the insufficiently separated components. After comprehensive evaluation and validation, the ANF platform successfully identified 12 compounds as potential TIs, including 8 flavonoids and 2 organic acids. Additionally, a comparison study was conducted using two other ligand fishing approaches, i.e., capillary monolithic and magnetic beads-based trypsin-immobilized enzyme microreactors, which successfully identified 8 identical flavonoids as TIs. Importantly, the molecular docking study showed the molecular interactions between enzymes and inhibitors, strongly supporting the experimental results. Overall, this work has fully demonstrated the feasibility of the established ANF platform for screening TIs from Cotinus coggygria Scop., and proved its great prospects for screening bioactive components from natural products.

Keywords: Trypsin inhibitors, At-line nanofractionation, Natural product, Bioactive screening

OP-035

Peptide Assays: An Ounce of Pre-Analytics is Worth a Pound of Cure

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The recent increase of peptidomimetic-based medications, and the growing interest in peptide hormones has brought new attention on the quantification of peptides for diagnostic purpose. Indeed, blood circulating concentrations of peptide hormones provide a snapshot of the body's state, eventually leading to the detection of particular health conditions.

Although very useful, the quantification of such molecules, preferably by liquid chromatography coupled to mass spectrometry (LC-MS), might be quite tricky to handle. First, peptides are subject to hydrolysis, glycosylation, oxidation, and other post-translational events, and, most importantly, they are substrates of specific or aspecific proteases in biological matrices. All these events might continue after the sampling, changing the peptide hormone concentrations. Second, because of their structure

with positively and negatively charged groups and hydrophilic or hydrophobic residues, they have interactions with their environment that might lead to a local change of the measured concentrations. Phenomenon such as adsorption have sometimes tremendous effect on the concentration and need to be controlled with particular care. Finally, the circulating levels might be low (femtomolar range), increasing the impact of the abovementioned effects, and inducing the need of highly sensible instruments and well optimized methods.

Thus, despite the extreme diversity of these peptides and their matrix, a common challenge is shared by all the assays: the need to keep concentrations unchanged from the sampling to the analysis. While great efforts are often placed in the optimization of the analysis, few studies consider in depth the impact of pre-analytics on study results.

Going through practical examples, this talk will address typical pre-analytical challenges encountered during the development of a peptide assay from the standpoint of a clinical laboratory. Tips and tricks to avoid pitfalls as well as strategies to guide all new developments will be on the corner, with the final goal to spread a pre-analytical awareness that will be profitable for all upcoming peptide assays.

Keywords: Pre-analytics, Peptide assays, Mass spectrometry, Adsorption, Stability, Proteases

OP-036

An Overview in the Chiral Separation of Azole Compounds Using Some Analytical Methods

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We were interested in this study in the enantiomeric analysis of the metabolites of some chiral drugs with antifungal effects in the urine using a simple direct isocratic high-performance liquid chromatographic process and capillary electrophoresis (CE). Particularly, in enantiomeric separations for determining of enantiomeric purity and analysis of ten imidazole and azole compounds (imidazole and triazole) in biological fluids and their therapeutic monitoring. Chiral separation was performed and optimized by HPLC using six polsaccharides coated derived chiral stationary phases and two covalently immobilized polysaccharide based chiral selectors with different mobile phases under different modes. And by CE using different cyclodextrins with different concentrations, temperatures and voltages. The Rapid, simple, accurate and a baseline separation of antifungal drugs was achieved. Parameters influencing chiral separation include mobile phase, chemical nature of the chiral selector and structure of antifungal drugs, were found to be highly efficient for the chiral separation. These processes were found suitable for rapid enantiomeric purity analysis and useful for quality control in pharmaceutical formulation, pharmacokinetics, pharmacology, pharmacodynamics and drug toxicology.

Keywords: Azole, HPLC, cellulose-amylose CSPs, immobilized-coated CSPs, CE

OP-037

Enhancement of Apixaban's Solubility and Dissolution Rate by Inclusion Complex (β-Cyclodextrin and Hydroxypropyl β-Cyclodextrin) and Computational Calculation of their Inclusion Complexes

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Apixaban (AP) is a factor X inhibitor, an orally active drug that inhibits blood coagulation for a better prevention of venous thromboembolism. It has poor solubility, dissolution rate and low bioavailability. The aim of this study was to improve the aqueous solubility and dissolution rate of oral AP as a step to enhance its bioavailability by preparing it as an inclusion complex with beta and hydroxy propyl beta cyclodextrin. A simple rapid method of analysis of AP was developed using ultraviolet spectrophotometry (UV) and partially validated in terms of linearity, precision and accuracy, recovery, and robustness. AP was prepared as complex with beta cyclodextrin (βCD) and hydroxy propyl beta cyclodextrin (HPβCD) in ratios 1:1, 1:2, and 1:3 by kneading, solvent evaporation and spray drying methods and characterized by Fourier Transfer Infra-Red (FTIR), Differential Scanning Calorimetry (DSC), and percent drug content in each of the prepared

complexes. A phase solubility study of AP was conducted using different molar concentrations of HPβCD and the stability constant was calculated. Using the computer simulation, the interaction of AP with βCD and HPβCD were investigated. The complex that gave the best results in evaluation was then tested for AP release and then used to prepare hard gelatin capsules in addition to a reference capsule containing simple formula of AP with additives and their dissolution was compared. The results showed that AP can successfully form an inclusion complex with both βCD and HPβCD with high percent yield (>82%) and high drug loading efficiency (>88%). DSC and FTIR support the formation of the inclusion complexes. The phase solubility study showed that solubility of AP was greatly enhanced from 54×10-3 mmol /L to 66 mmol/L using HPβCD with acceptable stability constant. Computer docking supports the formation of a stable 1:1 complex between AP and CDs. The dissolution test results showed that the complex gave significantly higher percent of drug release (95%) over one hour compared to the free AP (60%) ($p<0.05$). Hard gelatin capsule formulation containing AP-HPβCD gave also higher amount of AP release with first order kinetics than the formula that contains free AP with excipients. As a conclusion, AP- HPβCD complex in ratio of 1:2 (w/w) can improve the solubility and in vitro dissolution rate of AP significantly.

Keywords: Apixaban; HPβCD, βCD, capsule, bioavailability, solubility

OP-038

The Lipidomic Landscape of Different Mutations in Human Brain Tumors

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Brain tumors are one of the most dangerous neoplastic lesions with high mortality rates. Among them meningiomas and gliomas are the most common. Meningiomas are benign brain tumors but sometimes they can evolve to a higher grade. Gliomas, on the other hand, are malignant lesions with high heterogeneity at the histological, genetic, and metabolic levels. Therefore, basic research is essential to understand the relationship between genetic mutations and metabolic alterations to better characterize these lesions and improve further treatment. Thus, the aim of this study was to analyze the influence of genetic changes on the lipid composition of brain tumors. As a sampling method chemical biopsy was applied as a low-invasive technology that could be complementary to clinically available tests.

Brain tumors were obtained during neurosurgical procedures in 10th Military Research Hospital and Polyclinic. Then, directly after lesions excision sampling using solid-phase microextraction (SPME) fibers with 7 mm C18 coating was performed. After the collection of the whole batch of samples, desorption using an isopropanol-methanol solution was performed. Subsequently, instrumental analysis was carried out using liquid chromatography coupled with high-resolution mass spectrometry, Q_Exactive Focus. The remaining part of the lesion was stored as paraffin tissue blocks and then genetic testing towards the presence of mutations in the following genes: NF2 in meningiomas as well as IDH1 and IDH2 in gliomas was performed.

Genetic profiling of meningiomas revealed that the majority of lesions had a mutation in the NF2 gene. Application of two types of chromatography: hydrophilic interaction chromatography (HILIC) and reversed-phase chromatography (RPLC), in positive and negative modes, enabled selection of 26 analytes differentiating meningiomas with different NF2 statuses. Thus, it was proven that lipidomic phenotype was related to the NF2 status. In regard to gliomas, the most common mutation refers to gene coding isocitrate dehydrogenase (IDH). The chemometric analysis of obtained lipidomic data did not clearly reflect the differences in IDH mutation status. However, it was possible to select a few statistically ($p<0.05$) important phospholipids and sphingolipids distinguishing these two genetic types of brain tumors. Brain tumors with worse clinical outcomes were usually associated with alterations in the level of lipids involved in the phospholipids metabolism and especially their transition to lysophospholipids as well as changes in sphingolipid homeostasis. The changes in the acylcarnitine shuttle system were also observed.

To sum up, the study revealed that genetic changes have an impact on lipidome of given neoplasms. Therefore assessment of lipid composition using a low-invasive method such as a chemical biopsy could be an important step in the personalized therapy of brain tumors.

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Keywords: Brain tumor, spme, IDH, NF, meningioma, glioma

OP-039

Synthesis of Novel Oxime and Benzofuran Chemical Frameworks Possessing Potent Anticholinesterase Activity: A SAR Study Related to Alzheimer Disease

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Abstract

Alzheimer's Disease (AD) is a neurological disorder that causes the brain to shrink and brain cells to eventually die. Main symptom of AD is dementia. Today, approximately 50 million people worldwide are estimated to have AD. Population suffering from AD is getting increased all over the world over time. However, there is not a determined cure known for AD today. There are just four drugs against AD on the market. Unfortunately, none of them can totally destroy AD. Thus, discovering new molecules having capability to treat AD is required and significant. Benzofurans and oximes demonstrate a number of biological effects including anti-inflammatory and brain-barrier penetrating. For this reason, a number of novel compounds bearing oxime and benzofuran skeletons besides some known compounds were designed and synthesized, and their structural elucidation was carried out by spectroscopic techniques, especially nuclear magnetic resonance (NMR). Their anticholinesterase activities were investigated *in vitro* by the Ellman method. Based on the results, several molecules demonstrated very strong anticholinesterase activities against both AChE and BChE enzymes, even much better than the reference drug molecule, galantamine. Besides, structure-activity relationship (SAR) studies belonging to these chemical cores were revealed through this study for the first time. It is clear from the SAR results that benzofuran-oxime-phenylcarbamoyl framework is essential for the activity. Various derivatives with different types of selective activities were obtained, which brightens the detailed SAR tendency of these molecules. This study can contribute to discover a novel lead drug molecule against AD, which is an urgent need.

Keywords: Alzheimer, anticholinesterase, oxime, structure-activity relationships.

I. Introduction

AD is a progressive neurodegenerative disorder, and it has been reported that the protein level of acetylcholine receptors which is responsible for memory is reduced in AD and that dysfunction of cholinergic signal transmission could be responsible for the symptoms of AD. AD is a type of dementia, which mortally affects more than 44 million people in the world (Cunningham et al., 2015). No effective treatment exists to kill AD. For this reason, searching for novel potent ChEIs is still in progress.

Molecules bearing benzofuran moiety possess various pharmacological effects such as antibacterial, antimicrobial, anti-inflammatory, and antitumor activities (Abdel-Aziz et al., 2009). Due to its biological features, the benzofuran rings has recently gained much interest as a synthetic core (Bellur and Langer, 2005). An oxime moiety that might cross the blood-brain barrier and re-activate acetylcholinesterase in the central nervous system, which cause longer survival and prevent seizures with a possible neuropathology. This is an excellent and precious feature since brain penetrating is essential for a drug lead against neurological diseases.

In the light of all these chemical and biological information mentioned above, in the present study, we succeeded to synthesize 19 novel compounds (**5-23**) bearing oxime and benzofuran skeletons, besides 4 known compounds (**1-4**) through the synthetic methods in our previous studies (Koca et al., 2022). Also, their anticholinesterase activity was investigated. This work attributes to background regarding to the potential role of oximes and benzofurans in the drug development against neurological diseases.

II. Material and method

Synthesis of the ketones 1 and 2 was done according to the literature (Koca et al., 2022). Structural elucidation was done by using an Agilent VNMRS 500 MHz NMR spectrometer. The inhibition of AChE and BChE enzymes by samples was determined by the Ellman method (Yilmaz et al., 2012).

III. Results and discussion

As for structure-activity relationship (SAR), molecules composing of benzofuran framework bearing a halogen atom or not (**1**, **2**) were completely dead in anticholinesterase activity. Inser-

tion of an oxime moiety instead of a keto group (**3**, **4**) was not enough to make a change in the activity. However, an addition of a benzoyl moiety to the oxime group gave an opportunity to make an active chemical core for anticholinesterase effect. Modification with an electron-withdrawing nitro (-NO_2) group on the para position of this inserted benzoyl group gave two active molecules (**7** and **9**) against AChE enzyme selectively with IC_{50} values, 27.16 and 22.04, respectively. Non-modified or p-methoxy modified benzoyl molecules (**5**, **6** and **8**) were inactive, and this may be attributed to electron-donor/mesomeric methoxy group or the lack of an electron-withdrawing group such as nitro.

Along with the insertion of an ethyl group between the oxime and benzene groups, benzofuran-phenylacetyl-oxime skeleton formed. This formation did not cause activity (**10**), but 5-bromobenzofuran analogue of this framework (**11**) was selectively active against AChE (IC_{50}: 22.14). Substitution of benzoyl group with a chloronicotinoyl group resulted in activity against AChE enzyme with 18.28 of an IC_{50} value (**12**). However, 5-bromobenzofuran derivative of this core (**13**) was not active against any of the enzymes.

Insertion of a secondary amine group instead of ethyl group between the oxime and benzene groups led to formation of benzofuran-(phenyl)carbamoyl-oxime framework. Substitution of a methyl group into the para position of phenylcarbamoyl moiety gave molecule **15** which is selectively active against BChE enzyme with an IC_{50} value of 8.48. In the same chemical core, 5-bromination of benzofuran ring formed **20**, a selectively potent molecule against AChE with an IC_{50} value of 12.89. **20** differs from **15** with just the bromine in the position 5 on the benzofuran ring, which converts selectivity of the activity among the enzymes. Insertion of a meta-chlorine instead of para-methyl group on the (phenyl) carbamoyl moiety generated molecule **23** which is selectively most active molecule against AChE (IC_{50}: 8.05) same as **20**. Comparing 3,4-dichlorinated molecules **17** and **22** revealed that 5-bromination on benzofuran ring increases the anti-AChE activity more than 3 folds while the anti-BChE activity remains same. Comparing two derivatives **14** and **18** bearing non-substituted benzofuran moiety disclosed some SAR features. Substitution with different groups at the same positions on the phenyl ring are not tolerated and causes different activities. 3-Chlorobenzene derivative (**18**) possesses IC_{50} values of 10.82 (AChE) and 25.86 (BChE) while 3-methylbenzene derivative (**14**) holds IC_{50} values of 20.37 (AChE) and 7.49 (BChE), respectively. According to these results, it can be observed that in the meta position of the phenyl ring for this kind of chemical core, chlorine is preferred for the anti-AChE activity while methyl group is much more favored for the anti-BChE activity. **16**, **19** and **21** are the potent molecules against the both enzymes. Among them, **21** is the most active molecule with IC_{50} values of 12.79 (AChE) and 7.37 (BChE). This data concluded that chlorine in the para position on the phenyl ring and bromination in the position 5 on the benzofuran ring are the most favorable derivatizations for the activity against the both enzymes. In addition, **23** is the most active compound against AChE, selectively. This information is precious in terms of SAR, because the only difference between **21** and **23** is the position of chlorine on the phenyl ring. Switching the chlorine from para position to meta position makes the molecule dead against BChE but selectively most active molecule against AChE enzyme. **15** is interestingly the only molecule that is selectively active against BChE enzyme. As for its chemical structure, **15** possesses benzofuran-(phenyl)carbamoyl-oxime framework as **21** and **23** do. However, **15** does not bear bromination on the benzofuran ring and chlorination on the phenyl ring unlike **21** and **23**, instead it holds a methyl group which is an electron donor group in the para position on the phenyl ring.

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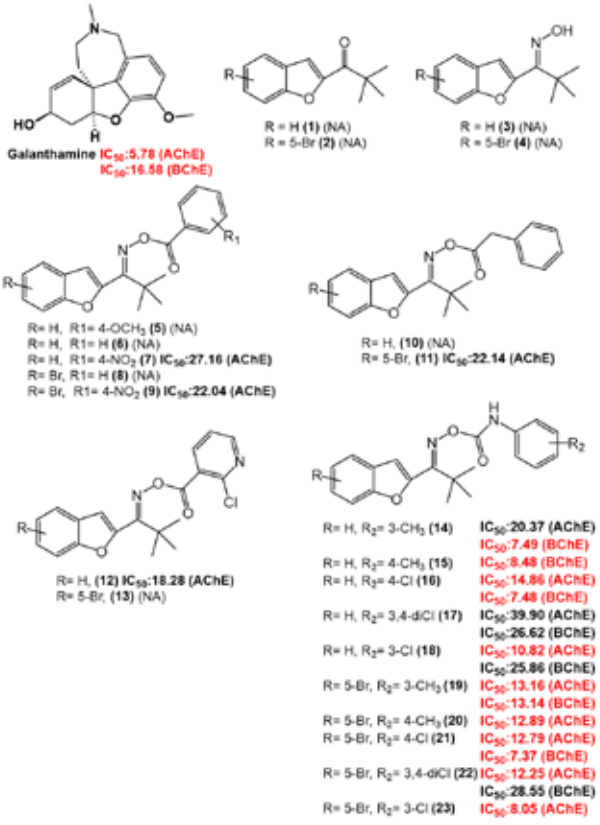

*All enzyme inhibition activity values are given as IC_{50} ± standard deviation. Strong activities are given in red color while moderate activities are given bold. NA means not active. Galantamine is the standard compound.

Fig. 1: Synthesized compounds and their anticholinesterase activity results

IV. Conclusion

As conclusion, benzofuran-(phenyl)carbamoyl-oxime framework is essential for the activity. Analogs bearing bromobenzofuran, chlorinated-phenyl or methyl-phenyl functional groups (**14,**

16, **17**, **18**, **19**, **21** and **22**) are potent against both cholinesterase enzymes. Moreover, various derivatives possessing chlorophenyl, nitrophenyl, methylphenyl or chloropyridine groups are selectively active against AChE enzyme while the only selectively active molecule against BChE is **15** which is bearing a non-substituted benzofuran ring + para-methylphenyl ring. This study can contribute to discover a novel lead drug molecule against AD, which is an urgent need. As future perspective, the synthesized molecules can go further steps and their *in vivo* activity against AD can be investigated.

Acknowledgements: Thanks to Faculties of Pharmacy of Adiyaman University, Trakya University and Dicle University, Faculties of Science and Letters of Adiyaman University and ITU.

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OP-040

Quantification of Carbamazepine Profile in Human Plasma by GC-MS

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Carbamazepine is an antiepileptic drug most prescribed as a first-line drug for the treatment of partial and generalized tonic-clonic epileptic seizures (1). The drug carbamazepine is metabolized by carbamazepine-10,11-epoxide. The carbamazepine is converted to %5 to %15 the carbamazepine-10,11-epoxide which has pharmacological activity and can have a toxic effect (2). Given that only the free (non-protein bound) concentration of an antiepileptic drug crosses the blood-brain barrier and reaches the brain, producing an antiepileptic effect, it is important to know and measure the free drug fraction. (3). Due to the narrow therapeutic index range, routine TDM is required for epilepsy patients clinically treated with carbamazepine (4). This study, it was aimed to develop a validated GC-MS test for therapeutic monitoring of the carbamazepine profile in human plasma.

Carbamazepine, carbamazepine epoxide, and internal standard carbamazepine-d10 were extracted from plasma by using the salt-assisted liquid–liquid microextraction (SALLME) method. Free carbamazepine was extracted from plasma by ultrafiltrate method. The test method has been validated according to the European Medicines Agency (EMA) Bioanalytical method validation guidelines (5).

In the bioanalytical method we developed to measure the carbamazepine profile in human plasma, the lower limits of quantification of carbamazepine, and carbamazepine epoxide were determined as 1 µg/mL and 0.4 µg/mL, respectively. The calibration curve of carbamazepine and carbamazepine epoxide for the method was validated between 1 and 15 µg/mL for carbamazepine and between 0,4 and 8 µg/mL for carbamazepine epoxide, showing correlation coefficients >0.99. At the same time, the developed method was used to measure carbamazepine profiles in real patient plasma.

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Keywords: GC-MS, Carbamazepine, Carbamazepine epoxide, Free Carbamazepine, SALLME

OP-041

Vulpinic Acid Induces Ferroptosis by Inceasing ROS Level and Changing Expression Profile of Ferroptosis Related Genes in Breast Cancer

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Breast cancer (BC) is the most common invasive cancer in women worldwide, and the number of women diagnosed with breast cancer has increased significantly over the past 10 years. BC is a genetically heterogeneous disease with multiple subtypes including estrogen receptor (ER)+ (MCF-7) and (ER)- BC. Surgical resection, adjuvant chemotherapy, radiotherapy, and hormone therapy represent the main treatment options for early-stage BC. However, more proficient therapeutic agents and strategies are needed due to development of resistance to chemotherapy and endocrine therapy. Ferroptosis is a recently described form of regulated cell death based on the iron-dependent accumulation of lipid peroxidation and is taking part in different diseases such as cancer. Targeting ferroptosis with drugs approved by FDA is a promising strategy to be used in cancer treatment. Ferroptosis is an iron-dependent programmed cell death and it differs from necrosis apoptosis, and autophagy. In the course of ferroptosis, the accumulation of intracellular iron and lipid peroxides results in the rise of reactive oxygen species (ROS), which promotes oxidative cell death. Researches indicated the promising anti-cancer function of ferroptosis in multiple cancers including BC. It was discovered that some molecules stimulate or suppress ferroptosis. Small molecules derived from biological organisms can be considered novel strategies to contribute to the treatment of cancer. Vulpinic acid (VA) is a secondary metabolite obtained from lichens and has anti-proliferative, anti-angiogenic and, anti-cancer effects. It was shown that VA inhibited cell viability of BC cells showing anti-proliferative effect on mainly MCF-7 cells in our previous study. Moreover, another study showed that VA has anti-cancer effect in MCF-7 cells through TrxR1, which is anti-cancer drug target and related to ROS increase and ferroptosis. Despite evidence indicating anti-cancer effect of VA, the mechanism underlying this effect has not yet been completely clarified. In this study, it was aimed to reveal the relationship between the anti-cancer effect of VA on MCF-7 cells and the ferroptosis mechanism. For this reason, MCF-7 cells were treated with VA and induced ferroptosis activity. As a result of the study, the level of lipid reactive oxygen species (ROS) detected by Dichlorodihydrofluorescein diacetate (DCFH-DA) increased to 66.68% in MCF-7 cells treated with VA ($p<0.05$). Moreover, after treatment of MCF-7 cells with VA, ferroptosis related gene expression profile which was determined by qRT-PCR was significantly changed. While expression levels of *LPCAT3, ALOX15, PTGS2, TF, NCOA4, TP53, VDAC2* genes, especially *ACSL4* gene, significantly increased in VA-treated MCF-7 cells, *HMOX1, SLC7A11, GPX4, NFE2L2* gene expression levels significantly decreased ($p<0.05$). These results indicate that VA effectively promotes ferroptosis by generating excess ROS. These findings suggest that therapeutic interventions mediated by the use of VA-induced ferroptosis could be used as a potentially effective method for the treatment of BC.

Keywords: Breast cancer, ferroptosis, vulpinic acid

OP-042

Green Synthesis and Characterization of Silver Nanoparticles Using *Pyracantha Coccinea M.J. Roem* and Their Antibacterial, Antibiofilm and Anti Quorum Sensing Activities

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Abstract

Green synthesis of nanoparticles has increased their use in recent years over chemical synthesis techniques with significant advantages such as high biosecurity, environment friendly, and lack of toxic environmental benefits. In this study, *Pyracantha coccinea* M.J. Roem was used as a reductive agent for silver nanoparticle synthesis. *Pyracantha coccinea* M.J. Roem plant contains phenolic substances that have allowed the use of nanoparticle synthesis. Synthesized nanoparticles were characterized using UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Zetasizer and Scanning electron microscope (SEM). The surface plasmon resonance peak of the synthesized nanoparticles was observed at 417 nm. Furthermore, Fourier transform infrared spectroscopy analysis confirmed that the plant extract not only acts as a bio-reducer but also functions as a lock-

down ligand to stabilize the surfaces of NPs within the solvent. The prepared silver nanoparticles were well distributed and the particle size was less than 100 nm. Antibacterial, antibiofilm and anti-quorum sensing activities were examined for the synthesized silver nanoparticle and good antibiofilm activity was observed.

Keywords: *Pyracantha coccinea* M.J. Roem, green synthesis, silver nanoparticle, antibacterial activity, antibiofilm activity, anti-quorum sensing activity

I. Introduction

Pyracantha coccinea M.J. Roem, also known as fire thorn, is a member of Rosaceae (Rosaceae). It is an evergreen, spiny, perennial shrub native to Southeast Europe and Asia. (Gül and Altuntaş, 2021). In addition, the fire thorn plant is rich in phenolic acids, flavonoids, vitamins, carotenoids and anthocyanins (Sarikurkcu and Tepe, 2015). Nanotechnology is the science that generally deals with the study of materials in the nanoscale ranges between 1 and 100 nm. There are different physical, chemical, and green methods for successfully synthesizing nanoparticles (NPs). Green methods have proved more likely to succeed in producing NPs than other methods and are more effective at lower cost and characterization benefits. Green synthesis methods are significantly attractive because of their potential to reduce the toxicity of NPs (Gour and Jain, 2019). Plant-induced phenolic acids have been used for green synthesis of metallic or metallic oxide nanoparticles (NPs). Phenolic acids play a role in both reductive substances and stabilizers in the process of NP synthesis. The use of phenolic acids represents a repeatable, simple, profitable and cost-effective strategy for synthesizing metal NPs (Amini and Akbari, 2019). Antibiotic resistance is one of the most important health problems of our time. New compounds that are effective antibacterially are urgently needed to treat infections caused by resistant bacteria. The failure to discover new antibacterias that can be used in treatment and the unavoidable resistance to existing antibiotics have led scientists to explore alternative treatment options (Murray et al. 2022). It is thought that finding new molecules to inhibite the mechanisms involved in pathogenicity would be effective in solving the resistance problem. The aim of this study is to green synthesis and characterize silver nanoparticles using *Pyracantha coccinea* M.J. Roem and investigation of their antibacterial, antibiofilm and anti-quorum sensing activities.

II. Material and method

In this study, the fruits of the *Pyracantha coccinea* M.J. Roem plant were collected on the Beşevler campus of the Ankara University. 10 grams of fruit weighed from the cleansing and it is added to a mixture of 250 ml methanol:water (1:4). It was mixed in a magnetic stirrer at 30 min 300 rpm. Whattmann filtered the extract using No:2. 100 ml of 50 mmol silver nitrate solution was prepared, 100 ml of extract was added and the solution's pH was set to 10. The solution was incubated at 90 °C for 2 hours, and sediment was obtained in black. The solution was filtered using Whattmann No:2. The sediment was dried at 90 °C. The characterization of nanoparticles using the UV-Vis(Carry 60 UV-Vis, Agilent Technologies, Santa Clara, California), FTIR(Shimadzu Infinity FTIR spectrometer,Shimadzu, Kyoto, Japan), XRD(Ultima IV XRD, Rigaku, Austin, TX, USA), Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, Worcestershire, England) and SEM(EVO 40 SEM, Zeiss, Oberkochen, Germany).

In the antibacterial activity test, *Staphylococcus aureus* ATCC 29213 (methicillin-susceptible, MSSA), *S. aureus* ATCC 43300 (methicillin-resistant, MRSA), *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883 and *Pseudomonas aeruginosa* ATCC 27853 were used as test bacteria. The silver nanoparticle was dissolved in dimethyl-sulfoxide (10% DMSO). The broth microdilution method was used to determine minimum inhibitory concentration (MIC) values. Serial two-fold dilutions ranging from 0,25 mg/mL to 0,002 mg/mL were prepared in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA).

In antibiofilm activity test, 100 µl silver nanoparticle (subMIC value-0.0625 mg/mL) was transferred into the wells containing mature *P. aeruginosa* biofilms and the plates were incubated at 37°C for 24 h. After incubation, 100 µl of 0.5% crystal violet solution was added to each well for staining the biofilm cells. Then acetone-alcohol (30:70 v/v) solution was added into the wells to dissolve the bound dye within the biofilm matrix. The optical density of the dissolved crystal violet dye was measured by a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Vantaa, Finland) at 620 nm (OD 620 nm). The percentage biofilm inhibition values were calculated according to the following formula:

% Biofilm inhibition = [(OD (growth control) – OD (sample)) /OD (growth control)] x 100

The anti-quorum sensing activity was performed by the disc diffusion method using reporter bacteria *Chromobacterium violaceum* ATCC 12472. Sterile blank discs (6 mm diameter; Bioanalyse®, Ankara, Turkey) impregnated with twenty microliters of the silver nanoparticle solutions (1 mg/mL-0,5 mg/mL-0.250 mg/mL-0.125 mg/mL) were placed on the medium. The formation of an inhibition zone around the disc was noted as the potential anti-quorum sensing activity.

III. Results and discussion

After the synthesis of silver nanoparticle (AuNP), the solution observed a change in color over time. Observations showed that after pH adjustment of the silver solution, the color changed rapidly from dark orange to black. From UV-visible spectrum, the peak of AuNP and plant extract have been observed to occur at 417nm and 278nm, respectively.

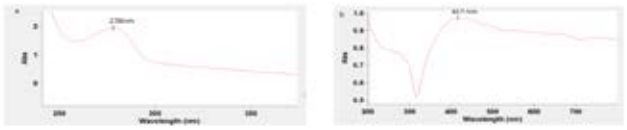

Fig. 1: UV spectrum a) plant extract b) green synthesis of silver nanoparticle.

FTIR analysis has identified possible functional groups of phytochemicals in the *Pyracantha coccinea* M.J. Roem extract contained in the synthesis of AuNP. Adsorption of the band at 1398 cm-1 shows amino acids and amide bonds covering the surface of the nanoparticles. The adsorption of the band at 1101 and 1041 cm-1 shows the C-N stretching vibration. The peaks observed at 729, 644, and 617 cm-1 correspond to the C-H stretching of the alkenes.

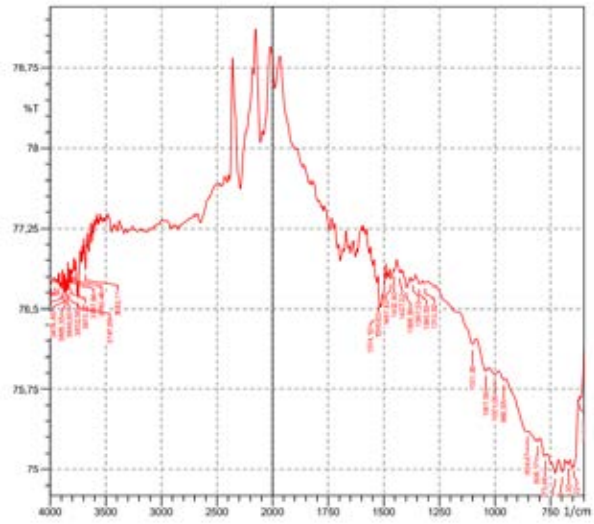

Fig. 2: ATR-FTIR analysis of silver nanoparticle green synthesised by *Pyracantha coccinea* M.J. Roem

XRD spectra clearly show that silver nanoparticles synthesized using P. coccinea fruit extract are in crystalline structure. The Bragg reflections of silver nanoparticles are observed at 2θ values of 38.09°, 44.24°, 64.43°, and 77.26°, corresponding to the (111), (200), (220) and (311) planes, respectively, to the facets of face-centered cubic (fcc) crystal structure of silver (JCPDS, No. 04-0783).

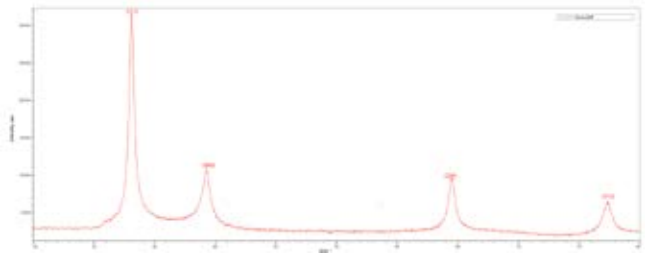

Fig. 3: XRD analysis of green synthesized silver nanoparticle by using fruit *Pyracantha coccinea* M.J. Roem**.**

The stability of AgNPs is confirmed by the value of the zeta potential. The resulting value is -35.3 mV , indicating that AgNPs are negatively charged in their dispersed aqueous solutions, thus AgNPs are long-term stable due to negative-negative repulsion.

SEM is one of the tools used to determine the shape and size of nanoparticles. The silver nanoparticle green was synthesized using P. coccinea is mostly spherical in shape and the size of the nanoparticles is smaller than 100 nm. A scanning electron microscope with energy dispersive X-rayspectrometer (SEM-EDX) was employed to determine the silver concentration of the nanoparticles. From the EDX analysis, the distinct peak detected at 3 keV confirmed the presence of elemental silver in the nanoparticle. A silver concentration of 83.97 ± 2.7% was detected.

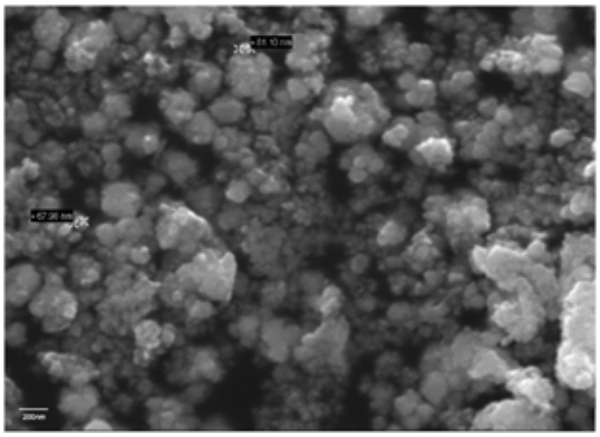

Fig. 4: SEM image of synthesized silver nanoparticle by using fruit *Pyracantha coccinea* M.J. Roem

Based on the MIC results, it was found that the silver nanoparticle showed antibacterial activity only against Gram-negative bacteria. However, the most notable activity was observed against *P. aeruginosa* ATCC 27853, with a MIC value of 0.125 mg/mL. The percentage biofilm inhibition value of silver nanoparticles was determined as 88.77%. All the silver nanoparticle solutions that were tested did not exhibit any anti-quorum sensing activity.

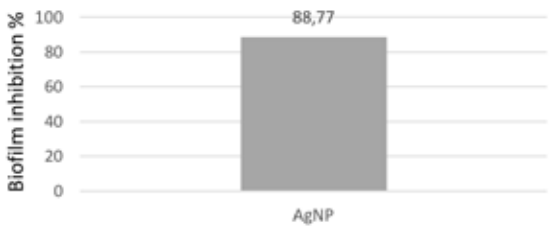

Fig. 5. Antibiofilm activity of silver nanoparticles

IV. Conclusion

The characterization studies have shown that AuNPs are synthesized. AgNP showed the best antibacterial activity against *P. aeruginosa* ATCC 27853. The MIC values of the AgNP against *P. aeruginosa* ATCC 27853 was found to be 0.125 mg/ml. The

synthesized AuNP showed good antibiofilm activity.

Acknowledgements: Authors were a Zerrin Sezgin Bayındır who helped him study. Authors acknowledge ODTÜ Merlab for XRD analyses and Ankara University Institute of Nuclear Sciences for SEM and EDS analysis.

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OP-043

HILIC-MS-Driven Optimization of Protein Glycosylation via Disuccinimidyl Linker for Pure Glycoconjugate Vaccine Synthesis

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Abstract

Glycoconjugate vaccines are obtained by covalent linkage of sugar antigens to carrier proteins. Conjugation via disuccinimidyl homobifunctional linkers is reported in literature as a convenient approach to producing glycoconjugate vaccines. However, its use is generally supported by an incomplete characterization of the synthesized glycoconjugates, resulting in products with unproven identity and purity.

A hydrophilic interaction liquid chromatography (HILIC)-MS-based monitoring of the conjugation procedure reported in literature revealed that the non-optimal purification of activated saccharides and the high tendency to hydrolysis of disuccinimidyl linkers result in side-reactions and non-pure glycoconjugates. On the bases of these data, the glycosylation approach was revised and optimized. As model reaction, 3-aminopropyl mono- to trimannose saccharides were conjugated, via disuccinimidyl glutarate (DSG), to a commercial protein, ribonuclease A (RNase A). Through a detailed characterization of synthesized glycoconjugates, purification protocols and conjugation conditions have been modified with a dual aim: ensure high sugar-loading and avoid the presence of side reaction products. An alternative purification approach based on HILIC allowed to elude the formation of glutaric acid conjugates and a design of experiment approach permitted to optimized glycan loading. The developed conjugation strategy was applied to the synthesis of glycoconjugates developed as antitubercular vaccines. A recombinant protein antigen, Ag85B-dm, and a nanoparticle candidate vaccine, β-E50I60, were considered. Together these results suggest that, with an adequate protocol, conjugation via disuccinimidyl linkers can be a valuable approach to produce high sugar-loaded and well-defined glycovaccines.

Keywords: Glycoconjugate vaccines, hydrophilic interaction liquid chromatography (HILIC), anti-tubercular vaccines, glycoconjugate characterization

I. Introduction

The development of glycoconjugate vaccines requires the chemical conjugation of glycans to an immunogenic carrier protein. The choice of the conjugation strategy is extremely important since it strongly influences the quality of the final glycoconjugates in terms of site-specificity, glycan loading and purity/homogeneity. One of the most common approaches for the covalent coupling of oligosaccharide epitopes to carrier proteins entails the use of disuccinimidyl bivalent linkers (such as disuccinimidyl glutarate, DSG), able to react with amino-functionalized oligosaccharides to form reactive ester (Micoli *et al.*, 2018). However, the high tendency to hydrolysis of disuccinimidyl-esters may represent a significant drawback for this conjugation chemistry. This instability can hamper an extensive linker purification, leading to side reactions between protein lysine residues and residual linker, to form conjugates of the corresponding carboxylic acid. The effectiveness of the purification approaches reported in literature has not been proven as the reported analytical methods only provides the average glycan loading, while no information of any side reactions. The use of this conjugation chemistry to produce glycovaccines thus requires the development of most efficient purification protocols and detailed analytical characterization of produced glycoconjugates. In this context, the aim of this work is to set up an experimental protocol that, being addressed by a proper analytical characterization, allows to obtain glycoconjugates with satisfactory sugar loading, and also adequate purity, suitable to be used as therapeutic agents. HILIC-UV-ESI-MS/MS is a powerful

tool for qualitative and quantitative analysis of glycans, glycopeptides and intact glycoproteins. Thus, HILIC was used to elucidate the neo-glycovaccine structures.

II. Material and method

3-aminopropyl small-sized (from mono- to tri-) mannose saccharides were ad-hoc synthesized by adapting known synthetic methods. Antigenic protein Ag85B-dm was produced as recombinant proteins in *Escherichia coli* as previously described (Rinaldi *et al*., 2018) Elastin-like block corecombinamer-based nanoparticles (**β-E50I60) were provided by Prof. F. Javier Arias from University of Valladolid (García-**Arévalo *et al*., 2013).

The preparation of the glycoconjugates involved two different steps. First, the aminopropyl saccharides are modified through the formation of an amide bond between their amino group and one of the terminals of the homobifunctional DSG linker. Then, the obtained active esters, purified from linker excess, are reacted with the ε-amino group of lysine residues on protein surface. The purification step represents a critical point to obtained pure glycoconjugates. The procedure reported in literature consists in the precipitation of active esters by washing with ethyl acetate. The effectiveness of this procedure was evaluated by monitoring in MS the composition of the reaction mixture during purification and by HILIC-UV-MS analysis of the resulting glycoconjugates. The alternative purification approach, herein developed, entails the use of semipreparatieve HILIC chromatography to collect the active ester in purified form. Analytical HILIC was thus used to confirm the purity of produced glycoconjugates and to address an optimization, by Design of Experiment approach, of the reaction conditions to increase glycosylation yields.

III. Results and discussion

Small sized mannose saccharides were used to study the glycosylation of a model protein, RNase A. At first, the purification procedure reported in literature was reproduced to verify its effectiveness. LC-MS monitoring revealed the presence of linker trace even after the purification. Moreover, the HILIC-UV-MS intact protein analysis showed the formation of glutaric acid conjugates as side products. Once proved that the reported method was unable to produce pure and defined glycoconjugates, an alternative purification procedure was developed. The final method entails the use of semipreprartive HILIC chromatography. This approach allowed to obtain pure glycoconjugates without impurities of acidic products. Their absence was also proved by glycopeptide mapping bottom-up approach. However, glycosylation yields were still unsatisfactory, being the unmodified protein the main species. Intact protein HILIC-UV was thus used as rapid tool to addres an optimization of the reaction condition based on a DoE approach. Four parameters were considered (sugar/protein molar ratio, temperature, protein concentration and buffer pH), by studyng their effect on the average number of incorporated saccharides. The optimization allowed to significantly increase the yields, as an example the average protein loading of RNasi A with ManMan saccharide increased from 0.6 to 7.9 ± 0.1 mol/mol. HILIC mode was also used to perform glycopeptide mapping analysis, that revealed the predominant glycosylation of N-terminal position.

The optimized glycosylation protocol was then used for the glycosylation of recombinant protein Ag85B-dm, candidate carrier for the development of a novel anti-tubercular vaccine, obtaining ≥ 99.5% pure glycoconjugates. The predominant glycosylation of N-terminal amino group allowed also the modification of a protein nanoparticle structure developed as nanovaccines. Its succesfull modification with mannose saccharides should increase its immunogenic properties.

IV. Conclusion

In the present work, a common conjugation chemistry, based on the use of a disuccinimidyl functional linker (namely DSG), was exploited for the synthesis of glycoconjugates. A detailed analytical characterization, entairly based on HILIC chromatography, confirmed the limitations of this conjugation approach, due to the intrinsic tendency of hydrolysis of the linker, and addressed us in the development of a novel, most selective, purification method based on preparative HILIC chromatography. The proposed purification method, together with a detailed structural characterization and a rational optimization of glycosylation conditions, made this conjugation approach suitable for the synthesis of pure and high-loaded glycoconjugates, to be considered as carbohydrate-based vaccine.

Acknowledgements: We thank Prof. F. Javier Arias from University of Valladolid for providing the elastin-like block corecombinamer-based nanoparticles (**β-E50I60).** This work was partially supported by the Italian Ministry of Health (Project Immunoter-655 apia: cura e prevenzione di malattie infettive e tumorali (Immuno-HUB), project number T4-CN-656 02).

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OP-044

Development of a Fluorescence-Based Nucleic Acid Biosensor for the Detection of Bacterial Protease Enzyme

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Abstract

Bacterial protease enzymes are essential for breaking down proteins inside bacterial cells. By cleaving peptide bonds, these enzymes enable bacteria to absorb vital nutrients from diverse protein sources. Bacterial proteases are also important for cell division, gene expression control, and the synthesis of virulence factors, which are all physiological processes essential for bacterial survival and pathogenicity. By helping germs invade and evade the host immune system, bacterial protease enzymes contribute significantly to the pathogenicity process. These enzymes can break down host defense proteins, interfere with cell adhesion, and encourage tissue damage, which helps infection progress. Understanding the pathogenicity potential of pathogens, creating efficient diagnostic tools, and creating tailored therapeutics to decrease their activity depends on detecting bacterial protease enzymes, opening up possibilities for managing and treating bacterial infections. In this study, unique ssDNA nucleotide sequences were designed as a specialized recognition element for the fluorescent-based detection of a particular protease enzyme from a bacterial species. For this purpose, the binding of the *Acinetobacter baumannii* protease protein by the relevant ssDNA sequence was determined by associating it with a fluorescence signal by using the ssDNA-based special recognition element marked with fluorescent molecules.

Keywords: ssDNA-based detection, Fluorescent biosensor, *Acinetobacter baumannii*

I. Introduction

Protease enzymes in bacteria are crucial for the digestion of cellular proteins. Bacterial protease enzymes play a crucial role in pathogenicity by facilitating microbial invasion and immune evasion. These enzymes can disrupt host defense proteins, disrupt cell adhesion, and promote tissue damage, all aiding the spread of infection. Proteases are known to significantly impact the pathogenicity and antibiotic resistance of bacteria, as they contribute to the survival and virulence of these microorganisms (Li et al, 2023). Pathogenic bacteria can generate proteases that can break down host defense proteins, including antibodies and complement factors, thereby diminishing the effectiveness of the immune response. The enzymes in question can degrade extracellular matrix components, thereby facilitating the invasion of tissues and dissemination of the infection (Ingmer and Brøndsted, 2009).

Furthermore, proteases play a role in the mechanisms of antibiotic resistance. Proteases synthesized by bacteria can specifically target and break down antibiotics, thereby leading to their loss of efficacy. Furthermore, proteases can alter or degrade host-produced antimicrobial peptides or impede the effectiveness of antimicrobial proteins.

Opportunities for monitoring and treating bacterial infections can be opened by detecting bacterial protease enzymes, which can be used to understand pathogens' pathogenicity potential better, develop effective diagnostic methods, and create targeted therapies to reduce their activity.

In this research, a specific bacterial protease enzyme was detected by fluorescence. Hence a unique ssDNA nucleotide sequence was created as a recognition element. Using the ssDNA-based special recognition element labeled with fluorescent molecules, we determined the protease protein's binding by the relevant ssDNA sequence.

II. Material and method

All compounds used were of analytical purity and were purchased from Sigma Aldrich (USA). Solutions were prepared using Elga PURELAB flex purified water equipment, weighed with a Shimadzu Corporation ATX224 instrument, and measured using a Fisher Scientific pH meter.

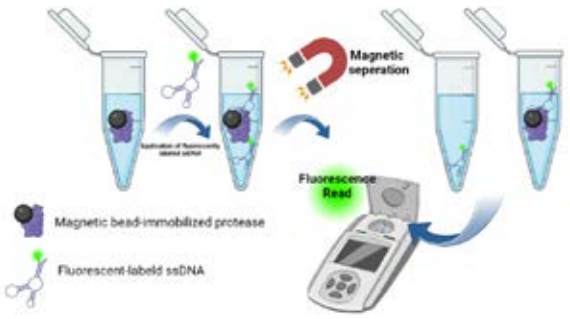

Fig. 1: Schematic representation of the developed biosensor system

Fabricating of the fluorescent-based biosensor

Fluorescence-labeled ssDNA sequences were used to perform fluorescence measurements. First, the protease protein to be detected was prepared in a pH 7.0 phosphate buffer in the nM-μM range. Then, 100 pmol of fluorescently labeled ssDNA was incubated with protein samples independently for 30 minutes with

shaking. After incubation, unbound sequences were removed by applying a magnetic field. After 2 consecutive washing steps, fluorescence values of bound and unbound ssDNA sequences were read and plotted against protein concentration.

III. Results and discussion

The experimental methodology summarised in the text showcases the development of a linear association between the fluorescence signal and protein concentration. This correlation facilitates the measurement of protein levels within the nanomolar concentration range to micromolar. The application of fluorescently labelled single-stranded DNA recognition elements enables the binding of the intended protease protein, thereby identifying and quantifying its concentration.

The magnetic separation process enables the selective isolation of ssDNA-protein complexes that are specifically bound, thereby separating them from unbound sequences. The acquisition of fluorescence spectra within the emission wavelength range of 500-550 nm, with excitation at 495 nm, yields significant insights into the fluorescence properties of the specimens.

The percentage of fluorescence changes computed for both the wash samples and the bound samples provide valuable insights into the nature of the interaction between the protein of interest and the fluorescently labeled single-stranded DNA. The observed reduction in the percentage of fluorescence changes in the wash samples as protein concentrations increases the effective elimination of unbound sequences. On the other hand, the observed linear increment in the percentage of fluorescence alterations in the bound samples implies a direct correlation between the fluorescence indication and the protein concentration.

Using the calibration curve derived from the obtained data, significant analytical parameters such as the limit of detection (LOD) and limit of quantification (LOQ) can be ascertained. The parameters above provide insights into the fluorescence biosensor's sensitivity and specificity for detecting Acinetobacter baumannii protease.

The results indicate that the fluorescence-based biosensor is a reliable and effective method for detecting the target protease with high sensitivity and specificity. These findings suggest that the biosensor has potential applications in research and clinical settings for detecting and monitoring bacterial protease enzymes. Enhancing the biosensor's applicability and validation through further optimization could contribute to advancing diagnostic tools for bacterial infections.

IV. Conclusion

In conclusion, the involvement of bacterial protease enzymes in the pathogenicity process is of utmost importance as they aid in nutrient acquisition, invasion promotion, and evasion of the host immune system. The present investigation employed distinct single-stranded DNA sequences as specialized recognition elements to detect a particular protease enzyme from *Acinetobacter baumannii*, a bacterial species that hold clinical significance, through a fluorescent-based approach. The fluorescence signal observed upon association of the pertinent ssDNA sequence indicated the binding of the protease protein, thereby facilitating the creation of a highly sensitive detection technique. The discoveries, as mentioned earlier, exhibit potential for enhancing the administration and therapy of bacterial infections by providing significant perspectives into the identification and suppression of bacterial protease enzymes.

Acknowledgements: This work was supported by TUBITAK project number 221Z345.

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OP-045

Chiral Separation and Absolute Configuration Assignment of Racemic Bioactives Molecules

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The determination of enantio-separation, absolute configuration and chiral recognition mechanism is a very significant aspect in medicinal, pharmaceutical, agricultural and chemical science. The aim of this work is to apply a simple methods for enantioresolution and determine of absolute configuration of some racemic bioactive molecules by using direct isocratic high-performance chromatography correlated by modeling process. Rapid, simple, accurate and a baseline chiral separation was achieved by using seven polysaccharide-derived chiral stationary phases (Chiralcel® OZ-3, Chiralcel® OD-H, Chiralcel® OD, Chiralcel® OJ, Chiralpak® AD, Chiralpak® IA and Chiralpak® IB) and different mobile phases. Also the most important parameters studied which control enantiomeric resolution, are composition of mobile phase, the effects of nature and the concentration of the alcoholic modifiers, the pH of mobile phase by adding chemical additives, flow rate, temperature and effect of other parameters on the separation of enantiomers for optimum resolution. The absolute configuration

of the eluted enantiomers of the reported drugs were determined by comparing way with available pure enantiomers is investigated. For new synthesis bioactive molecules some racemic iminoflavan derivatives were synthesized by simple condensation in position C4 of the flavanone. All new compounds were characterized by using UV-Vis, IR and NMR as spectroscopic techniques. Chromatographic analysis of racemic mixtures was performed by direct chiral high-performance liquid chromatography using Daicel Chiralcel® OD-H as chiral stationary phase, and online coupled with electronic circular dichroism (ECD) detector. The correlations of experimental ECD traces with quantum chemical ECD calculations with time-dependent density functional theory made it possible to elucidate the absolute configuration for each enantiomer, and establish the elution order. Furthermore, molecular docking was performed to confirm of absolute configuration, elution order and analyze the binding modes of R- and S-enantiomers. Moreover the stereoselective and the chiral recognition mechanism of racemic bioactive molecules on Chiralcel® OD-H chiral stationary phase (CSP) have also been researched via modeling studies. This process was found to be suitable for rapid enantiomeric purity analysis and a quality control of racemic compounds.

Keywords: Absolute Configuration, Racemic, Chiral Separation, Electronic Circular Dichroism, TD-DFT Calculations.

OP-046

Electroanalytical Biotools to Advance Food Allergy at Different Molecular Levels

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Precision nutrition uses information about individual characteristics to develop specific nutritional advice, products or services to help individuals achieve lasting, health-enhancing dietary behavior change; more specifically it addresses the influence of nutrients and foods present in a normal diet on the development of food allergies or intolerances[1]. Most of these important food allergens share a number of common characteristics, such as being water-soluble and relatively heat-stable glycoproteins, acids and proteases. For example, soy is among the most common human food allergens, known as the "big eight" because they account for 90% of all food allergies. Despite this, it is widely used as an ingredient in meat, dairy, and bakery products, cheese analogues, desserts, soups, etc. Food allergy also does not exclude the main rival of soy-based or vegan diets, and meat allergy should also be considered. Alpha-gal (α-Gal) syndrome is a severe food allergy to red meat, which occurs most commonly in adults bitten by certain types of ticks, its symptoms range from mild to severe and can vary over time, and has recently been reported to adversely affect cancer therapy, demonstrating the need for research into the relationship between nutrition and health[2].

Food analysis is a continuously evolving analytical methodology aimed at developing increasingly competitive, simple, sensitive and cost-effective technologies to ensure food safety, quality and traceability in accordance with food legislation and individual consumer needs. Following this wave, our most recent investigations have addressed the development of two electrochemical immunoplatforms for interrogating dually the main allergenic protein targets of soy (glycinin and β-conglycinin)[3], and individually the main mammalian oligosaccharide (α-Gal) associated with allergy to red meat. Both methodologies ingeniously combine the use of immunoassay formats, suitable antibodies and tracers, magnetic particles (MP), and amperometric transduction of horseradish peroxidase (HRP)-assisted and hydroquinone (HQ)-mediated H_2O_2 reduction.

Both bioplatforms demonstrate very attractive analytical and operational characteristics in terms of sensitivity and selectivity for the amperometric determination of standards and potential for discriminating samples incurred with as little as 0.0005 ppm of soy flour in model cookie extracts and for the determination of α-Gal in different mammalian meats both raw and processed using simple, fast and easily applicable protocols at the point of care. These unique features and their versatility to profile allergens at different molecular levels (protein or carbohydrate) and origin (vegetable or animal) deserve further exploitation to ensure food safety for manufacturers, distributors and consumers and to advance in precision nutrition research and application.

Acknowledgments: Financial support of PID2019-103899RB-I00 and Grant S2018/NMT-4349.

Keywords: Food allergy, soybean, red meat, electrochemical immunoplatform

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OP-047

Smart Sampling: Next-Generation DBS Samplers for LC-MS Based Protein Analysis

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Sampling of blood and other biological fluids on paper (Dried Blood Spots – DBS and Dried Matrix Spots – DMS) have been used in newborn screening for half a century. Collection of blood in this sustainable manner has become popular as it allows for sampling of tiny amounts (newborn, test animals). In addition, sampling can be performed at home without the need of trained personnel and the samples can be transported without the necessity of refrigeration.

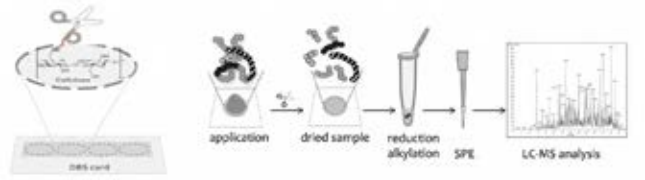
Compared to low molecular substances there is less knowledge available on determination of proteins from dried samples. Especially protein determinations based on liquid chromatography mass spectrometry (LC-MS) from these samplers are still in its infancy. Not only are these analyses advanced, also a lot of time and effort needs to be put into the sample pretreatment to get satisfactory results.

The research in our group focuses on targeted and shotgun analysis of proteins extracted from the dried state using smart samplers. Two pretreatment steps are essential in analysis of proteins from biological matrices by LC-MS: enzymatic digestion for bottom-up analysis and selective affinity capture using monoclonal antibodies. Incorporating these steps in an initial stage of the sampling workflow saves both time and simplifies the work to be carried out in the laboratory. Smart samplers are designed such that sample preparation starts already during blood collection and transport. The samplers are prepared through covalent binding of trypsin (see figure) or monoclonal antibodies to cellulose (and other relevant materials).

This presentation gives an overview of our efforts made to develop the concept of smart samplers for both bottom-up analysis as well as affinity capture for sample clean-up. Although this concept is in an exploratory phase, it shows to have potential for advanced protein analysis from minute amounts of dried blood.

Keywords: DBS, protein determination, LC-MS, paper integrated sample preparation, proteolysis, affinity extraction

Example of a smart proteolysis sampler.



Trypsin is covalently bound to cellulose. This allows proteins to be proteolysed during the sampling and drying of the sample. Only little sample handling is needed for bottom-up LC-MS based protein analysis when the sample arrives at the lab.

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OP-048

Colorimetric Aptasensor for Paraquat Detection Based on Truncated DNA Aptamer and Gold Nanoparticles

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Paraquat poisoning is one of the major medical problems in many countries in Asia, often found in farmers and farmworkers who are exposed to this herbicide during working in agricultural fields. Since paraquat poisoning can cause a high fatality rate due to its inherent toxicity and lack of effective treatment, monitoring an exposure level may help to reduce the risk of severe effects in humans. Thus, this work is interesting to develop a simple and sensitive colorimetric aptasensor to detect paraquat. The truncated DNA aptamer was excised from its full length and analyzed for its selective interaction with paraquat by molecular dynamic simulation. The aptasensor developed from this short DNA aptamer and gold nanoparticles exhibited the limit of detection (LOD) for paraquat of 2.76 nM, which was more sensitive than the aptasensor derived from the full-length aptamer (LOD of 12.98 nM). This aptasensor selectively detected paraquat, but not to other tested herbicides; ametryn, atrazine, difenzoquat, 2,4-D-dimethyl ammonium, and glufosinate. These results suggest the potential

applications of this developed aptasensor to monitor an exposure paraquat level of farmers and farmworkers as well as to detect paraquat residues in the environment and agricultural products.

Keywords: Aptamer, Biosensor, Gold nanoparticles, Herbicide, Paraquat

OP-049

Development of Selective and Sensitive MIP-Based Electrochemical Sensor for Quercetin Determination from Rubus sanctus, Fragaria vesca Extracts and Some Herbal Supplements

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Abstract

In this work, a novel molecularly imprinted polymer (MIP)-based electrochemical sensor was fabricated for the selective and sensitive determination of the important flavonoid quercetin (QUE). The photopolymerization (PP) technique was applied using tryptophan methacrylate (TrpM) as the functional monomer on the glassy carbon electrode (GCE). Electrochemical and morphological characterizations of the developed sensor (TrpM@QUE/MIP-GCE) using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM) were performed after the optimization process. Determination of QUE was performed in the standard solution, in the linear concentration range between 1 pM and 25 pM, with the calculated limit of detection (LOD) and limit of quantification (LOQ) values of 0.18 pM and 0.61 pM, respectively. Recovery studies were performed using two brands of commercial supplement products containing different amounts of QUE. Great recovery% results (101.25% and 100.23%) demonstrated the TrpM@QUE/MIP-GCE sensor's applicability and accuracy. Imprinting factor analysis using similarly structured compounds (caffeic acid, luteolin, naringenin, hyperoside, apigenin, p-coumaric acid, and rutin hydrate) confirmed the sensor's excellent selectivity performance. Quantification of QUE was made in methanol extracts prepared from *Rubus sanctus* stem, leaves, flowers, and *Fragaria vesca* fruits. The performance verification of the sensor was tested by adding QUE of known concentration to the extracts. Lastly, the non-imprinted polymer (NIP) based GCE was utilized to control and verify the TrpM@QUE/MIP-GCE sensor's performance.

Keywords: Quercetin, electrochemical sensor, molecularly imprinted polymer, *Rubus sanctus*, *Fragaria vesca*, herbal supplements

I. Introduction

Quercetin (QUE) is a type of flavonoid found in high concentrations naturally in foods such as onions, cabbage, broccoli, apples, strawberries, grapes, cherries, artichokes, green tea, garlic, and citrus fruits. Flavonoids are plant-derived compounds with polyphenolic structure; in addition to QUE, rutin, naringenin, apigenin, kaempferol, and myricetin are also included in this group. The most significant features of QUE are antioxidant, anti-inflammatory, and antiviral effects and its ability to support the immune system. Apart from the presence of QUE in various fruits and vegetables, it is included in the content of many supplements as a commercial product, thanks to its critical effects on health. Therefore, the development of a sensitive, selective, accurate, and precise sensor for determining QUE has great importance (Akbari et al., 2022; Dias et al., 2021; Li et al., 2016; Oluwole et al., 2022). This work combines the sensitivity, low cost, and easy application advantages of electrochemical sensors with the superior selectivity feature of molecularly imprinted polymers (MIPs). MIP was fabricated on the glassy carbon electrode (GCE) surface with photopolymerization (PP) using tryptophan methacrylate (TrpM) as the functional monomer, QUE as the template, 2-hydroxyethyl methacrylate (HEMA) as the basic monomer, ethylene glycol dimethacrylate (EGDMA) as the crosslinker, and 2-hydroxy-2-methylpropiophenone as the initiator. This study aims to meet the need for selective and sensitive analysis of QUE in commercial and natural products.

II. Material and method

The stock solution of QUE was prepared as 1.0 mM in methanol. PALMSENS (Netherlands) potentiostat was utilized for all electrochemical measurements (CV and DPV) using 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution as the redox probe. For the sample application, commercial capsules were weighed, then the powder contents of the commercial capsules were separated, and the empty capsules were weighed. Crude extracts were prepared by powdering dried plant materials and applying ultrasonic extraction with methanol solvent. The methanol extracts were dried by removing

the solvent under reduced pressure. By calculating the difference, the required amount was taken to prepare 1.0 mM QUE solution and dissolved in methanol. Critical parameters affecting MIP (monomer to template ratio, dropping volume, PP time, removal solution, removal time, and rebinding time) were optimized.

III. Results and discussion

Electrochemical characterization of the molecularly imprinted polymeric was performed with CV and EIS methods during the different steps of the sensor preparation process. While CV directly evaluated the peak currents of the redox probe, the EIS method evaluates the charge transfer resistance (R_{ct}). Analytical performance evaluation and validation of the newly developed TrpM@QUE/MIP-GCE sensor was carried out in standard solution by DPV method. TrpM@QUE/MIP-GCE gave a linear response in the concentration range between 1 and 25 pM, with the calibration graph of ΔI values (difference between peak currents obtained after removal and after rebinding) versus QUE concentrations. The LOD and LOQ values were calculated as 0.18 pM and 0.61 pM, respectively. The regression equation was found as ΔI (μA) = $1.81x10^{12}$ C (M) + 92.68 (r^2 = 0.999). Excellent repeatability and reproducibility values confirmed the sensor's precision. For the recovery analysis, two different commercial supplement products containing different amounts of QUE were selected. The QUE amounts stated in the content of the products have been verified. In addition, spiked sample studies gave good RSD% and recovery% results. This demonstrates the applicability and accuracy of the sensor. QUE was quantified using solutions prepared at certain concentrations from methanol extracts prepared from *Rubus sanctus* stem, leaves, flowers, and *Fragaria vesca* fruits. The standard of quercetin with a certain concentration was added to the extracts and the accuracy of the sensor was proved by quantification. Imprinting factor (IF) calculations were made for the selectivity evaluation, which is the most prominent feature of the TrpM@QUE/MIP-GCE sensor. For this purpose, the effects of caffeic acid, luteolin, naringenin, hyperoside, apigenin, p-coumaric acid, and rutin hydrate, molecules with similar structures to QUE, which may affect selectivity, have been studied. As a result, the TrpM@QUE/MIP-GCE sensor has a very high affinity for QUE. As the last step, the non-imprinted polymer (NIP)-based GCE was fabricated and used to control and confirm the TrpM@QUE/MIP-GCE sensor's performance.

IV. Conclusion

This study explains the most sensitive and selective MIP-based electrochemical sensor application for the determination of an important flavonoid QUE in the standard solution and commercial and natural products. Acquired very low LOD and LOQ values verified the sensor's high sensitivity performance. Additionally, the results of imprinting factor studies demonstrated the excellent selectivity of TrpM@QUE/MIP-GCE. Furthermore, PP-based polymerization technique stands out with good stability, enabling the sensor to be used for up to 7 days. In conclusion, TrpM @QUE/MIP-GCE is an advantageous option for QUE analysis in commercial and natural samples.

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OP-050

Recessed Solid-Phase Microextraction Acupuncture Needle for in Vivo Tissue Sampling and Direct Analysis by Mass Spectrometry via Automated Microfluidic Open Interface

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Microfluidic open interface (MOI) is a sample injection interface which operates under the concept of flow-isolated sample injection chamber with the solvent delivery system. MOI-MS is an ideal interface for direct coupling solid-phase microextraction (SPME) to MS for rapid analysis (less than 50 s per sample). The interface provides a small desorption chamber and constant liquid flow to MS with stable ionization. Recessed solid-phase microextraction (SPME) chemical extraction acupuncture needles are in vivo sampling devices based on medical-grade stainless-steel acupuncture needles. A specialized extraction phase, designed for a broad analyte coverage without the need for solvent activation, is chemically deposited into a recession behind the tip of the needle where it will be physically protected during sampling. Herein, the in vivo SPME using recessed acupuncture needle was direct coupled with MS by the automated MOI-MS interface for potential rapid analysis of the drug concentration during the surgery.

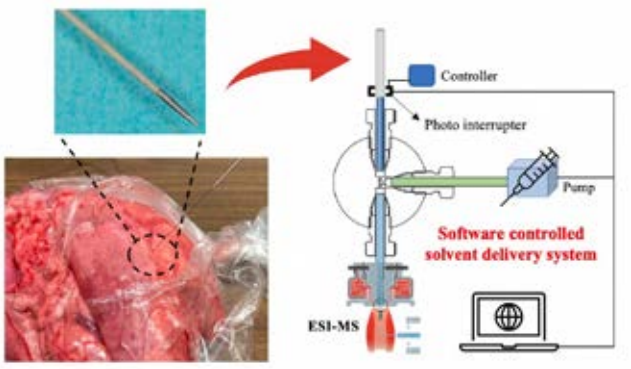
Recessions on the stainless-steel needles are created by ac-

id-etching inside a precision-machined mold which determine the dimensions of the coating containing the extraction phase. The new developed particles which called wettable hydrophilic-balanced (HLB) was used to make coating materials which doesn't require activation after autoclave. The recessed SPME acupuncture needle is ideal for in vivo tissue sampling as it is minimally invasive and robust with the protection from the recession. An automated MOI-MS interface was developed by using a homemade software controlled solvent delivery system. The combination of in vivo SPME with automated MOI-MS technique allowed users to obtain quantitative results within a minute.

The new design with standard chromatography-used three-port tee and tubes makes the interface can be easily manufactured and connected with different ESI interfaces tested on Shimadzu, SCIEX and Thermo MS instruments. The automation of the MOI-MS interface can not only save labor time, but also significantly improve the reproducibility of the SPME-MOI-MS method with the RSD% ≤ 7% (n=9). The MOI-MS interface provides a continuous and stable electrospray fluid flow to the MS without generating any bubble, a feature that we exploit to introduce the concept of multi-segment injection for the determination of multiple samples in a single MS run. For in vivo SPME, we demonstrate both the physical and analytical robustness of the in vivo SPME sampling needles. Various puncturing tests are performed, and microscopic images of the needles show the coating remains unaffected. The extraction efficiency of the new wettable HLB (wHLB) is also compared to conventional HLB and commercial C8-sulfonate mixed mode phases, but don't need organic solvent activation after autoclave. As a proof-of-concept testing, the matrix-match calibration curve of a chemotherapeutic agent, doxorubicin (DOX), was performed using homogenized lamb lung tissue using the SPME acupuncture needles. The end-point detection of DOX was performed by automated MOI-MS, which shows LODs of 3 mg/kg. The further demonstration of the new method and system will be performed with our collaborators at Roswell Park Comprehensive Cancer Center.

Keywords: microfluidic open interface, in vivo SPME, recessed acupuncture needle, mass spectrometry

In vivo SPME-MOI-MS



Recessed SPME acupuncture needle for in vivo tissue sampling and then use automated MOI-MS for rapid analysis

OP-052

Chloroformate Derivatization Combined with HS-SPME-GC/MS for Determination of SCFAs in Mice liver: A Proof of Concept

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It has become evident that the gut microbiome plays a crucial role in health and disease. Short-chain fatty acids (SCFAs), those with fewer than 6 carbon atoms, are produced in the colon by bacterial fermentation of dietary fibers and resistant starch. These SCFAs also present important roles in the highly interconnected gut-brain axis, such as modulating neurotransmission, affecting the levels of neurotrophic factors, and participating in serotonin biosynthesis. Additionally, SCFAs can cross the epithelial barrier and enter the circulation via the hepatic portal vein, which connects the gastrointestinal tract, spleen, and liver. In fact, in humans, it has been reported that portal vein concentrations of SCFAS are quite higher than those encountered in peripheral blood. Therefore, it can be inferred that the liver act as a major sink for gut-produced SCFAs, where they can be metabolized via β-oxidation, used for the synthesis of ketone bodies, or converted to acetyl coenzyme A. Because of their high polarity and volatility and low concentration in biofluids, the determination of SCFAs is not easy. Conventional methods employ polar stationary phases in gas chromatography for the determination of native SCFAs, which presents considerable drawbacks. For this reason, most strategies for SCFAS determination by GC/MS employ derivatization prior to analysis. However, some of these derivatization protocols can be quite cumbersome, such as requiring anhydrous extracts, which difficults matters when handling biological samples. In this sense, this work presents a comprehensive study of chloroformate derivatization prior to extraction of SCFAs by HS-SPME in mice liver. Once developed, SPME protocols are easy to implement; however, the development of a competent quantitative SPME protocol capable of rendering accurate results requires a deep understanding of the fundamentals governing the entire process, which includes not only the chemistry of the coating but also the inherent composition of the matrix being studied. Therefore, herein, we not only optimize but also discuss in detail the effect of factors such as derivatization reagent volumes, fiber coating type, HS volume, liver homogenate pre-treatment, and

extraction parameters. In addition to the importance of greener sample preparation workflows, we optimize the usage of solvents during the derivatization step, in order not only to decrease organic solvent waste but also to minimize the displacement effects in the fiber coating due to the high concentration of solvents in the HS. Moreover, we discuss different quantitation strategies and matrix effects. The optimized protocol combined with GC/MS(-SIM) provided sub μM limits of quantitation for all the main SCFAs studied in mice liver (acetic, propionic, butyric, and valeric). The results obtained with the present method were also compared to those obtained by LC-MS/MS.

Acknowledgements: This research was funded by the European Union under Horizon MSCA Postdoctoral Fellowship (project 101064457), the Ministry of Science and Innovation of Spain (MICINN) and the European Regional Development Fund FEDER, grant number PID2021-122490NB-I00.

Keywords: Short chain fatty acids, solid phase microextraction, derivatization, gas chromatography, metabolomics, liver.

OP-053

Investigation of Binding Constants of Some Small Molecules to Deoxyribonucleic Acid in Solution Medium

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It is of great importance to experimentally determine the binding constant of any small molecule to a target molecule of biological importance. In particular, it is an essential experimental parameter in various studies such as drug-dsDNA interaction studies, estimation of drug efficacy or pharmacokinetic drug interaction. Quantitative aspects of target dsDNA and dsDNA binding small molecule interaction can be studied using a variety of methods using thermodynamic or kinetic approaches. In equilibrium reactions of the interaction, the binding constant (or affinity) of the dsDNA-small molecule reaction is expressed as: K = [AB]/[A][B], where A represents free dsDNA sites, B is a free small molecule and AB is the dsDNA-small molecule complex. However, reproducible results cannot be obtained with this equation. This is because the binding of the small molecule to dsDNA is not from a single site, but rather dsDNA has two or more binding sites with different intrinsic affinity. From the application of the law of mass action to the dsDNA-small molecule reaction, a variety of methods have been developed which facilitate experimental calculations of binding constants for the interaction between dsDNA-binding small molecule and target dsDNA. These include spectrophotometry, fluorescence quenching, thermal denaturation, and voltammetry. In this presentation, these methods and how to calculate the coupling constant using them will be discussed.

Keywords: dsDNA binding, binding constant, spectrophotometry, fluorescence quenching, voltammetry

OP-054

On the Transport of an Anticancer Drug, Leflunomide in Human Blood Circulation as Studied by Multi-Spectroscopic and Computational Investigations

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Abstract

Leflunomide (LEF) is a potent anticancer agent, which has manifested appreciable clinical success to treat various cancers such as metastatic breast cancer, melanoma and gliomastoma. In this study, we aimed to characterize the biomolecular recognition of LEF with the major transport protein in the blood plasma, human serum albumin (HSA) using fluorescence, absorption and voltammetric techniques as well as computational analyses. The fluorescence, absorption and voltammetric spectral results endorsed the complexation between LEF and HSA. The Stern-Volmer constant values and UV-vis absorption spectral results revealed the quenching of HSA fluorescence as static quenching. Value of binding constant (K_a = 2.76–4.77 × 10^4 M^{-1}) for LEF–HSA interaction defined the binding strength between LEF and HSA as intermediate. Thermodynamic (entropy change, ΔS= + 19.91 J mol^{-1} K^{-1} and enthalpy change, ΔH = – 20.09 kJ mol^{-1}) as well as molecular docking data predicted the involvement of hydrogen bonds, hydrophobic and van der Waals forces. Although microenvironmental perturbation around HSA fluorophores were noticed upon LEF binding to the protein, it significantly defended the protein's temperature-induced destabilization. Competitive ligand displacement as well as molecular docking findings revealed the binding preference of LEF to Sudlow's Site I (subdomain IIA)

of HSA. The stability of the LEF–HSA complex was hinted by the molecular dynamics simulation assessment.

Keywords: Leflunomide; human serum albumin; drug–protein interaction; fluorescence quenching; computational analyses.

I.Introduction

Cancer continues to be one of the biggest risks to life worldwide despite the impressive advancement found in the expansion of novel treatments recently. Conventional cancer treatments (chemotherapy, radiation, and surgery) can effectively eradicate cancer cells, but severely harm healthy tissues and have unfavorable side effects. Targeted therapies with bioactive compounds have drawn significant attention from researchers looking to overcome the limitations of traditional therapies due to their anticancer potency, lesser side effects and toxicity (Guo et al., 2019). LEF is a promising anticancer drug that has shown significant clinical effectiveness to treat various carcinomas (melanoma, gliomastoma and breast cancer). Following ingestion, LEF is completely converted to its active metabolite, teriflunomide that has encouraging pharmacological anti-tumor properties. LEF also acts as tyrosine kinase inhibitor by competing with oncogenic catalytic domains of tyrosine kinases for the same ATP-binding site and halting cell proliferations (Zhang & Chu, 2018). Drug–protein interactions are crucial because they directly affect the drug's pharmacokinetics and pharmacodynamics in human body. To better understand the drug's pharmacokinetics, quantitative investigation of drug–protein interaction is essential (Kragh-Hansen et al., 2002). HSA is a major transport protein because of its unique biochemical and pharmacological characteristics. HSA can interact reversibly with numerous ligands due to having two well-known binding sites, *i.e.,* Sudlow's sites I and II in its hydrophobic pockets (Sudlow et al., 1976). Therefore, we investigated the interaction mechanisms of the LEF–HSA complex regarding the quenching process, binding strength, intermolecular forces engaged, and localization of LEF binding in HSA.

II.Experimentals

Fluorescence spectra of 3 μM HAS with increasing LEF concentrations (0–60 μM at 5 μM intervals) were recorded upon excitation at 295 nm individually at 288, 298 and 308 K. Fluorescence data for LEF–HAS interaction were analyzed in accordance with the published methods (Kabir et al., 2023). The 3-D fluorescence signals of HAS (3 μM) and 5:1/10:1 LEF–HAS mixtures, and synchronous fluorescence signals of HAS (3 μM) with rising LEF (0–60 μM with 5 μM increments) were recorded following the published methods (Kabir et al., 2023). Absorption spectra of pure HAS (12 μM), pure LEF and LEF–HAS mixtures, involving rising LEF concentrations (0–60 μM with 5 μM increments) were also registered. Differential pulse voltammograms were scanned using 100 μM LEF with increasing HAS concentrations (0–5.0 μM). Fluorescence signals of HAS (3 μM) and 10:1 [LEF]:[HAS] mixture at advancing temperatures, 298–353 K (with 5 K increments) were monitored to assess thermostability of HAS upon LEF binding. Site-specific markers, *viz.*, warfarin and indomethacin (site I) and ketoprofen (site II) were utilized to detect the preferred LEF binding place in HAS. Titrations of HAS (3 μM) and 1:1 site-markers–HAS mixtures with rising LEF concentrations (0–60 μM with 5 μM increments) were performed. To identify the drug's binding pockets, interacting residues and participated interacting forces in LEF–HAS interaction, molecular docking analysis was performed. Assessment of molecular dynamics simulation was also made for determining the stability of LEF–HAS complex.

III.Results and discussion

Fluorescence quenching results of HSA without and with rising LEF concentrations were obtained at three different temperatures. A gradual reduction in the HSA fluorescence signals at emission peak was noticed upon increasing LEF concentrations to HSA mixtures. Such variations in the HSA fluorescence features can be caused due to the LEF–HSA complex formation. Decrease in the K_{SV} values (Table 1) upon increasing temperatures disclosed the involvement of a static quenching process in the LEF–HSA complexation. The complexation and participation of static quenching in the LEF–HSA interaction were further confirmed from alterations in the absorption spectrum of HSA upon LEF addition. Binding constant values ($K_{a\,=\,2.76}$–4.77 × 10^4 M^{-1}) demonstrated an intermediate binding affinity between LEF and HSA. Such binding strength seems suitable for the active transport of LEF through blood and therefore, release at the body's targeted locations. We found that hydrophobic interactions were involved in the LEF–HSA binding reaction, as indicated by the positive sign of ΔS (+ 19.91 J mol^{-1} K^{-1}), while a negative value of ΔH (– 20.09 kJ mol^{-1}) attested the role of H-bonds and van der Waals forces. Contributions of similar intermolecular forces were also predicted from our molecular docking analysis.

The differential pulse signal of LEF constantly declined by addition of rising HSA concentrations. This result revealed that addition of HSA in the LEF–HSA mixture governed to reduce the LEF peak current because of lowering in the equilibrium concentration of LEF owing to the LEF–HSA complexation. Synchronous fluorescence signals of HSA without and with rising LEF concentrations were obtained, and fluorescence signals of HSA progressively reduced by adding LEF. Fluorescence signal of Trp residue was markedly larger than Tyr residue, suggesting that Trp residue was mainly responsible for the action of LEF on the HSA fluorescence signal. A pattern of decreases in the 3-D fluorescence signals of HSA were observed upon addition of LEF. Such variations in the spectral features were suggestive of changes in the microenvironment near Trp and Tyr residues of HSA upon LEF binding. This finding was well-supported by intrinsic and synchronous fluorescence results. The LEF–HSA mixture produced a lesser decline in fluorescence intensity value compared to pure HSA within the investigated temperatures range, which indicat-

ed increased the protein's thermostability due to the LEF–HSA complexation.

In competitive displacement experiments, fluorescence signals of site markers–HSA (1:1) mixture that were acquired without and with increasing amounts of LEF. Fluorescence signals of site markers–HSA mixtures decreased gradually when LEF was added to solutions. The reduction was lesser in the WFN/IDM–HSA mixtures than those found with the HSA and KTN–HSA mixture, which clearly detected the favored of LEF binding to site I of HSA. These results further well-supported by our molecular docking analysis. Based on the molecular dynamics simulation inspections, it was observed that LEF and HSA structures were stable, and the complex retained compact with crucial interactions throughout the simulation periods.

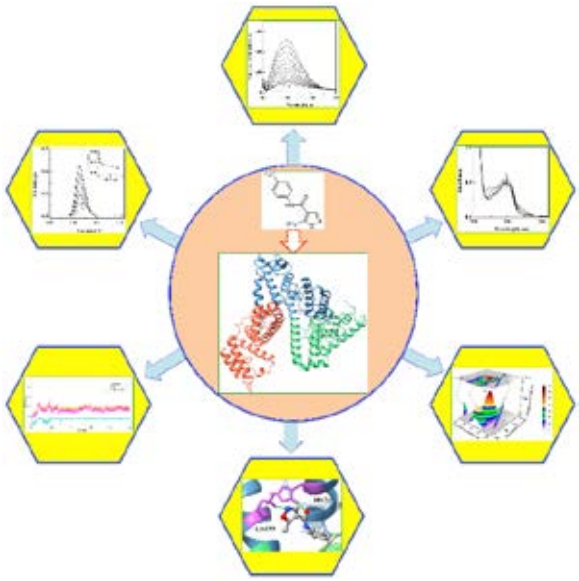

Fig. 1. Schematic views of LEF–HSA Interaction

Tab. 1. Binding characteristics and thermodynamics of LEF–HSA binding reaction.

T (K)	$K_{sv} \times 10^4$ (M^{-1})	$k_q \times 10^{12}$ (M^{-1} s^{-1})	$K_a \times 10^4$ (M^{-1})	ΔS (J mol^{-1} K^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})
288	5.45 ± 0.07	9.73	4.77 ± 0.05			– 25.82 ± 0.10
298	3.79 ± 0.06	6.68	3.74 ± 0.04	+ 19.91 ± 0.07	– 20.09 ± 0.09	– 26.02 ± 0.08
308	2.27 ± 0.04	4.05	2.76 ± 0.04			– 26.22 ± 0.11

IV.Conclusion

The complex formation between LEF and HSA was confirmed by wet-lab procedures and computational approaches. Hydrophobic interactions, H-bonds, and van der Waals interactions were predicted to stabilize the complex. With addition of LEF, microenvironmental disruption around HSA fluorophores was seen, but the protein's thermostability was considerably improved. Although, site I was considered to be the more beneficial location, it was discovered that HSA sites I and II were the location of LEF binding. These results are important for understanding the pharmacokinetics and mechanism of LEF, which may be helpful in the future clinical research of LEF as the drug's binding to transport proteins in the human circulation is crucial.

Acknowledgements: The Scientific and Technological Research Council of Türkiye (TÜBİTAK) provided Research Grant [2236-TÜBİTAK project number: 121C051] for this work.

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OP-055

Carbon Based Electrochemical Immunosensor for Malignant Tumors Marker Anti-SOX2 Detection

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SOX2 is a transcription factor comprised in the maintenance of embryonic stem cell pluripotency and multiple progressive processes containing lung branching morphogenesis. In tumor gene expression studies, analyses of genes that are active in progression and differentiation have shown that SOX2 is overexpressed in poorly differentiated cancer subtypes. SOX2 is amplified and

overexpressed in various malignant tumors such as lung, prostate, breast, colon, glioblastoma, ovarian, cervical, and pancreatic cancer (1). An immunosensor (affinity biosensor) is based on interactions between an antigen and a specific antibody immobilized on a transducer surface. Immunosensors possess high selectivity and sensitivity due to the specific interaction between antibodies and corresponding antigens. For this reason, it makes a suitable bioplatform for several applications, especially in the medical and bioanalysis fields (2).

This work describes a simple, rapid, and sensitive electrochemical immunosensor for determining anti-SOX2 antibodies. Multiwalled carbon nanotubes modified electrodes(DRPMWCNT) were used in this study. First, the DRPMWCNT surface was activated in a freshly prepared 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxy succinimide (EDC/sulfo-NHS) solution in humid room conditions. Then, SOX2 protein at different concentrations was dropped onto the surface and incubated. Next, the surface was blocked with 1% BSA for 30 minutes to prevent the interaction of the antibodies with the proteins of different surface configurations. In the last step, anti-SOX2 antibody was dropped onto the surface and incubated. The required optimization parameters were performed to select the best value. The performances of the immunosensor were evaluated by drawing a calibration curve.

Keywords: Biosensor, cancer, electrochemical

OP-057

Electrochemical Design of Two New Sensors Based on Graphene Nanoparticles and Polymer Resin -Modified Carbon Paste Electrodes for the Determination of Tenofovir and Entecavir

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Entecavir and Tenofovir, nucleos(t)ide analogs (NUCs), the combination is recommended first-line therapy in multidrug-resistant hepatitis B patients due to good tolerance, lower side-effect profile, and high genetic barrier. This study reports the fabrication of a novel polymer resin and graphene-modified carbon paste electrodes for the simultaneous determination of the anti-HBV drugs entecavir (ETV) and tenofovir (TEV) in bulk and dosage form by differential pulse voltammetry (DPV). The electrodes were specified through cyclic voltammetry, and scanning electron microscopy (SEM), and the analytical parameters were optimized. Both drugs displayed clear oxidation peaks on the polymer resin and graphene-modified carbon paste electrodes in the Britton-Robinson buffer. The proposed method was successfully applied to the simultaneous determination of ETV and TEV in commercial tablet dosage forms, exhibiting good accuracy and precision. Moreover, the addition of many excipients in the simultaneous analyzes of TEN and ENT confirmed the anti-interference properties of the fabricated sensors, as they did not affect the electrochemical behavior of the substances.

Keywords: Tenofovir, entecavir, voltammetry

OP-058

Production of a Hybrid Structure Consisting of Polycaprolactone Electrospun Nanofiber and Poly(2-Hydroxyethyl Methacrylate)-Based Cryogel for 5-Fluorouracil Release

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Cryogels are a subclass of hydrogels made up of networks of polymer chains that have a high capacity to absorb water while maintaining structural integrity. Hence, cryogels offer a hydrophilic surface which enhances the biological response of the cells. On the other hand, electrospun nanofibers are materials exhibiting higher mechanical strength compared to hydrogels and cryogels. Antineoplastic drug 5-fluorouracil (5-FU) is utilized to treat solid tumors that cause carcinomas in the gastrointestinal tract, breast, liver, brain, and other organs through chemotherapy. Poly(hydroxyethyl methacrylate) is one of the most widely used polymers in various biomedical applications such as drug delivery systems (DDSs), contact lenses tissue engineering, and so on because of its advantageous traits like high water content, high tissue- and blood-compatibility, and low toxicity. The objective of this study is to develop DDSs based on the electrospinning of polycaprolactone (PCL) and copolymerization of 2-hydroxyethyl methacrylate (HEMA) and vinyl imidazole (VIM). The advantages of electrospun nanofibers and cryogels were combined in this DDS as a hybrid structure to increase the mechanical properties of cryogels and enhance the biocompatibility and hydrophilicity of PCL

nanofibers. VIM is used as a copper ion chelating ligand to supply metal-ion mediated drug loading. The physical and chemical properties of hybrid DDSs were investigated using various characterization methods including gelation yield and swelling studies, field emission scanning electron microscopy, Fourier-transform infrared spectroscopy, water contact angle measurements, and so on. In vitro release studies were carried out to analyze the effects of medium pH and temperature, and drug content on release rate. The cryogel layer of the hybrid DDS has a macroporous structure while the nanofiber layer has relatively small pores. In the release profiles of the electrospun nanofiber/cryogel hybrid DDS, a biphasic release was observed, first a burst release followed by a slower and sustained release. The cumulative release rate of 5-FU from hybrid DDS was higher at more acidic conditions. Since the release rate of 5-FU changed by changing the medium pH because the Cu(II) ion acts as a Lewis acid, and the drug molecule, i.e. 5-FU acts as a Lewis base resulting in pH-responsive release for the hybrid DDS.

Acknowledgements: This study was supported by HORIZON EUROPE (Project acronym: REGENEU, Grant Agreement ID 101079123).

Keywords: Cryogelation, drug delivery systems, electrospinning, nanofibers, in vitro release, 5-fluorouracil

OP-059

A Label-Free Electrochemical Magneto Immunosensor for Celiac Disease

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Abstract

A label-free electrochemical magneto immunosensor was designed for ultrasensitive detection of anti-transglutaminase antibody in human real samples. The immobilization process was performed on the iron oxide magnetic particles. In this way, by the advantage of large surface of iron oxide particles, the antigens were immobilized on this polymer modified magnetic particles. To obtain a good performance of the biosensor, the conditions affected the biosensor response was optimized. Under optimal conditions, the immunosensor was responsive to anti-transglutaminase antibody concentrations in a wide detection range and a low detection limit. The reliability of the biosensor in clinical analysis was proved by successful quantification of anti-transglutaminase antibody levels in serum samples. Moreover, the precision, selectivity and storage-stability of the sensor were evaluated, and the results of this studies verified the acceptability of the proposed assay.

Keywords: Celiac disease, anti-transglutaminase antibody, impedimetric detection.

I. Introduction

Celiac disease is a gluten-dependent autoimmune disorder, which damages the small intestine. This disease is triggered and sustained by the ingestion of gluten in genetically susceptible individuals. The ingestion of some peptides originated from wheat, barley, rye, oats, and hybrids of these grains causes intestinal and extraintestinal symptoms. Chronic diarrhea, exhaustion, and weight loss are usually observed symptoms and they are variable in individuals (Habtamu et al., 2015). Furthermore, some individuals may also exhibit extraintestinal signs, but silent cases are also quite prevalent (Giannetto et al., 2014; Scherf et al., 2016).

To diagnose the celiac disease, gastrointestinal endoscopy is usually utilized, and, in this method, tissue samples are collected for mucosal biopsies. The collection of mucosal biopsies illustrates the potential abnormalities. A relationship is found between celiac disease and human leucocyte antigen HLA-DQ2 and HLA-DQ8 (Scherf et al., 2016). The utilization of gluten-free diet provides the elimination of these antibodies from human blood and therefore, they are utilized as biomarkers of celiac disease (da Silva Neves et al., 2010). Other celiac disease detection techniques contain serum testing for gliadin-induced antibodies using an enzyme-linked immunosorbent assay. This technique is high-cost, and it requires specialized laboratory, experienced personnel, and long analysis duration. Therefore, easy, low-cost, and rapid systems are required toward celiac disease diagnosis (Habtamu et al., 2015).

In this study, an impedimetric immunosensing tool based on modified magnetic particles was developed for the determination of anti- transglutaminase antibody. The analytical determination of anti-tissue transglutaminase implemented on the carboxyl groups modified iron oxide magnetic particles.

II. Material and method

Ferric chloride hexahydrate, ferrous chloride tetrahydrate, N-Hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), potassium chloride, sodium di-hydrogen phosphate, di-sodium hydrogen phosphate, 2-(N-morpholino)ethanesulfonic acid (MES), bovine serum albumin, anti-transglutaminase antibody and transglutaminase antigen were purchased from Sigma-Aldrich. The buffer solutions (50 mM phosphate buffer, pH 7.4; 50 mM MES buffer, pH 6.0) were prepared with an ultrapure water from a Milli-pore

MilliQ purification system (18.2 MΩ cm).

All electrochemical measurements were performed in a traditional three-electrode cell including an indium tin oxide (ITO) electrode, a platinum wire and Ag/AgCl as a working, a counter, and a reference electrode, respectively. These experiments were performed in the presence of ferri-ferro redox indicator and a Gamry Potentiostat (Reference 1000, USA) was employed for electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) analyses. The used potentials for CVs were between – 0.5 V and 1 V. EIS spectra were recorded in the frequency range from 0.5 Hz to 50 kHz.

III. Results and discussion

The low-cost and flexible ITO electrodes were utilized to build the magnetosensor for determination of the anti-transglutaminase antibody. Schematic illustration of the fabrication process of the sensor is demonstrated in Fig. 1. First of all, modified magnetic nanoparticles were synthesized by the coprecipitation method under mild reaction conditions. Then, the synthesized particles were modified with conjugated polymer containing carboxyl groups. Before the biomolecule immobilization the end groups were activated with NHS and EDC chemicals. After that, transglutaminase antigens were immobilized onto the modified particles. The free ends were blocked with BSA and thus, the immunosensor was ready to analyze the anti-transglutaminase antibody.

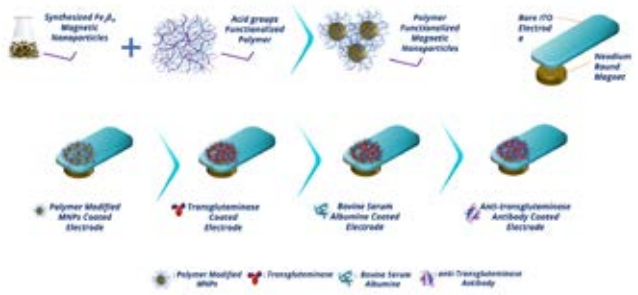

Figure 1. The modification protocol and measurement strategy of proposed magnetosensor.

The biosensor preparation procedure could be analyzed based on the electron transport of redox probe through the modified electrode. The electrochemical measurements of the immunosensor were performed using electrochemical impedance spectroscopy and cyclic voltammetry methods. EIS could offer further information on the impedance variations on the electrode surface during the modification procedure. In EIS, the semicircle diameter could illustrate the electron-transfer resistance, which represent the electron transfer kinetics of the ferri-ferro couple at the electrode interface. The magnetic attachments of polymer modified, antigen coated, BSA modified, and antigen coated magnetic particles on the electrode surface increased the electron-transport resistances. A pair of well-defined redox peaks observed after modifications and after magnetic attachments of polymer modified, antigen coated, BSA modified, and antigen coated magnetic particles on the electrode surface caused decreases in peak currents. The impedance experiments for prepared electrodes were in good agreement with the results by cyclic voltammetric analyses.

IV. Conclusion

A simple method was utilized to fabricate magnetic particles attached electrodes. The electrochemical and morphological characterizations of modified electrode were performed. With the use of ferri-ferro redox indicator, the fabricated magneto biosensor had a low detection limit and a wide linear range toward the detection of anti-transglutaminase antibody. Moreover, this biosensor preparation strategy could be utilized for extensive applications for different areas.

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OP-060

The Affinity of Dopamine and Serotonin to Serum Albumin: Capillary Electrophoresis-Frontal Analysis and In-Silico Molecular Docking Approaches

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Plasma protein-drug binding interactions have an important role in many biochemical and physiological processes since only

the free drug passes across the biological membrane and gets to its specific site of action. Albumin due to its high concentration in plasma controls not only drugs but also free concentrations of bioactive constituents taken to the body with food. Dopamine is also used to treat symptoms of low blood pressure, low cardiac output, and improve blood flow to the kidneys. In recent years, it has been accepted as a potential candidate for the treatment of neurological disorders such as Alzheimer's and Parkinson's. The stability of the interaction between dopamine and serum albumin provides important information for albumin to transport dopamine from the blood system to the cells. The determination of the interaction of dopamine and serotonin with serum albumin is important for therapy and similar drug design. In this study, the interaction of BSA with dopamine and serotonin was investigated for the first time in the literature by capillary electrophoresis-frontal analysis (CE-FA) method. The binding constants of dopamine and serotonin to BSA were calculated. The separations were conducted under physiological conditions with the CE-FA method. In the electropherogram, the plateau-shaped free dopamine and serotonin peak was well separated from the BSA-protein complex peak. Protein-bound concentrations of free dopamine and serotonin and these neurotransmitters were obtained by monitoring the height of their free plateaus. The number of binding sites (n) and binding constant (Kb) were determined using Scatchard graph. Moreover, an in-situ molecular docking method has been performed to predict the preferred orientation of serotonin and dopamine to albumin.

This study (Project No: 121Z888) was supported by TUBITAK.

Keywords: Albumin, binding constant, dopamine, frontal analysis, serotonine

OP-061

Molecularly Imprinted Electrochemical Sensor for the Selective and Sensitive Determination of Venetoclax in Pharmaceuticals and Human Serum

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In this study, a new electropolymerized molecularly imprinted polymer (MIP) film was synthesized on a glassy carbon electrode (GCE) by photopolymerization method using acrylamide (ACR) as a functional monomer and venetoclax (VEN) as a template molecule. Optimization of the MIP film was carried out using $[Fe(CN)_6]^{3/4-}$ as a redox probe. The removal and rebinding of the template molecule were investigated by differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) methods. The analytical performance of ACR@MIP/GCE was evaluated by comparing the electrochemical reaction of MIP with non-imprinted polymer (NIP). The limit of detection (LOD) and limit of determination (LOQ) of the VEN on ACR@MIP/GCE were determined as 0.016 pM and 0.055 pM, respectively, and the linearity range was found between 0.1 pM and 10 pM. The feasibility and validity of the developed sensor was proved by applying it to the artifical serum. The selectivity of the sensor was compared by examining molecules with similar structure to the binding of VEN. The developed ACR@MIP/GCE sensor exhibited high sensitivity and high selectivity for VEN and is the first method reported to be used in the electroanalysis of VEN.

Keywords: Venetoclax, molecularly imprinted polymer; electrochemical determination; photopolymerization

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PBA2023

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POSTER ABSTRACTS & PROCEEDINGS

PM-001

Electrochemical Nanosensor Development for the Determination of VEGFR Tyrosine Kinase Inhibitor Drug Axitinib

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Abstract

This study explains the electrochemical determination of the anticancer drug axitinib (AXI) using a multiwalled carbon nanotubes/iron(III) oxide nanoparticle–chitosan nanocomposite (MWCNTs/Fe_2O_3@CHIT) based nanosensor. The nanomaterial-modified surface of the glassy carbon electrode (GCE) was characterized by electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM). The electrochemical behavior of AXI on MWCNTs/Fe_2O_3@CHIT/GCE was investigated by various studies after showing the synergistic enhancing effect of the nanomaterial combination on the peak current of AXI. Sensitive determination of AXI was performed in the linear range between $6x10^{-9}$ and $1x10^{-6}$ M with the LOD value of $9.04x10^{-11}$ M using adsorptive stripping differential pulse voltammetry (AdSDPV) method. Pharmaceutical and biological sample application studies yielded good recovery% and RSD% values and demonstrated the MWCNTs/Fe_2O_3@CHIT/GCE's accuracy and applicability. The electron transfer mechanism and interactions were explained by density functional theory (DFT) calculations.

Keywords: Axitinib, electrochemistry, determination, nanosensor, drug analysis

I. Introduction

Axitinib (AXI) is a vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor used in the treatment of renal cell carcinoma, the most common kidney-associated cancer. It can be used as a combined therapy option with immune checkpoint inhibitors (McGregor et al., 2021). Based on the literature search, in addition to the electrochemical studies performed by our group (Cetinkaya, Kaya, et al., 2021; Cetinkaya, Topal, et al., 2021), there are also studies based on other methods for the determination of AXI (Albiol-Chiva et al., 2018; Likar et al., 2011; Shi et al., 2019)lapatinib and afatinib in plasma is reported. The sample pretreatment was a simple 1/5-dilution in a pure micellar solution, filtration and direct injection, without requiring extraction or purification steps. The three drugs were resolved from the matrix in 17 min, using an aqueous solution of 0.07 M sodium dodecyl sulfate – 6.0% 1-pentanol, buffered at pH 7 with 0.01 M phosphate salt as mobile phase, running under isocratic mode at 1 mL/min through a C18 column. The detection was performed by absorbance at 260 nm. An accurate mathematical relationship was established between the retention factor of each drug and the surfactant/organic solvent concentration in the mobile phase, achieved with a limited number of experiments, in order to optimize these factors. A binding behavior of the analytes face to the micelles was found out. The method was successfully validated by the guidelines of the European Medicines Agency in terms of: selectivity, linearity ($r^2 > 0.9995$. However, unlike other electrochemical studies, this work combines great conductivity, electrocatalytic activity, and stability features of selected nanomaterials (multiwalled carbon nanotubes/iron(III) oxide nanoparticle–chitosan nanocomposite) with affordability, good sensitivity, easy application, and environmentally friendliness features of electrochemical sensors. This study describes the development, characterization, and validation of the electrochemical nanosensor (MWCNTs/Fe_2O_3@CHIT/GCE) for AXI determination.

II. Material and method

AXI was prepared as 10^{-3} M stock solution in methanol. AUTOLAB potentiostat/galvanostat was used for electrochemical measurements. MWCNT was prepared as dispersion (1:1) in N,N-Dimethylformamide. Chitosan dispersion (0.1%, w/v) was prepared in 1% acetic acid solution. After that, 2 mg of Fe_2O_3 nanoparticles were mixed with chitosan dispersion. While preparing the nanosensor, the nanomaterial mixture was dropped onto the glassy carbon electrode (GCE) surface at 0.5 µL to be dried in a vacuum oven for 15 min. Electrochemical activation was applied to the MWCNTs/Fe_2O_3@CHIT/GCE with 15 cyclic voltammetry (CV) cycles in 0.1 M H_2SO_4 solution.

III. Results and discussion

Firstly, in order to show the changes in the surface characteristics of GCE after nanomaterial modification, electrochemical impedance spectroscopy (EIS) technique was used. Lower R_{ct} values demonstrate that electron transfer is faster and easier. The order of the obtained R_{ct} values is as follows: Bare GCE>Fe_2O_3@CHIT/GCE>MWCNTs/GCE>MWCNTs/Fe_2O_3@CHIT/GCE. These results confirmed the catalytic effect of the developed nanosensor. Scanning electron microscopy (SEM) and SEM energy dispersive spectrometry (SEM-EDX) analyses were performed for the morphological surface characterization. SEM results demonstrated the three-dimensional and large active

surface area of modified GCE. EDX analysis results verified the presence of selected nanomaterials on the surface. After that, pH and scan rate effects were observed on the AXI's electrochemical behavior. The optimum pH media was found as 0.1 M H_2SO_4 solution, and the scan rate study revealed that oxidation of AXI is adsorption-controlled. After optimization, MWCNTs/Fe_2O_3@CHIT amount, accumulation time, and potential were selected as 1.5 µL, 60 s, and 0 V, respectively. In the standard solution, the MWCNTs/Fe_2O_3@CHIT/GCE sensor showed a linear response between $6x10^{-9}$ and $1x10^{-6}$ M with the LOD and LOQ values of 90.4 pM and 301 pM, respectively. The same linear range was applied for the serum sample with LOD and LOQ values of 144 pM and 483 pM, respectively. Recovery studies in serum and tablet dosage form samples resulted in good recovery% and RSD% values, demonstrating the MWCNTs/Fe_2O_3@CHIT/GCE sensor's accuracy and applicability. Lastly, interference studies using ascorbic acid, dopamine, uric acid, Na^+, K^+, Mg^{2+}, SO_4^{2-}, NO_3^-, and Cl^- confirmed the sensor's interference-free performance. Additionally, density functional theory (DFT) calculations proved the enhanced charge transfer activity and the interactions between AXI and nanomaterials.

IV. Conclusion

This is the first study that evaluates the electrochemical determination of AXI using a nanomaterial-based sensor. Very high sensitivity with LOD values at the 10^{-11} M level was obtained for both standard and serum solutions thanks to the catalytic effect of MWCNTs/Fe_2O_3@CHIT. Electrochemical behavior evaluation and characterization studies were also performed. Pharmaceutical and biological sample application studies showed the potential for routine analysis of AXI. Consequently, this newly developed nanosensor is an advantageous option for AXI determination.

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PM-002

Simultaneous Determination of Hydroquinone and Catechol Using Highly Conductive Ketjen Black Screen–Printed Electrodes Treated With Electrical Discharge

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Hydroquinone (HQ) and catechol (CC) are environmentally hazardous dihydroxybenzene isomers, by virtue of their low degradability and high toxicity, which are released into aquatic environments during various industrial activities. The US Environmental Protection Agency and the European Union have considered these compounds as priority environmental pollutants as even small amounts of them could be seriously harmful to both the water environment and humans. Thus, the digging up of a rapid, selective, and sensitive method for their determination is of great importance. The similar structure and close redox potentials of HQ and CC render their simultaneous determination by conventional plain electrodes difficult. Therefore, the development of sensors that enable the simultaneous determination of HQ and CC at environmentally relevant concentrations is of immense importance.

Herein, we report on the development of a low–cost graphite screen–printed electrode (SPE) modified with Ketjen Black (KB, EC600JD), a highly conductive carbon black, and carbon nanomaterials generated by spark discharge [1-3] for the determination of HQ and CC. Specifically, Ketjen Black aqueous suspension was drop–cast onto a SPE, and the resulting modified surface was further treated by a direct KB/SPE-to-graphite pin electrical discharge. Sparked KB/SPEs were characterized

by scanning electron microscopy, Raman spectroscopy, cyclic voltammetry, and electrochemical impedance spectroscopy. Results demonstrated that the designed sensor exhibits outstanding electrocatalytic properties, very well-resolved oxidation peaks for HQ and CC, and enables their simultaneous determination in real world samples at the sub millimolar concentration range.

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Keywords: Electroanalysis, screen-printed sensors, green electrode modification process, water quality

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PM-003

SARS-CoV-2 Detection By A Novel QTF Based Immunosensor

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Abstract

A case of pneumonia of unknown origin was reported in Wuhan, China, in December 2019. Subsequent research found a new coronavirus, SARS-CoV-2, similar to previous outbreaks of coronavirus infection in humans. There has been a significant worldwide demand for technologies that quickly and precisely identify such an infectious virus to stop and manage its spread. It is very important to treat patients promptly and identify asymptomatic carriers immediately. In this study, a new, low-cost, portable, all-screen frequency analyzer is used for the first time. Simple, sensitive, and selective mass-sensitive detection of COVID-19 was performed with the modified QTF sensor. Quartz tuning fork (QTF)-based sensors are portable, fast, and can be integrated into point-of-care devices, making them accessible to a wider range of people and can deliver fast results, often within minutes. Modification of OTF surfaces is important for the success of the immobilization step. In addition, the effect of concentration and incubation time on the surface of functionalized QTFs on the sensor design was checked. The electrode surface was characterized with SEM and AFM techniques.

Keywords: COVID-19, QTF, mass-sensitive biosensor

I.Introduction

COVID-19 is a highly contagious respiratory disease caused by the SARS-CoV-2 virus. The virus was first identified in December 2019 in Wuhan, China, and has since spread worldwide, causing a global pandemic. The virus is primarily spread through respiratory droplets when an infected person coughs or sneezes. (Ciotti et al., 2020)

Diagnosis of COVID-19 involves a combination of clinical evaluation and laboratory testing. The most common diagnostic test for COVID-19 is PCR, which detects the genetic material of the virus in a sample of respiratory secretions. PCR testing is typically performed in a laboratory and can take from a few hours to a few days for results to come back. (Tahamtan et al., 2020)

Biosensors are analytical devices that can detect and measure certain biological or chemical analytes by converting a biological or chemical signal to an electrical or optical signal. In the context of COVID-19, biosensors have emerged as a powerful tool for rapid and accurate diagnosis of disease. Mass-sensitive biosensors, in particular, are a type of biosensor that can measure changes in mass, typically in the nanogram range, as a result of the binding of a target analyte to a sensing surface. One of the main advantages of mass-sensitive biosensors is their speed and ease of use. (Chadha et al., 2022)

In this study, SARS-CoV-2 protein was analyzed for the first time with a portable device by modifying the QTF electrode, which has the potential to be ideal for mass-sensitive biosensors. The biosensor, which emerged with the analyzer designed by our team, showed excellent analytical properties for SARS-CoV-2 protein determination.

II. Materials and Methods

All compounds used were of analytical purity. Bovine serum ort he (BSA) was purchased from 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS), Sigma Aldrich (USA). MBS355892 COVID-19 Nucleocapsid Coronavirus Recombinant Protein, MBS355887 anti-SARS-CoV-2 COVID-19

Nucleocapsid Humanized Coronavirus Monoclonal Antibody were obtained from MyBioSource. Solutions were prepared using Elga PURELAB flex purified water equipment, weighed with a Shimadzu Corporation ATX224 instrument, and measured using a Fisher Scientific pH meter. QTFs were acquired via Shoulder Crystal (China).

Fabricating of QTF-based biosensor

Mercapto or thiol (-SH) groups are often used to modify the surface of gold electrodes in biosensor design. Using mercapto for gold surface electrodes creates a stable and highly reproducible surface for measurements.

As seen in Figure 1, this study aims to successfully fix the SARS-CoV-2 antibody covalently using the EDC/NHS couple to the QTF/mercapto electrode. Therefore, the activated carboxyl groups of mercapto bind to the amino groups of the antibodies. A solution containing antibodies that specifically bind to the SARS-CoV-2 protein is then incubated with the electrode ort he optimized time. During this time, the antibodies absorb the gold surface via covalent bonding. After the formation of the antibody layer, the surface is typically blocked with a blocking agent such as BSA to prevent the non-specific binding of other proteins to the surface. Thus, the immunosensor design was completed. When the biosensor is exposed to a sample containing the SARS-CoV-2 protein, the protein will specifically bind to the immobilized antibodies on the biosensor's surface. The binding event is read as the frequency change observed depending on the mass on the electrode surface, and the presence of SARS-CoV-2 protein is detected.

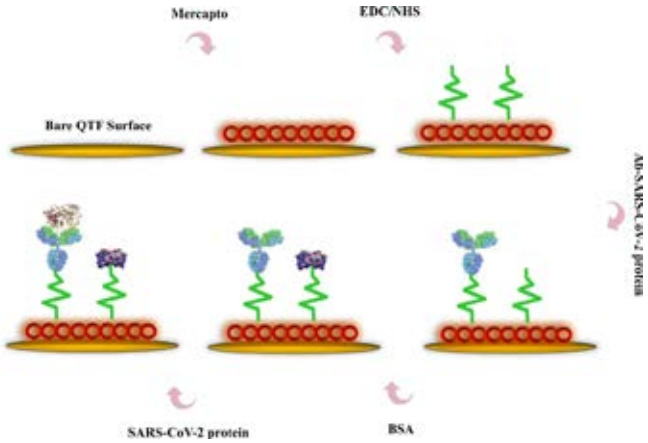

Figure 1. Schematic illustration of the SARS-CoV-2 immünosensor preparation.

III. Results and Discussion

Quartz crystal microbalance (QCM) is the most preferred crystal type in mass-sensitive biosensors. The sensitive data obtained in this study support recent research into the usefulness of QTFs operating on a similar basis to QCM, and it is thought that these forks could replace QCM. The binding of the target analyte to the electrode surface causes a change in the mass of the tuning fork, which affects the QTF resonance frequency. This change in frequency was measured using a portable frequency analyzer developed by our team. This measurement result is converted into a graph and processed by a reading system. When the anti-SARS-CoV-2 antibody (Ab-SARS-CoV-2) and SARS-CoV-2 interacted, the detected frequency difference formed the calibration curve of the proposed QTF-based immune sensor. When the graphics in When Figure 2 is examined, it is seen that the developed portable analyzer can precisely measure the presence of SARS-CoV-2 protein at ng levels.

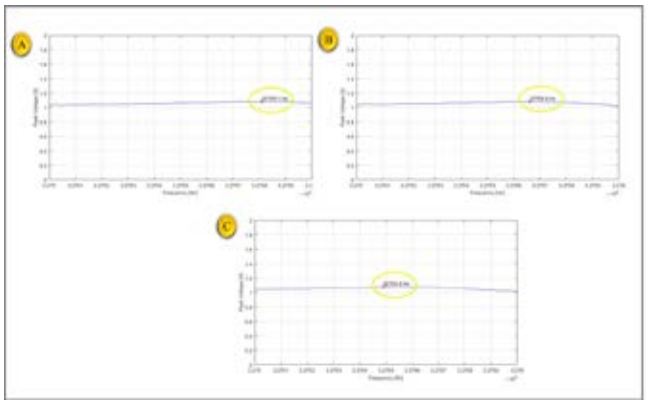

Figure 2. Frequency spectra of QTF (A) Bare, (B) Mercapto/ EDC-NHS /Ab-SARS-CoV-2 /BSA modified, (C) Mercapto/ EDC-NHS /Ab-SARS-CoV-2 /BSA/SARS-CoV-2.

In the optimization study of the biosensor, the effect of Ab-SARS-CoV-2 concentration was investigated. Three different concentration values, 50, 100, and 200 ng/mL, were tested. (Figure 3) Each step of immobilization is characterized by mass sensitive technique with a frequency analyzer. Standard curves were plotted with the difference in frequency shift obtained.

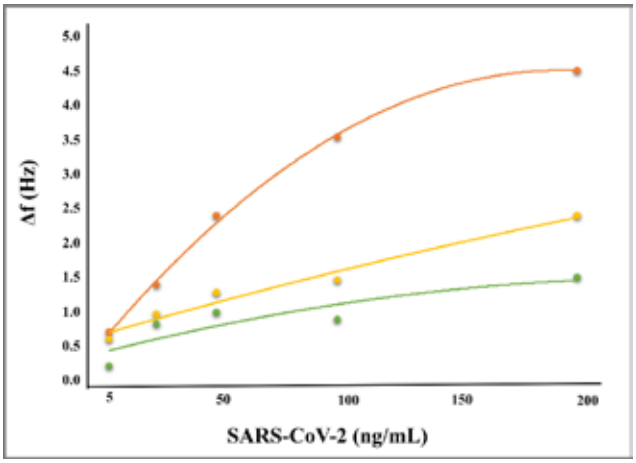

Figure 3. SARS-CoV-2 antibody concentration optimization study.(yellow; 50 ng/mL, green; 100 ng/mL, orange;200 ng/mL)

IV. Conclusion

The aim of this study is to develop a portable biosensor for early diagnosis of COVID-19 disease. Parameters were optimized

for the creation of the desired biosensor and characterization studies were carried out extensively. This biosensor is promising for clinical research due to its sensitivity, wide detection range, and low cost.

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PM-004

Construction of a Sensitive Electrochemical Magneto-Biosensor Based on Functionalized Magnetic Particles for Anti-Transglutaminase Antibody Detection

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Celiac disease is a complicated immune-mediated disorder triggered by dietary gluten in people with a genetic predisposition. The predicted worldwide prevalence of celiac disease is relatively high (1–1.5%) (Ramírez-Sánchez et al., 2020). The diagnosis of celiac disease is still based on histological variations in small intestinal mucosa (Habtamu et al., 2015). Other celiac disease detection techniques contain serum testing for gliadin-induced antibodies using an enzyme-linked immunosorbent assay (Pasinszki & Krebsz, 2018). This technique is high-cost, and it requires specialized laboratory, experienced personnel, and long analysis duration. Early diagnosis and treatment with a gluten-free diet decreases mortality and the prevalence of celiac disease. Therefore, easy, low-cost, and rapid systems are required toward celiac disease diagnosis (Scherf et al., 2016).

In this study, a novel platform based on 3-phosphonopropionic acid modified iron oxide magnetic particle was constructed and utilized for sensitive detection of anti-transglutaminase antibody detection. 3-phosphonopropionic acid was coated on the magnetic particles and they had a large surface area for transglutaminase antigens immobilization. The modified particles were placed on the electrode surface via magnetic forces. Upon binding of anti-transglutaminase antibodies to the biosensor, the electron transfer resistance was increased. A linear relationship between impedance and anti-transglutaminase antibody concentration was found and the developed biosensor had a low detection limit. Furthermore, modified magnetic particles attached electrode was successfully applied to the determination of anti-transglutaminase antibody in human serum with a good reproducibility. The developed system offers several advantages including low cost, low sample volume requirement, and rapid analysis relative to traditional methods, allowing the platform to be used as an alternative tool. In addition, this biosensor was a promising approach for the determination of biomarkers for celiac disease diagnosis.

Keywords: Celiac disease, anti-transglutaminase antibody, disposable electrode.

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PM-005

Impact of the Solid State on The Sterilization of Indomethacin

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In recent years, 70%–90% of potential drugs and 40% of marketed drugs have demonstrated a low solubility. Micronization is a widely spread approach to increase the dissolution rate. A subset of micronized products require sterilization, but published studies on the effects of sterilization on micronized products are currently lacking. Therefore, the effect of sterilization on the

micronized active pharmaceutical ingredient indomethacin was explored. The sterilization methods in scope were one photon-based method using gamma irradiation and one gas-based method with nitrogen dioxide gas. Indomethacin was micronized using two micronization techniques, cryomilling and spray drying. Different conditions were used for cryomilling where the number of grinding balls in the ball mill and the degree of filling were varied. The solid state of all samples was evaluated after micronization, and only the effectively micronized samples were selected for sterilization with gamma rays and nitrogen

dioxide. Gamma irradiation was performed with the active pharmaceutical ingredient stored at −80°C at a commonly used industry standard target dose of 25 kGy. Nitrogen dioxide sterilization took place at 21°C, a concentration of 10 mg/L, a relative humidity of 30% and using two NO_2 pulses. Before and after sterilization, all samples were analyzed, whereby the assay of indomethacin was examined as well as the peak purity and the formation of impurities. In comparison to the non-micronized reference, both sterilization methods demonstrate a significant decrease of content of micronized samples and an increase of the impurity profile. The non-micronized sample showed no significant difference after sterilization. It could be observed that micronized indomethacin samples demonstrate more degradation and are subsequently more susceptible to degradation upon sterilization with gamma rays and nitrogen dioxide gas, driving towards the need for assessment of the micronization impact combined with sterilization approach.

Keywords: Sterilization, micronization, indomethacin, HPLC

PM-007

The First Report for the Electrochemical Investigation of a New Tyrosine Kinase Inhibitor Vandetanib: Its Voltammetric Determination in Biological Samples with the Presence of Anionic Surfactant Using a Carbon Paste Electrode

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A sensitive, rapid, and inexpensive electrochemical method with bare carbon paste electrode (CPE) was developed for the first time for voltammetric determination of tyrosine kinase inhibitor vandetanib (VAN) is used as an anti-tumor drug in various tumor types, including medullary thyroid cancer. VAN showed an irreversible oxidation peak at 0.1 M HN03 at approximately +1.17 V (vs. Ag/AgCl, 3 M NaCl) by the cyclic voltammetry technique. The electrode reaction takes place by a controlled dual mechanism of diffusion and adsorption. The effects of anionic surfactant, sodium dodecyl sulfate (SDS), instrumental parameters, pH, and the nature of the supporting electrolyte are shown on the oxidation peak of VAN. It was shown that the sensitivity of stripping voltammetric measurements increased with the addition of 9×10^{-4} M SDS.

Using the suitable values of square-wave adsorptive stripping voltammetric parameters, the CPE showed good linearity in the concentration ranges of 1.05×10^{-7} – 1.6×10^{-5} M for VAN. The limit of detection and limit of quantification were determined to be 2.7×10^{-8} and 9.0×10^{-8} M for VAN, respectively. The developed electrochemical method has been successfully applied for the detection of VAN in the spiked model human urine and serum samples.

Keywords: Vandetanib, tyrosine kinase inhibitor, sodium dodecylsulfate, carbon paste electrode, biological sample

PM-008

SERS Based Exosome Detection Using Paper Substrates

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Exosomes are small extracellular vesicles that are released from cells to the whole body and provide intercellular communication. They carry a variety of cell-specific structures in which they are released. Examples of these are specific microRNAs, proteins, and metabolites. Exosomes can circulate in the body with the help of various fluids. Examples of these fluids are blood, saliva, breast milk, amniotic fluid, cerebrospinal fluid, and urine.

Disposable paper-based electrodes have also helped us in this study because of their excellent qualities, including portability, inexpensive fabrication, simplicity of use, and low sample volume requirements a brief analysis period. In addition, we added gold nanoparticles onto the paper electrodes to boost sensitivity. Rapid analysis of exosomes with proteins or other biomarkers requires complex methods and has a relatively high cost. In the present study, we describe the construction of a sensor via immunological recognition techniques for the detection of exosomes at low concentrations. The implementation of the biosensor consists of three steps: (i) functionalization of paper based electrodes with anti-CD9 antibodies, (ii) capturing exosomes by the electrode surface (iii) by interacting with the antibody-modified SERS tag. Calibration plot was created against different exosome concentrations.

Acknowledgement: This research was funded by Gazi University Scientific Research Project (FCD-2021-7119).

Keywords: Exosome, paper substrate, SERS

PM-009

Selective, and Sensitive Voltammetric Detection of Antibacterial Drug Linezolid Using Designing and Fabrication of Electrochemical Nanosensor TiO_2 Nanoparticles and MWCNT-COOH Modified Glassy Carbon Electrode in Environmental Samples

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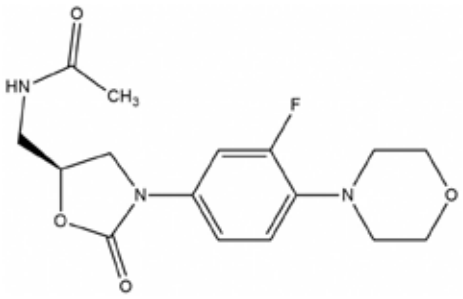
Antibiotics are widely used in many fields, such as medicine, animal husbandry, agriculture, and aquaculture. These uses of antibiotics have led to their presence in environmental samples, including water, soil, and sediment affected by wastewater, sewage sludge, or animal manure. Therefore, accurate, precise, fast, selective, and sensitive analytical methods are needed for the determination of antibiotics mixed with environmental samples. Up to now, there has been no study in the literature regarding the determination of linezolid with environmental samples. Linezolid (LNZ), the IUPAC name of N-((3-(3-fluoro-4-morpholinylphenyl)-2-oxo-5-oxazolidinyl)methyl)acetamide, is a synthetic antibacterial agent in the group of oxazolidinones class of antibiotics (Scheme 1). In this study, a sensitive, selective, and applicable electrochemical method based on the oxidation of linezolid active substance was developed using a bare glassy carbon electrode and modified TiO_2/MWCNT-COOH/GCE. The surface characterization of the developed TiO_2/MWCNT-COOH/GCE nanosensor was examined by scanning electron microscopy (SEM) and SEM-energy-dispersive spectrometry (SEM-EDX). The electrochemical properties of the substance were investigated with cyclic voltammetry, and linezolid determination was carried out by differential pulse and absorptive stripping differential pulse voltammetry technique. Under optimized conditions, the linearity range was determined as 1-20 µM and 0.2-2.0 µM with the bare electrode and TiO_2/MWCNT-COOH/GCE nanosensor, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were found for the bare GCE as 36 nM and 121 nM, for TiO_2/MWCNT-COOH/GCE nanosensor 0.69 nM and 2.31 nM, respectively. Interference studies were carried out with inorganic and organic substances that may affect LNZ determination using the proposed sensor. The developed nanosensor was applied for the determination of LNZ in soil, tap water, and natural spring water samples, and the accuracy of the proposed methods was proven in recovery studies with values of 99.90%, 100.94%, and 101.17%, respectively. Finally, determining the LNZ active ingredient in real samples was successfully performed with modified electrodes with high recovery, low relative error, and high selectivity.

Acknowledgements: This work was produced as a part of Nida Aydogdu's Ph.D. thesis.

Keywords: Linezolid, nanosensor, voltammetry, electrochemical, environmental samples

Scheme 1. Linezolid molecule structure



PM-010

Electrochemical Enzymatic Biosensor Based on a Novel Conducting Polymer and NH_2 Functionalized Quantum Dots for The Sensitive Detection of Catechol

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Conducting polymers has attracted many researchers' interest as alternating single and double-bond configurations in the polymer backbone chain allow conjugated polymers to conduct electricity up to a specific limit, and this enables them to possess interesting intrinsic properties beneficial for electronic applications. Among numerous electron-deficient core groups like derivatives of diketopyyrolopyyroles, quinoxalines, and perylenes, benzoxazoles and thienopyrroledione moieties are used to lower the LUMO levels with the available position for substituents for further tuning the electronic, optic and morphological properties. On the other hand, among numerous electron-rich core groups like carbazoles, thiophenes, and fluorenes, benzodithiophenes are considered a good donor candidate with their fused structures, good hole transport abilities, an available position for solubilizing the polymer, and further tuning of properties. Thienopyrroledione has unique imide bonds with available nitrogen atoms for substituents, making them a good candidate for acceptor moiety to be used in conjugated systems, and there is not enough literature for biosensor applications. Moreover, quantum dots offer new platforms in biosensor design. Taking advantage of quantum dots, such as large surface area, low cost, excellent optical and electrochemical properties, and the ability for attractive surface modification, in recent years, quantum dots have been regarded as one of the most promising materials in biosensor construction. In this work, a novel multi-purpose amperometric nanobiosensor based on tyrosinase enzyme for the determination of catechol was suggested using three moieties of benzoselenodiazole, thienopyrroledione, and benzodithiophene containing selenium-bearing conjugated polymer. This novel nanobiosensor was prepared from Tyrosinase enzyme immobilization in a novel selenium-bearing conducting polymer and NH_2 functionalized quantum dots matrices. Parameters affecting biosensing were optimized, such as NH_2 functionalized quantum dots amount, pPSe amount, tyrosinase amount, and glutaraldehyde percent. Under optimized conditions, catechol was determined in the range of 0.1-88 µM with a limit of the detection value of 0.023 µM and a limit of quantification value of 0.07 µM.

Keywords: Tyrosinase, catechol, electrochemical biosensor, conducting polymers, quantum dots

PM-011

Disposable Electrochemical Sensor for the Analysis of Cholesterol

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Cholesterol is an essential lipidic component of mammalian cell membranes, which plays important roles in organisms. Regardless of this importance, an inadequate level of cholesterol is associated with severe health problems. Thus, high levels of cholesterol in blood may cause life-threatening vascular and cardiac diseases, while low levels are related to the appearance of hyperthyroidism or anemia. Therefore, the determination of cholesterol level in blood is important to achieve the diagnosis of the serious diseases mentioned and, in addition, the evaluation of cholesterol content in food may be interesting to select an adequate diet for low cholesterol intake [1]. Different methodologies have been developed for the analysis of low quantities of this molecule, including the advantageous electrochemical methods, associated with high sensitivity and selectivity, as well as the low cost and portability of their instrumentation [2]. A large number of these sensors are based on the incorporation of enzymes which provide high selectivity and sensitivity in the determination of cholesterol, however, they usually present problems related to the progressive loss of enzymatic activity with use and the great influence that exert parameters such as temperature or pH on stability. To avoid these drawbacks, equally sensitive and selective non-enzymatic systems based on the modification of the working electrode with different nanomaterials have been used [2].

In this work, screen-printed carbon electrodes modified with graphene oxide (GPHOX/SPCE) to enhance the electrochemical active area have been used for the analysis of cholesterol following the oxidation response obtained by means of differential pulse voltammetry (Fig. 1a). The developed sensor was adequate for the determination of cholesterol in different samples, showing a linear range from 296 to 666 µg/L (Fig1. b), a reproducibility of 11.4 % and a capability of detection (CC_{β}) value of 296 µg/L, under optimized conditions.

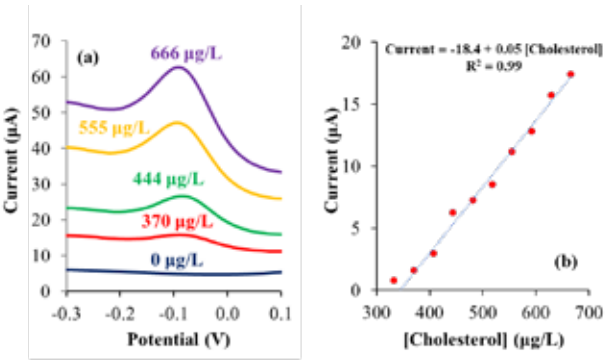

Fig. 1: (a) Differential pulse curves and b experimental points and calibration plot obtained under optimized experimental conditions using a GPHOX/SPCE (Briton Robinson, pH 9).

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PM-013

Development of Lateral Flow Immunosensor for Detection of Growth Hormone: An Ongoing Study

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Biosensors used in many fields having more attention especially in recent years. One of the paper-based tools used as biosensors, lateral flow assay (LFA) has the increasing as they comply with REASSURED (Real-time connectivity, Ease of specimen collection and environmental friendliness, Affordable, Sensitive, Specific, User friendly, Rapid/robust, Equipment-free, and Delivered) criteria (Land et al., 2019). LFAs are used in many fields. One of these areas is hormone determination. Amount of GH which can be described as the vital hormone, in the blood cause multiple functional changes in the body (Kopchick et al., 2020). Depending on the basal GH amounts in the blood and anomalies, different physiological diseases such as Dwarfism, Gigantism and Acromegaly can be seen (Lu et al., 2019). Consequently, it is important to detect potential irregularities in GH secretion due to the direct problems posed by both its deficiency and excess. Considering all these effects, monitoring the level of this hormone in the blood is very important in the early diagnosis and treatment process. Although there are different assay methods for GH determination by optical or magnetical methods (Belén González-Guerrero et al., 2016; Ramanaviciene et al., 2022), LFA design for GH determination has not yet been performed. In this study, a novel LFA tool will be developed for growth hormone (GH) determination. The optimization studies of 4 main components of lateral flow immunoassays were carried out in the designed sensor. Sample pad, glass fiber, and membrane surface optimization studies have been completed and the necessary conditions for qualitative and quantitative analyzes have been determined. Quantitative and qualitative determination limits for GH will be determined after optimization studies. As a result, the rapid diagnosis kit to be developed can be used for the determination of a GH in healthcare applications.

Acknowledgement: This study was supported by Necmettin Erbakan University Scientific Research Projects Coordination Unit (221?MER03004).

Keywords: Lateral flow assay, hormone detection, point of care, growth hormone

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PM-014

Voltammetric Determination of Cytotoxic Antineoplastic Drug Cladribine

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Cladribine (Fig) is a purine analog, antineoplastic agent used in the treatment of hairy cell leukemia and B-cell chronic lymphocytic leukemia [1,2]. After Cladribine is taken into the cell, it is metabolized to nucleotidecladribine triphosphate by the enzyme deoxycytidine kinase. Activated Cladribine participates in the DNA synthesis pathway where it impairs DNA synthesis and repair, causing DNA strand breaks [3,4,5].

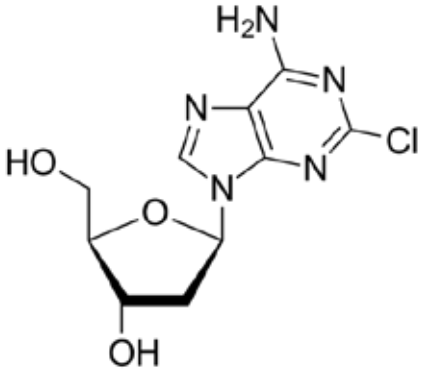

Fig. Chemical structure of Cladribine

In this study, voltammetric method were developed for the determination of Cladribine. First of all, pH scanning was performed for the drug. A calibration curve was obtained at this pH, which gave the highest current value. The quantitative determination of the drug from the pharmaceutical form was carried out using the developed method. In addition, the method has been fully validated.

Keywords: Cladribine, voltammetry, determination

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PM-015

A Systematic Protocol for GC-MS and LC-MS based Metabolomics and Lipidomics Analysis from Tissue Samples: By a Holistic Multi-Omics Optimization Approach based on Design of Experiment (DOE)

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In systems biology, establishing direct causal and functional links between genotype and phenotype requires a thorough analysis of intermediate molecular levels such as proteins, metabolites, and lipids [1, 2]. Comprehensive metabolomic, lipidomic, and proteomic analyzes because of the physicochemical diversities of biomolecules need efficient sample preparation steps, optimized analysis conditions, and the integration of orthogonal analytical platforms. Compared with high variation sample types such as plasma, urine, or stool, it is now recognized that tissue samples surrounded by tumor tissue play a major role in objectively classifying malignancies, predicting prognosis, and deciding personalized therapeutic strategies [3]. Here, we proposed a simple, rapid, and robust high-throughput analytical protocol for comprehensively analyzing the heterogeneous tissue samples surrounding the high-potential biomarkers such as metabolites, lipids, and proteins. This protocol presents a systematically DoE-based strategy for performing the most comprehensive analysis with integrated GC-MS and LC-qTOF-MS of many molecules having diverse physicochemical characteristics from tissue samples. Firstly, the extraction efficiency of solvents with different polarities on tissue samples was investigated, and it was determined that the methanol-water-dichloromethane (2:1:3, v/v/v) mixture was superior for the simultaneous extraction of metabolites, lipids, and proteins from tissue samples. Secondly, significant factors that affected the sample preparation step (derivatization protocol) of the GC-MS and LC-qTOF-MS-based metabolomics analyses, and then chromatographic parameters of LC-qTOF-MS-based metabolomics and lipidomics

analyses were determined and optimized. The incubation time and temperature of the silylation reagent (120 min at 70 °C), as well as the interaction of methoxyamine concentration (10 mg/mL)-extraction solvent (methanol-water; 2:1, v/v), and the reconstitution solvent (%0.1 FA in acetonitrile), were found to have a positive effect for the derivatization reaction and the LC-qTOF-MS-based metabolomic analysis sample preparation step, respectively. The percentage of eluent additive (formic acid, %) and column temperature were observed to be critical and significant parameters for high throughput metabolomics and lipidomics study using LC-qTOF-MS. Optimized conditions were verified by analyzing pooled brain tissue samples (QC), and a total of 675 lipids and 172 metabolites were identified from QC samples.

Keywords: Tissue, Design of Experiment (DoE), metabolomics, lipidomics, multi-omics, sample preparation

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PM-017

Development and Characterization of Mucoadhesive Buccal Film Formulations Containing Tamoxifen

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Abstract

Tamoxifen is a BCS Class II non-steroidal triphenylethylene derivative with low solubility and bioavailability. Our study aimed to develop a buccal film formulation to increase the systemic bioavailability of Tamoxifen. Preformulation studies were carried out with buccal film formulations containing different polymers and plasticizers at different concentrations. The obtained formulations were evaluated in terms of parameters such as film forming properties, removal from petri dish and film thickness, and final formulations were selected. Films were prepared using solvent casting method, and were evaluated with regard to drug content, thickness, weight variations, tensile strength, adhesion properties and percentage of in vitro drug release. It was concluded that formulations containing HEC were more favorable than others with regard to uniformity, flexibility, rate and percentage of drug release.

Keywords: Tamoxifen, buccal film, mucoadhesive buccal film

I. Introduction

Tamoxifen was found for emergency contraception in 1966, and as a result of studies carried out in 1973, it was released in the UK for the treatment of breast cancer. Tamoxifen is a non-steroidal triphenylethylene derivative that shows a complex spectrum as an estrogen antagonist and has effects similar to estrogen agonist in different tissues (1). It is a BCS Class II drug. It has low solubility in water and is therefore used in the form of the citrate salt. It has poor oral bioavailability due to precipitation as a free base in the acidic environment of the stomach and also due to extensive hepatic and intestinal first-pass metabolism (2,3).

Mucoadhesive buccal films are single or multiple layers of suitable materials. Mucoadhesive buccal films are more advantageous than other forms because of their high elasticity, low side effects, more accurate dosing than drop or syrup formulations, and a large surface area for drug absorption (4). These thin films accelerate the onset of action of the drug and contribute to the improvement of the efficacy and safety profile of some drugs (5).

The aim of the study is to develop a potentially safe and better bioavailability mucoadhesive buccal formulation of tamoxifen active ingredient, which has various difficulties such as low hydrophilicity, poor bioavailability and dose-related toxicity.

II. Material and method

II.I. Materials

Hydroxypropylmethylcellulose provided by DuPont (US), Hydroxyethylcellulose X, TAM Y. All other chemicals and reagents used were of pharmaceutical and analytical grade.

II.II. Method

II.II.I. Ultraviolet (UV) Spectrum

TAM was dissolved in 40:60/MeOH-artificial saliva medium (ASM), and the wavelength at which it formed the highest absorbance was determined at a wavelength of 200-400nm.

II.II.II. Differential scanning calorimetry (DSC) analyzes

Thermograms of the samples were taken between X °C at a heating rate of 10°C/min.

II.II.III. Fourier Transform Infrared (FTIR) Spectrum

The characteristic peaks were examined by taking the spectra of the polymers used in the production of the active substance and the film.

II.II.IV. Analytical method

Validation procedures were carried out to show that the analysis method is valid and reliable. The validated method was used in quantification studies.

II.II.V. Preparation of Film Formulations

For preformulation studies, formulations were carried out with polymers (HPMC, HEC, HPMC-HEC and chitosan) and plasticizers (PEG 400 and Glycerin) at different concentrations. The obtained formulations were evaluated in terms of parameters such as film forming properties, removal from petri dish and film thickness. Characterization studies were carried out by adding the active substance to the suitable formulations.

II.II.VI. Characterization of buccal films

Thickness of the films was measured at 6 different locations using calipers.

To control the weight uniformity, 6 separate sections of 1x1 cm were taken from various parts of the films and weighed.

pH 6.8 buffer was added on the 1x1cm cut film formulations and pH measurement was carried out after 2 hours.

The films were placed in a hot air oven at 50°C for 24 hours to calculate the percent moisture content (%MC).

Texture analysis (TA) was used to measure tensile properties. Thickness and width of the films were measured and stress and strain values were calculated based on these values.

Cow cheek was used in the studies carried out with the Texture Analyzer device in order to examine the adhesion properties of the films.

II.II.VII. Drug content

40:60/MeOH- ASM was added to the film formulations cut in 1x1cm size, after mixing for a while, they were diluted and 3 different determinations were made in UV spectrophotometer.

II.II.VIII. Dissolution Studies

The films were studied with a dissolution device by providing sink condition in 40:60/MeOH- ASM at 37°C. Samples were taken at predetermined time intervals and a dissolution profile was created.

II.II.X. Calculation of Kinetic Parameters

The DDSolver Program (DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles) was used to find out which kinetics the release from the TAM dosage form fits.

III. Results and discussion

III.I. UV Spectrum

In the TAM scan, it gave the highest absorbance at 237 nm.

III.II. DSC analyzes

When the DSC thermograms of TAM and polymers were examined, it was seen that there was no interaction between them.

III.III. FTIR Spectrum

There are no new peaks generated and no significant peak shifts are observed although there might be possible interaction between drug and film components.

III.IV. Analytical method

Since the determination coefficients of the active substance standard curves are high and the % RSD values are greater than 2, the calculated line equations were used in the quantification. The recovery values obtained by the studies were found to be more than 95% at different concentrations. In repeatability studies, the % relative deviation was found below % 2. It was observed that substances other than TAM did not give any peak in the working environment. The limit of quantification values for tamoxifen were found to be 1 and 3 µg/ml, respectively.

III.IV. Preparation of Film Formulations

Appropriate preparation method has been developed with preformulation studies. HPMC, HEC and HPMC-HEC mixture were dissolved in some distilled water and mixed in a magnetic stirrer. A plasticizer was added to the methanol-ethanol mixture, and the remaining part of the water followed by the alcohol phase was added to the swollen polymer. Formulations with chitosan were obtained by adding the acid to a mixture of chitosan-distilled water, mixing until gelation and then adding the alcohol phase mentioned above. Film formulations were dried at 50 °C for 24 hours.

The formulations obtained as a result of the preformulation studies were evaluated in terms of parameters such as film forming properties, removal from the petri dish and film thickness. The formulations given in the table 1 were found suitable for these parameters and films were formed by adding TAM to these formulations.

Tab. 1: Formulation of buccal films

	F1	F2	F3	F4
TAM (g)	*0,03*	*0,03*	*0,03*	*0,03*
HPMC (g)	*0,25*		*0,25*	
HEC (g)		*0,1*	*0,025*	
Chitosan (g)				*0,2*
PEG 400 (g)	*0,1*	*0,1*	*0,1*	
Glycerine (g)				*0,2*
Methanol (g)	*1*	*1*	*1*	*1*
Ethanol (g)	*1.5*	*1.5*	*1.5*	*1.5*
Distilled Water (g)	*7.5*	*7.5*	*7.5*	*7.5*

III.V. Characterization of buccal films

All formulations were evaluated for physicochemical characterization and results are tabulated in table 2.

The results obtained showed that the lightest films were the formulations prepared with chitosan (0.0049±0.0006 g) and the heaviest films were the formulations prepared with HPMC-HEC (0.0122±0.0010). The film formulation with the highest pH change was found to be F4. This situation was associated with the acid added to the formulation.

Tab 2: Formulation characteristics of buccal films

Formulation	Weight	SD	pH	SD	% Moisture	SD
F1	*0,0128*	*0,0012*	*6,98*	*0,005*	*2,499*	*0,710*
F2	*0,0046*	*0,0002*	*6,99*	*0,005*	*7,310*	*1,339*
F3	*0,0122*	*0,0010*	*7,00*	*0,005*	*2,701*	*1,047*
F4	*0,0049*	*0,0006*	*7,01*	*0,005*	*14,863*	*3,834*

The results obtained from the tensile strength test of the films showed that formulations containing HPMC had the highest tensile strength and the HPMC-HEC had the lowest elongation. However, the buccal films prepared by HEC showed the maximum elongation percent and the minimum tensile strength among the formulations. It was observed that the best mucoadhesive property belonged to the formulation containing HEC (F2), and the adhesion property decreased with the HPMC-HEC mixture.

Tab 3: The mechanical properties of the gel formulations measured with texture analyzer.

Formulation	Tensile Strength (Mpa)	Elongation at Break (%)	Muco Work mJ/cm2
F1	*6,46±0,96*	*19,71±2,34*	*0,016±0,02*
F2	*0,32±0,08*	*71,85±23,15*	*0,095±0,018*
F3	*0,66±0,30*	*8,66±2,12*	*0,045±0,009*
F4	*1,28±0,23*	*43,20±18,67*	*0,058±0,009*

III.VI. Drug content

The active substance determination results of the films other than chitosan were found to be over 90%. These findings were satisfying the pharmacopeia recommendations.

III.VII. Dissolution Studies

In the study, it was observed that after 2 hours, formulations containing 5mg TAM prepared with a mixture of HEC and HPMC-HEC released 96% and 98% of the active substance, respectively. It was determined that this rate was 82% in HPMC and 70% in the formulation prepared with chitosan.

III.VIII Calculation of Kinetic Parameters

After the drug release studies, kinetic models were found. Equations of kinetic models are listed in Table 4. Among the values selected as criteria; as the R2 adj approaches 1, as the AIC decreases and as the MSC increases, the fitness of the mathematical model to the release profiles increases. According to these values obtained from DDsolver Software, the most suitable mathematical model was found to be the Weibull model for F1 and F4 formulations, Korsmeyer-Peppas model for F2 and Higuchi model for F3.

Tab 4: Values we obtained from DDsolve

Mathematical Models	Compliance Criteria	Formulations			
		F1	F2	F3	F4
Zero Order	*r2adj*	*-1,058017*	*-1,822142*	*-0,009223*	*-0,368443*
	AIC	*87,522894*	*92,238736*	*91,270320*	*81,966479*
	MSC	*-2,039715*	*-3,044667*	*-0,932938*	*-1,303205*
First Order	*r2adj*	*0,448248*	*0,720230*	*0,615626*	*0,586630*
	AIC	*75,675295*	*71,437171*	*82,582449*	*71,192696*
	MSC	*-0,723315*	*-0,733382*	*0,032381*	*-0,106118*
Higuchi	*r2adj*	*0,359506*	*-0,038117*	***0,834893***	*0,671191*
	AIC	*77,017564*	*83,237945*	***74,977242***	*69,132907*
	MSC	*-0,872456*	*-2,044579*	***0,877404***	*0,122748*
Korsmeyer-Peppas	*r2adj*	*0,920194*	***0,995876***	*0,691693*	*0,912255*
	AIC	*59,071963*	***34,281400***	*81,395978*	*58,041730*
	MSC	*1,121500*	***3,395037***	*0,164211*	*1,355100*
Hixson-Crowell	*r2adj*	*0,234052*	*0,095480*	*0,036039*	*0,418789*
	AIC	*78,627442*	*83,722007*	*90,857360*	*74,259640*
	MSC	*-1,051331*	*-2,098364*	*-0,887053*	*-0,446890*
Weibull	*r2adj*	***0,964211***	*0,998129*	*0,481104*	***0,960636***
	AIC	***52,467114***	*27,779831*	*86,694085*	***51,440049***
	MSC	***1,855372***	*4,117433*	*-0,424467*	***2,088621***

IV. Conclusion

Mucoadhesive buccal films of TAM were prepared by solvent casting method using different materials including HPMC, HEC and chitosan. FTIR spectra and DSC thermograms showed good compatibility between TAM and other components of film formulation. In vitro drug release profiles, mechanical properties, and mucoadhesive characteristics of TAM films were investigated. Considering all these parameters, it was found that the optimised formulation was the F2 formulation.

Overall, this research can be an innovative and promising work for TAM delivery in breast cancer.

Acknowledgements: The authors thank … for providing tamoxifen citrat as a gift sample.

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PM-018

Chitosan Modified Filter Paper for Colorimetric Based Non-Invasive Detection of Glucose

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Biofluid samples, such as saliva and interstitial fluid, offer a less invasive alternative for glucose analysis and can be collected with minimal discomfort to the patient.

In the proposed method, filter paper acts as a substrate for sample collection and analysis. Glucose oxidase (GOX), an enzyme that specifically reacts with glucose, is immobilized on the filter paper. When the biofluid sample is applied to the paper, the glucose present in the sample interacts with GOX, leading to the generation of hydrogen peroxide (H_2O_2). To detect the presence of glucose, a colormetric reaction is employed. Tetramethylbenzidine (TMB) and horseradish peroxidase (HRP) are incorporated into the system. The H_2O_2 produced in the presence of glucose triggers the enzymatic reaction between TMB and HRP, resulting in the formation of a colored product. The intensity of the color is directly proportional to the glucose concentration in the biofluid sample.

To enhance the color intensity and sensitivity of the system, chitosan-modified paper is utilized. Chitosan, a biocompatible polymer with excellent adsorption properties, is applied to the filter paper surface. This modification facilitates the immobilization of GOX and enhances the glucose detection process, leading to improved color intensity and accuracy of glucose measurements.

In summary, the integration of GOX, TMB, HRP, colormetric analysis, and chitosan-modified paper presents a promising solution for non-invasive glucose determination from biofluids. This approach has the potential to revolutionize glucose monitoring, enabling frequent and convenient measurements for individuals managing diabetes or related metabolic disorders.

Keywords: Non-invasive, paper, glucose determination, citosan, colormetric

PM-019

Synthesis of Fluorescent Carbon Dots from Anethum Graveolens by Hydrothermal Carbonization

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Carbon quantum dots (CQD) are hemispherical and their size are below 10 nm. CQDs are seen as a new member of the family of fluorescent nanoparticles. They are consisting of carbon atoms with many functional groups on the surface and whose carbonization degree can be changed. Carbon dots are of great interest due to their optical and chemical properties and have a wide range of uses as electrocatalysis, photocatalyst, bio-imaging, chemical-sensor, bio-sensor, nanomedicine, biomolecule/drug release, and luminescent diodes (1). Carbon dots have different superior properties compared to other quantum dots. While quantum dots containing heavy metals such as cadmium and lead have toxic effects on both the environment and humans, carbon dots are not toxic because they do not contain these heavy metals (2). Due to green synthesis from natural sources, CQDs are reproducible, simple, low cost, and environmental friendliness, have superior chemical and photo stability. For this reason, CQDs have often been considered a potential candidate in biosensing, bioimaging, and other biologically relevant applications (3). Various chemical carbonic compounds and natural sources are used in the synthesis of carbon quantum dots. Recently, the use of natural resources has become widespread. Many plants, fruits, and different organic materials can be used as a natural carbon source.

In this study, a simple and green technique was developed to synthesize carbon dots from Anethum Graveolens (dill), an easily available green plant. Anethum Graveolens is readily available, inexpensive, and can be easily converted to carbon dots with a simple hydrothermal technique. These carbon dots showed good optical and structural properties. After washing the dill leaves with deionized water, they were dried in an oven and cut into small pieces. 5 mL deionized water and 10 mL H_3PO_4 were added to 250 mg dried leaves. The mixture was heated in a magnetic heater at 80°C for different times (30,60,90,120,150,180, and 240 minutes). The solution was then cooled to the room temperature. For the characterization of synthesized carbon dots. UV–visible absorption spectrophotometer and fluorescence spectrophotometer were used. Obtained results showed that dried dill was successfully transformed into fluorescent carbon dots by hydrothermal carbonization.

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Keywords: Carbon quantum dots, spectrofluorimetry, dill, fluorescence nanoparticles, nanotechnology

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PM-020

Investigation of Cholinesterase Inhibition, Antimicrobial Activity and Volatile Contents of Eryngium campestre

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Eryngium L. genus (Hyacinthaceae) was represented by 317 taxa worldwide (1). Neurodegenerative diseases, i.e. Alzheimer's (AD) pervasive in the elderly population over the age of 65, have turn out to be one of the serious health problems. According to the cholinergic hypothesis, inhibition of acetylcholine esterase is treatment approach for this serious disorder (2). The aim of the study was to investigate inhibitory activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) of the plant and also to identify antimicrobial activity, volatile contents for evaluating medicinal potential. AChE and BuChE inhibitory activities were determined by colorimetric Ellman's method with some modifications and the antimicrobial activity was studied using agar diffusion method. Volatile compounds of the plant were identified using solid phase microextraction and gas chromatography coupled to mass spectrometry (GC-FID/MS). As a results of the study, The IC50 values for inhibitory activities of AChE assay and BuChE assay of E. campestre have been found as 131.82 µg/mL and 134.89 µg/mL, respectively. Antimicrobial activities were observed on *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Mycobacterium smegmatis and Candida tropicalis* . The major volatile compounds identified in the plant, benzaldehyde, myrcene, caprylaldehyde, naphthalene, farnesene, humulene, caryophyllene oxide, carotol and germacrene-D were specified by GC-MS. Inhibitory activities of AChE and BuChE of the plants were found significiant. According to the results, the plant may be the attractive source for treatment of AD.

Keywords: Antimicrobial, cholinesterase, eryngium campestre, GC-FID/MS

PM-021

Synthesis, Characterization and Optimization of Novel Phenylalanine-Coated Copper Nanoclusters with High Fluorescence Intensity

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Metal nanoclusters are composed of a few to hundreds of metal atoms. They are less than 2 nm in size. Metal nanoclusters have unique properties such as water-solubility, low toxicity, photostability, easy synthesis procedures, quantum-size effect, high photoluminescence efficiency, and high stokes shift. Therefore, CuNCs are used for many purposes such as pharmaceutical analysis, food analysis, molecular and ion sensing, bioimaging, and chemical catalysis. The synthesis of CuNCs is more economical because copper sources are more abundant than gold and silver sources. There are various synthesis methods of metal nanoclusters such as electrochemical, chemical reduction, photoreduction, template-based, microemulsion technique, and microwave-assisted method. Amino acids, proteins, DNAs, and various polymers are used as coating agents, and substances such as ascorbic acid, hydrazine hydrate, and sodium borohydride are used as reducing agents in metal nanoclusters (1,2). Coating agents not only protect the surface, but also contribute to the functionalization of the surface.

In this experiment, ascorbic acid and phenylalanine were used as a reducing agent and functionalization/coating of CuNCs surface, respectively. For synthesis, phenylalanine and ascorbic acid amount, temperature, time, pH optimization, and excitation

wavelength optimizations were tried. FT-IR, XPS, TEM, zeta potential measurement, UV-VIS spectrophotometer, and spectrofluorimeter were used for characterization studies.

For the optimization studies, the amount of phenylalanine (10 mg/mL), amount of ascorbic acid (25 mg/mL), temperature (80 C), time (4 hours), pH (9) and excitation wavelength (345 nm) were optimized. According to the optimization study, the synthesis procedure was determined as follows; 5 mL of 10 mg/mL phenylalanine solution was added into 500 µL of 10 mM $CuNO_3$ solution, then 5 mL of 25 mg/mL ascorbic acid solution was added dropwise. The synthesis was completed by mixing the solution for 4 hours in a water bath at 80 °C.The sizes of CuNCs were found to be 1.5-3 nm by TEM scaning. The zeta potential of CuNCs was found to be -31.5 mV. When the FT-IR spectrum was examined, the -NH_2 group of phenylalanine at 3000 cm^{-1} and the characteristic peak of the L-amino acid at 2113 cm^{-1} were not observed in the spectrum of the synthesized CuNCs. In addition, the COO- peak in the 1560 cm^{-1} band is not observed in the spectrum of the CuNCs, it can be said that phenylalanine interacts with CuNCs.

Metal nanoclusters are frequently used in various fields due to their many extraordinary properties. The functionalization of the surface of metal nanoclusters is very important for their interaction with different materials. In this experiment, a new, inexpensive, suitable for green chemistry and rapid method was developed for the synthesis of new phenylalanine-coated CuNCs with high fluorescence intensity.

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Keywords: Copper nanocluster, phenylalanine, fluorescence nanomaterials

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PM-022

Investigation of Asphodelus Aestivus as Potential Antioxidant, Antityrosinase and Anticollagenase Agent on Skin Aging

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Oxidative stress, which occurs due to high reactive oxygen species (ROS) concentrations in cells and insufficient antioxidant activity, is associated with skin aging. Collagenase (MMPs) has the ability to break down the structural molecules of collagen fibers in the skin tissue (1). Tyrosinase enzyme is an important enzyme in hyperpigmentation problems such as skin spots caused by excessive melanin synthesis in the body. Therefore, agents inhibit TYR enzyme can be used to treat hyperpigmentation problems (2). Collagenase and tyrosinase inhibitions are one of the preferred treatment approaches in skin aging and dermatological disorders.

Asphodelus species contain various secondary compounds such as steroids, anthranoids, phenolics, flavonoids, triterpenes, anthraquinones, arylcoumarins and glycosides (3). Among the people, these species are used in the treatment of problems such as acne and abscess, acne, psoriasis, burns, alopecia, wound healing, eczema. The antimicrobial, antifungal and antioxidant effects of the species have been reported in various studies (4, 5). In this study, it was aimed to determine the antioxidant, antityrosinase and anticollagenase effect potential of *Asphodelus aestivus* and to investigate its use in skin aging. Antioxidant capacity of the species was determined by total phenolic and flavonoid content determination, FRAP measurement, CUPRAC determination and DPPH radical scavenging activity assay . As a result, it was determined that the methanol extract obtained from the species showed strong antioxidant activity. It has also been observed that it has a high inhibitory effect on collagenase and tyrosinase enzymes.

Acknowledgement: We would like to acknowledge the Turkish Scientific and Technical Research Council for supporting the study.

Keywords: Asphodelus aestivus, collagenase, oxidative stress, tyrosinase

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PM-023

Investigation of the Use of Asphodelus Fistulosus as a Dermocosmetic Agent Against Skin Aging

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Dermocosmetic products are defined as care products containing active ingredients obtained from natural sources and applied to the skin, and evaluated between the category of medicine and cosmetics. Products obtained from herbal sources are used as free radical scavenger, anti-inflammatory, anti-aging and skin protector in the dermocosmetic field. Moreover, these products help to reduce skin problems such as allergic, excessively dry or oily, dehydrated, wrinkle, blemishes and acne (1).

Asphodelus genus (Liliaceae) are popularly called "çiriş otu" and "yalancı çiriş" as public (2). *Asphodelus* species contain several secondary metobolites such as steroids, anthranoids, phenolics, flavonoids, triterpenes, anthraquinones, arylcoumarins and glycosides (3). *Asphodelus* species, which have important biological activities thanks to their rich content, are used of treatment in various dermatologic disorders and wound healing (2, 4).

The purpose of study is to determine of the antioxidant capacity (total phenolic and flavonoid content determination, FRAP measurement, CUPRAC determination and DPPH radical scavenging activity assay), antityrosinase and anticollagenase activities of Asphodelus fistulosus as a dermocosmetic agent against skin aging. The result of the antioxidant activity studies on the methanol extract from the species, it was observed that the species had a high effect. In addition, the species was determined to have strong anticollagenase and antityrosinase activity.

Acknowledgement: We would like to acknowledge the Turkish Scientific and Technical Research Council for supporting the study.

Keywords: Asphodelus, collagenase, antioxidant, tyrosinase

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PM-024

A New Quartz Tuning Fork-Based Immunosensor for Analysis of Citrullinated Vimentin

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The development of biosensors that can detect specific biomarkers has been an active area of research in recent years. Citrullinated vimentin (Cit-Vim) is a biomarker associated with

several autoimmune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [1]. In this study, we have developed for the first time a biosensor capable of detecting Cit-Vim using a quartz tuning fork (QTF) electrode. The QTF electrode was immobilized with a mercapto compound and anti-Cit-Vim antibodies that bind to Cit-Vim. When a sample containing Cit-Vim is introduced into the biosensor, it binds to the antibodies on the surface of the QTF electrode, causing a change in the resonance frequency of the QTF. This change in frequency is proportional to the Cit-Vim concentration in the sample measured by the biosensor [2,3]. Optimization and characterization studies of this developed biosensor were carried out. This biosensor exhibited a sensitive detection limit and wide linear range. The biosensor also showed good reproducibility and stability. Overall, the QTF biosensor has the potential to be a valuable tool for the diagnosis and monitoring of rheumatoid arthritis and systemic lupus erythematosus, as well as other autoimmune diseases associated with Cit-Vim.

Keywords: QTF electrode, citrullinated vimentin, autoimmune diseases, biosensor

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PM-025

Selective and Sensitive Determination of Tolvaptan by Developing Molecularly Imprinted Polymer-Based Electrochemical Sensor

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Tolvaptan (TOL) is a selective vasopressin-2-receptor (V2R) antagonist used to treat heart failure, liver cirrhosis, and antidiuretic hormone secretion syndrome. Also, this drug is a p-glycoprotein substrate that interacts drug-drug with digoxin in vivo [1]. In the present work, an electrochemical sensor was developed using the photopolymerization method, one of the molecular imprinted polymer (MIP) methods. The developed MIP-based electrochemical sensor was made on the glassy carbon electrode (GCE) surface as a thin film layer. TOL showed high sensitivity and selectivity towards the template molecule in the designed sensor. The developed sensor used 4-aminophenol as a monomer, ethylene glycol dimetacrylate (EGDMA) as crosslinker for MIP synthesis and characterization of MIP-based electrochemical sensor using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), scanning electron microscope (SEM) and energy distribution X-ray spectrometry (EDX) was made. For the quantitative determination of TOL, 5.0 mM $[Fe(CN)6]3-/4-$ measurement with a redox probe was performed in solution using the differential pulse voltammetry (DPV) technique. The analysis of TOL in standard solution, commercial serum sample, and tablet dosage forms was successfully applied. After the optimization experiments, the calibration range was between 10 pM and 100 pM. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated as 1.54 pM and 5.16 pM, respectively. The accuracy of the sensor was approved by the recovery study and the recovery values were calculated as 103.0% and 102.0% in the tablet dosage form and commercial serum samples, respectively. Moreover, the selectivity of the sensor was determined using common interference agents. Imprinting factor (IF) were calculated using substances with similar molecular structures, such as sorafenib, regorafenib, imatinib, dasatinib, and nilotinib. The proposed method was proven highly sensitive and selective compared to other reported analytical methods.

Keywords: Drug analysis, electrohemical sensor, molecular imprinted polymer, tolvaptan

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PM-026

A Label-Free Electrochemical AGR2 Immunosensor Prepare Using a Hand-Made Disposable Electrode

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Abstract

In this study, a label-free AGR2 immunosensor was prepared for the sensitive, low-cost, and rapid detection of AGR2. First, the hand-made electrodes consisting of a working electrode (WE), a reference (RE), and a counter electrode (CE) were fabricated by a screen-printing method. The WE surface was modified with gold nanoparticles (AuNP). Morphological, chemical, and electrochemical characterizations of the AuNP-modified hand-made electrode were performed. The label-free AGR2 immunosensor was prepared using AuNP-modified hand-made electrodes, and electrochemically characterized. Experimental parameters (antibody concentration, antibody, and antigen incubation time) of the label-free AGR2 immunosensor were optimized. Analytical characterizations (linear range, detection limit, reproducibility, selectivity test) were performed by the DPV method. AGR2 determination in blood serum was applied with the developed AGR2 immunosensor.

Keywords: Hand-made electrode, AGR2 immunosensor, cancer biomarker

I. Introduction

Clinical studies have shown that anterior gradient homolog 2 (AGR2) is directly associated with various human cancers, including the esophagus, pancreas, breast, prostate, and lung (Li et al. 2017, Fritzsche et al. 2006, Fritzsche et al. 2007). AGR2 mRNA is co-expressed with ER (estrogen receptor) in human breast cancer cell lines and human breast cancer tissue (Zhao et al., 2010). It has been reported that plasma AGR2 concentrations are very high in stage II and III ovarian cancer patients and can be used as a new biomarker in ovarian cancer (Edgell et al., 2010). Therefore, sensitive detection of the AGR2 biomarker is of great importance in the early diagnosis of cancers. In this study, label-free AGR2 immunosensors were fabricated using hand-made electrodes.

II. Material and method

First, the hand-made electrodes were fabricated by a screen-printing method using templates with a cutter printer. Carbon ink was used for WE and CE, and Ag/AgCl ink was used for RE. The WE surface was modified with AuNP by CV (Bilgi Kamaç et al, 2023). Morphological characterizations of the prepared Au-NP modified hand-made electrode were carried out by Scanning Electron Microscopy; chemical analyses were performed with Fourier Transform Infrared Spectroscopy and X-ray Photoelectron Spectroscopy; electrochemical characterizations were made with cyclic voltammetry (CV) and differential pulse voltammetry (DPV). To prepare the label-free AGR2 immunosensor, the electrodes were modified with 6-mercapto hexanoic acid, EDC-NHS, Anti-AGR2, BSA, and AGR2, respectively. Electrochemical characterizations of the prepared AGR2 immunosensor were performed by CV and DPV.

III. Results and discussion

The preparation steps of the AuNP-modified hand-made electrodes and the label-free AGR2 immunosensor are given in Figure 1. The morphological, chemical, and electrochemical characterization results of the AuNP-modified handmade electrode proved the successful fabrication of the handmade electrodes and the deposition of AuNP on the electrode surface. A label-free AGR2 immunosensor was prepared using the AuNP-modified hand-made electrode, and each preparation step of the AGR2 immunosensor was characterized by CV and DPV. The peak currents in CVs and DPVs were found to decrease at each step. The antibody concentration, antibody, and antigen incubation time of the label-free AGR2 immunosensor were optimized by DPV. The linear range, detection limit, and repeatability of the developed AGR2 immunosensor were determined. AGR2 determination in blood serum with the developed AGR2 immunosensor was performed with high recoveries.

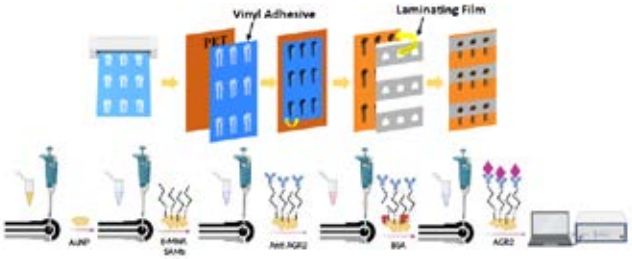

Fig. 1. The preparation steps of hand-made electrodes and label-free AGR2 immunosensor

IV. Conclusion

In conclusion, the disposable AuNP-modified hand-made electrodes and the label-free AGR2 immunosensor were successfully prepared. The developed AGR2 immunosensors

can be used for rapid and practical detection of AGR2 with high selectivity, sensitivity, and repeatability.

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PM-027

Preparation and Characterization of MXene-AuNP and MXene-COOH-AuNP Modified Screen-Printed Carbon Electrodes for Use in Biosensor

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Abstract

In this study, screen-printed carbon electrodes (SPCE) were modified with MXene/AuNP and MXene-COOH/AuNP, and then a label-free HE4 immunosensor was prepared as a biosensor application. First, the Al layers were separated from the MAX phase (Ti_3AlC_2). Next, -COOH groups were formed at the MXene ends. SPCEs were physically modified with MXene and MXene-COOH, then, AuNP was electrodeposited on SPCE/MXene and SPCE/MXene-COOH by cyclic voltammetry (CV). The fabrication steps of the SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP were characterized by scanning electron microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray diffraction analysis (XRD), differential pulse voltammetry (DPV) and CV. To prepare the label-free HE4 immunosensor, the SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP electrodes were modified with 6-mercapto hexanoic acid, EDC-NHS, Anti-HE4, BSA, and HE4, respectively. The prepared immunosensor was electrochemically characterized. Morphological, chemical, and electrochemical characterization results show that successfully prepared SPCE/MXene/AuNP, SPCE/MXene-COOH/AuNP electrodes and HE4 immunosensor.

Keywords: Screen-printed carbon electrode, MXene-COOH, gold nanoparticles, HE4 immunosensor

I. Introduction

MXene is a new two-dimensional material and consists of transition metal carbides and/or carbonitrides.

MXenes are prepared by etching element A (element A: 3A or 4A) from MAX phases (M: Ti, V, Nb, A: element 3A or 4A; X: C and/or N). They are formulated as $Mn^{+1}X_nT_x$ (T: -OH, -F and/or -O; n:1–3). MXene is used in the field of lithium-ion batteries, supercapacitors, catalysts, photocatalysis, field effect transistors, and biosensors (Satheeshkumar et al, 2016). MXene's features, such as large surface area, excellent electrical conductivity, compatibility with water and organic solvents, and easy fabrication at room temperature, make it increasingly widely used in sensors and biosensors. MXenes functionalize the surface when used with nanoparticles (gold, silver, etc.) (Medetalibeyoglu et al, 2020). Human epididymis 4 (HE4) is a biomarker with increased levels in ovarian, breast, lung, endometrial, stomach, and pancreatic cancers. Sensitive and accurate detection of HE4, an FDA-approved ovarian cancer biomarker, is important (Bilgi Kamaç et al, 2023). In this work, the SPCE/MXene-COOH/AuNP electrode was prepared, characterized, and used to prepare the label-free HE4 immunosensor.

II. Material and method

First, the Al layers were separated from the MAX phase (Ti_3AlC_2) (Mohammadniaei et al 2020). For this, 1 g of Ti3AlC2 powder (MAX phase) was mixed in 40% HF for 24 hours; the suspension was washed with distilled water, centrifuged, and the solid part was dried under vacuum at 50 °C (Liu vd., 2015; Mohammadniaei vd., 2020; Vasseghian vd., 2022). Next, -COOH

groups were formed at the MXene ends (Li et al, 2018). For this purpose, MXene powder and deionized water were mixed until a homogeneous mixture was obtained. Next, chloroacetic acid was added, incubated at 60°C for 3 hours, and freeze-dried (Li et al., 2018). The MXene suspension prepared in a DMF-H_2O mixture was used in the modification of SPCEs with MXene. Then, AuNP was electrodeposited on SPCE/MXene and SPCE/MXene-COOH (Bilgi Kamaç et al, 2023). The fabrication steps of the modified SPCE were characterized by SEM, FTIR, XRD, CV, and DPV. To prepare the label-free HE4 immunosensor, the modified electrodes were modified with 6-mercapto hexanoic acid, EDC-NHS, Anti-HE4, BSA, and HE4, respectively. Electrochemical characterizations of the prepared HE4 immunosensor were performed by CV and DPV.

III. Results and discussion

The preparation steps of SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP electrodes and the label-free HE4 immunosensors are given in Fig. 1. In the SPCE/MXene-AuNP and SPCE/MXene-COOH/AuNP electrodes, the peak currents in CV and DPV increased with each modification step. MXene, MXene-COOH and AuNP increased the electronic conductivity by creating a synergistic effect. A label-free HE4 immunosensors were prepared using SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP electrodes, and each preparation step of the HE4 immunosensors were characterized by CV and DPV. Due to decreased diffusion, peak currents in CVs and DPVs decreased at each immobilization step.

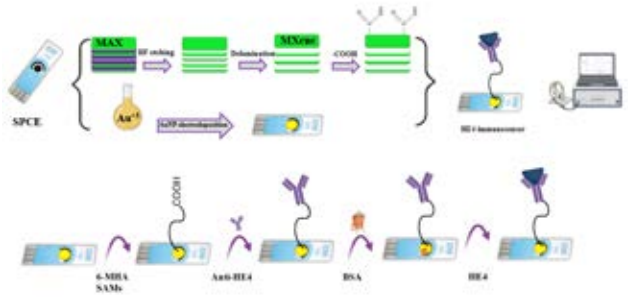

Fig. 1. Preparation steps of SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP electrodes and label-free HE4 immunosensors

IV. Conclusion

In conclusion, the SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP electrodes and the label-free HE4 immunosensors were successfully prepared. SPCE/MXene/AuNP and SPCE/MXene-COOH/AuNP electrodes can be used as transducers in sensors and biosensors.

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PM-028

A New Drug Candidate Molecule (3-((5-methylpyridin-2-yl)amino) isobenzofuran-1(3H)-one) Synthesis, Characterization and Antioxidant Activity

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In this work, the structure of a novel phthalide derivative, 3-((5-methylpyridin-2-yl) amino) isobenzofuran-1(3H)-one [1,2], was analyzed both experimentally and theoretically by X-ray

single crystal diffraction technique, IR spectroscopy, and quantum chemical computation. The X-ray diffraction analysis indicates that 3-((5-methylpyridin-2-yl) amino) isobenzofuran-1(3H)-one crystallizes in a monoclinic space group P21/n with unit-cell parameters a = 8.0712(7) Å, b = 6.6762(4) Å, c = 23.005(2) Å, β = 98.813(7)° and Z = 4. Additionally, DFT method at B3LYP level by using the hybrid functional with 6-311G (d, p) basis set have been used in the geometry optimizations and vibrational frequencies calculations of the title compound in ground state. The geometrical parameters obtained from XRD studies and the calculated values are in good agreement to each other. In addition, the electronic properties, such as HOMO and LUMO energies, and thermodynamic properties were calculated with the same method. The chemical reactivity estimation, the molecular electrostatic potential (MEP) surface map and PES scan of the related molecule were investigated with theoretical calculations at the B3LYP/6-31þG(d,p) and B3LYP/3-21G levels, respectively. The Folin-Ciocalteu's method have been used to determine the total phenolic (TP) content of the compound under study and it was found to be 0.14 (± 0.0) mg gallic acid equivalents (GAE)/g. Antioxidant activities were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity and ferric reducing anti-oxidant power assay (FRAP).

Keywords: Phthalide, crystal structure, DFT, antioxidant property

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PM-029

Quantitative Determination of Sunset Yellow (E-110) In Pharmaceutical Formulations by Micro Plate Spectrophotometry After Extraction with Hydrophobic Deep Eutectic Solvent

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Sunset Yellow (E110) is a coloring agent used in food, medicine and cosmetic products. E110 is one of the synthetic dyes commonly used in children's syrups and cold medicine. There are studies on the side effects and toxicological effects of excessive intake.

In this study, hydrophobic deep eutectic solvent based liquid phase microextraction method was developed for extraction of sunset yellow (SY) (E-110) in pharmaceutical formulations and quantitative determination of E110 was made by Biotek Synergy HT microplate reader. Extraction parameters such as pH, mol ratio of DES components, volume of deep eutectic solvent, sonication time and salt effect were optimized. Linear range in the optimum conditions was 50.0 –1000 ng mL^{-1} and the correlation coefficient was 0.995. The limit of detection (LOD) and the limit of quantification (LOQ) of this method were 5.0 and 15.2 ng mL^{-1}

The developed liquid phase microextraction method has advantages such as low cost, high extraction efficiency, and environmentally friendliness. This micro plate spectrophotometric method can be used for determination of SY in short time in routine analysis.

Keywords: Sunset yellow, hydrophobic DES, LPME

PM-030

Development of Highly Sensitive Fluorescence Aptasensor Based on Aptamer-Functionalized Magnetic Nanoparticles for 17β-Estradiol Detection

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17β-estradiol (E2) is a type of estrogen that plays a crucial role in regulating female characteristics and the reproductive system. The abnormal level of E2 links to human health problems such as menopause and amenorrhea, or breast and ovarian cancer. Consequentially, a simple, accurate, and sensitive method for E2 detection is in demand. This work aims to develop a fluorescence aptasensor using E2-specific aptamers functionalized with magnetic nanoparticles (MNPs) and fluorescence-labeled DNA probes. The MNPs were synthesized using the co-precipitation method and characterized by X-ray diffraction (XRD) analysis. To establish the E2-aptasenosor, the designed aptamer was linked with MNPs and hybridized with DNA probes. In the presence of E2, the hybridization was deconstructed by the competitive binding of E2 to the aptamer against the DNA probe, which

leads to high fluorescence intensity. The designed aptasensor can detect E2 in a linear detection range of 10^{-18}-10^{-10} M with a limit of detection of 2.92 aM under optimal conditions. Moreover, this assay can selectively detect E2 with slight cross-reactivity to other analogous-structure compounds: testosterone, genistein, bisphenol A, progesterone, and diethylstilbestrol. The results demonstrate that the developed assay has high sensitivity and selectivity, which could potentially be utilized to measure E2 for biomedical purposes.

Keywords: Aptamer, biosensor, estrogen, optical detection

PM-031

Production of Spinel Ferrite Nanoparticles for Latent Fingerprint Visualization

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In forensic investigations, a latent fingerprint is crucial evidence used to identify a criminal suspect. On the other hand, latent fingerprints on a crime scene are concealed from natural eyes and frequently contain low-quality or inadequate print information for comparison in the forensic database. As a result, a method for capturing the maximum amount of information from a finger imprint is critical for improving fingerprint analysis. This research aims to create and compare the efficacy of four spinel ferrites nanoparticles (SFNPs; $NiFe_2O_4$, $CuFe_2O_4$, $MgFe_2O_4$, and $MnFe_2O_4$) and magnetite nanoparticles (MNPs; Fe_3O_4) in detecting latent fingerprints. These metal nanoparticles were created using a sol-gel process, and their identities were determined using X-ray diffraction (XRD). The synthesized nanoparticles were used to visualize fingerprints on materials such as paper, plastic bags, glass, cardboard, and compact discs. The results demonstrated that the produced spinel nanoparticles could visualize fingerprints with good retention on a variety of surfaces. Among them, $NiFe_2O_4$ performed better in terms of seeing fingerprints with high contrast and anti-interference. As a result, $NiFe_2O_4$ is proposed as an alternative forensic magnetic power for fingerprint visualization applications.

Keywords: Biometric identification, Forensic, Magnetic nanoparticle, Nanomaterials, Sol-gel method

PM-032

New Approaches to Investigate the Antioxidant Activity and The Analysis of Radical Reactions Course 1HNMR Study

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There are many ways to analyze the antioxidant activity of chemical substances and drugs, i.e. spectrometric (DPPH, FRAP assays, EPR spectroscopy), electrochemical (cyclic voltammetry or amperometry) and chromatography (gas chromatography, HPLC) techniques [1]. Unfortunately, it is not always possible to use them and the results obtained may not be unequivocal. Therefore, it is important to look for new ways to search and analyze antioxidants.

The main aim of this study was to answer the following questions: (i) Is it possible to confirm the antioxidant activity of selected substances (not only well-known antioxidants) using nuclear magnetic resonance ^{1}HNMR spectroscopy?; (ii) Is it possible to determine using ^{1}HNMR which functional groups are involved in the radical reactions?; (iii) Which products are formed as a result of radical reactions?

MATERIAL-METHODS: The measurement protocol of 1HNMR was prepared on the basis of the work of López-Martínez et al. with the necessary modifications [2]. A DPPH solution (1mM) was used as a model free-radical. Five chemical compounds (5mM) were investigated (ascrbic acid, AA; gallic acid, GA; salicylic acid, SA; piperine, PI; caffeine, CA). (Methyl sulfoxide)-d6 was used as the solvent. ^{1}HNMR spectra were taken 30 minutes and 24 hours after the initiation of radical reaction between DPPH and chemical compound.

RESULTS: Based on changes in the number of protons and chemical shift of proton resonance signals it was possible to confirm that AA, GA and PI interact with DPPH, while for SA and CA no changes have been observed. The analysis also confirmed, which is very difficult with the use of other, singe technique, simultaneously the antioxidant activity of AA, GA and PI as well as the presence of the predictable products or radical reaction with DPPH.

CONCLUSIONS: Based on the ^{1}HNMR study it was possible not only to confirm that the selected substances react with DPPH, but also to identify the functional groups that

participate in the radical reactions and products formed as a result of radical reactions.

Acknowledgements: This research was funded by Grant PCN-2-012/K/2/F, PCN-2-030/K/2/F, PCN-1-022/K/2/F from the Medical University of Silesia, Poland.

Keywords: 1HNMR, antioxidants, DPPH, radical reactions

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PM-033

Spectroscopic Analysis of 9-amino-5-methyl-12(H)-chino[3,4-b][1,4]benzothiazine Chloride Interaction with Potential Proteins Carriers In Vitro Studies.

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Despite the existence of many drugs with anticancer activity and the progress of radiotherapy and surgical treatment, it is necessary to search for new substances with antiproliferative properties. Current treatments, particularly chemotherapy are associated with numerous side effects that can contribute to permanent damage to the human body. For this reason, it is very important to search for a new substances with potential anticancer activity, with the highest possible efficacy and the fewest possible side effects [1].

9-amino-5-methyl-12(H)-chino[3,4-b][1,4]benzothiazine chloride (AZMM-3) is a newly synthesized benzothiazine derivative with anticancer properties. AZMM-3 exhibits in vitro antiproliferative activity against HCT 116 and LLC cancer cell lines. The IC50 values were 5.1±1.1 $\mu g \cdot mL^{-1}$ and 8.9 ±2.2 $\mu g \cdot mL^{-1}$ for HCT 116 and LLC, respectively and the reference system was doxorubicin. Due to the significant harmfulness of anticancer drugs, it is necessary to determine the toxicity and therapeutic dose of the tested compound.

The aim of the study was the analysis of the interactions between 9-amino-5-methyl-12(H)-chino[3,4-b][1,4] benzothiazine chloride (AZMM-3) and potential proteins carriers - human serum albumin (HSA) and α1 acid glycoprotein (AGP). The assessment of structural changes in the secondary and tertiary structures of the studied proteins in the presence of AZMM-3 has been determined using spectroscopic techniques such as spectrofluorescence, UV-Vis spectroscopy and circular dichroism (CD) [2,3].

Using the Klotz equation, association constants (Ka $[M^{-1}]$) and the number of binding sites classes (n) in AZMM-3-HSA and AZMM-3-AGP complexes, at λex 275 nm and λex 295 excitation wavelength, were calculated. Spectral parameter A and second derivative of differential absorption spectra were used to evaluate environmental changes around aromatic amino acids residues in tertiary proteins structure while the analysis of CD spectra made it possible to evaluate changes in the secondary structure in the presence of AZMM-3.

AZMM-3 changes the secondary structure of HSA and AGP and binds to both proteins (a slightly higher affinity for AGP) molecules to a small extent, which can cause both a strong therapeutic and toxic effect (Ka ≈ 10^4 $[M^{-1}]$). Due to this phenomenon it is necessary to study other blood carrier proteins, such as human γ globulin.

Acknowledgments: This work was supported by Grants PCN-2-011/K/2/F, PCN-2-030/K/2/F, PCN-1-022/K/2/F, PCN-1-022/K/2/7 from the Medical University of Silesia, Poland.

Keywords: HSA, AGP, 9-amino-5-methyl-12(H)-chino[3,4-b][1,4]benzothiazine chloride, spectroscopy

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PM-034

Electrochemical DNA Biosensor Applications with Newly Developed Electrode Surfaces Based on Cu-ZrO_2 Nanoparticles

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In this study, electrochemical investigation of Cu-ZrO_2 modified pencil graphite electrode (PGE) for DNA biosensor applications was examined. Multiple production parameters were used in the laboratory to synthesize nanomaterials. Utilizing differential pulse voltammetry (DPV) methods based on the guanine signal, the effect of nanomaterial use on signal enrichment was determined. In order to get the best responses possible from each electrode modified with a nanomaterial, electrochemical activation techniques were used on the electrode surfaces at various pH levels. The activation process used on the Cu-ZrO_2 modified electrode was observed to have an impact on signal enrichment. In this study, synthetic probe and target DNA sequences were also used and it was investigated whether the developed biosensor could detect hybridization with high sensitivity.

Here, the performance of newly created electrode surfaces containing nanomaterial was evaluated according to their ability to detect DNA signal better than bare PGE electrode. As a result, the Cu-ZrO_2 nanomaterial modified biosensor exhibited %67 higher sensitivity performance in terms of guanine oxidation signal obtained at about 1.0V than the bare one.

Keywords: Electrochemical dna hybridization biosensors, Cu-ZrO_2 nanomaterials, hybridization

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PM-035

Electrochemical Characterization of Natural and Synthetic Molecules and Investigation of Their Interactions with DNA

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Determining the interaction between drugs and DNA is crucial for understanding the drug's mechanism of action and for the development of new drugs. DNA serves as an important target in drug development studies, and many molecules exert their effects by inhibiting or modifying DNA function upon interaction (Sirajuddin et al., 2013). However, the mechanism of interaction between many drugs and DNA remains unknown, making it essential to investigate this interaction to design potential drug molecules and predict their effects. Simple and rapid methods are needed to determine the mechanism of interaction between drug candidates and DNA. Electrochemical methods offer several advantages in this regard, including their ease of use, rapid analysis, and cost-effectiveness (Lu et al., 2021). Electrochemical techniques can provide valuable information such as the binding constant and binding mode of the molecule-DNA interaction.Therefore, they are valuable tools in the study of drug-DNA interactions, enabling researchers to gain insights into the molecular interactions and contributing to the design and development of novel drug molecules.

In this presentation, we will focus on the electrochemical characterization of both natural and synthetic drug molecules and drug candidates, along with their interactions with DNA. We will emphasize the significance of understanding the impact of these interactions on the overall drug effect. Experimental techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) will be employed to investigate the electrochemical properties of these molecules. Moreover, we will extensively explore the interaction between natural and synthetic molecules and DNA, investigating their behavior in both the solution phase and on the electrode surface. Specifically, we will examine the changes observed in the oxidation signal of guanine and/or adenine, particularly through DPV measurements. From these signal changes, we can calculate the toxicity percentage of the molecules on DNA, thereby providing insights into their potential toxic effects. Additionally, we will provide information regarding the potential mechanism of action of these molecules based on our findings.

This presentation aims to shed light on the electrochemical

characteristics of drug molecules, their interactions with DNA, and the implications of these interactions in terms of toxicity and potential mechanisms of action.

Keywords: Electrochemical sensors, DNA, drug candidates, interaction

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PM-036

Evaluation of the Electrochemical Characteristics of Triazolopyrimidinone Derivatives as Novel Drugs and Determination of Their Interactions with DNA

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Heterocyclic compounds, which consist of nitrogen-containing aryl substituents with five- and six-membered rings, have attracted significant interest due to their chemistry and various applications. Among them, triazolopyrimidinone is a class of fused pyrimidinone-triazole heterocyclic ring systems that have garnered significant attention in recent years (Istanbullu et. al., 2022). These compounds exhibit diverse biological activities, including anticancer, antimicrobial, anti-inflammatory, and antioxidant effects. The wide range of biological activities associated with triazolopyrimidinone makes them promising candidates for the development of novel drugs.

In this study, we synthesized and investigated the electrochemical properties of two drug candidates, which are derivatives of triazolopyrimidinone. We employed voltammetric techniques using pencil graphite electrodes to examine their behaviour. Experimental parameters such as pH, concentration, scan rate, and immobilization time were studied using Differential Pulse Voltammetry (DPV). We also determined the detection limit both derivatives and found them to be less than 10 µg/mL for both compounds. For CPD1, we observed the maximum peak currents at pH 5.6, while for CPD2, the maximum peak current was detected at pH 3.8. We also investigated the electrochemical interactions of the drug candidate molecules with single-stranded deoxyribonucleic acid (ssDNA) by examining the oxidation currents of DNA's guanine before and after the interaction. Furthermore, we evaluated the binding constant (K), toxicity (S%), and thermodynamic parameters, including Gibbs free energy (ΔG°), of the CPD1-ssDNA and CPD2-ssDNA complexes. Additionally, stability tests were conducted for each drug candidate under optimal storage conditions on different days to determine their shelf life. Based on our experimental data, we concluded that these molecules exhibit remarkable effects on DNA. This study provides valuable insights into the electrochemical characteristics of triazolopyrimidinone derivatives and their interactions with DNA, contributing to the development of novel drugs with promising therapeutic properties.

Keywords: Electrochemistry, triazolopyrimidinone, drug-DNA, drug molecule

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PM-037

Circular Dichroism Study of Albumin Nanoparticles

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Albumin is the protein found the most abundantly in plasma. One of its functions is to transport exo- and endogenous substances. According to mentioned before, albumin (human serum albumin, HSA) is used as a polymer to obtain various drug delivery systems, including nanoparticles. As a polymer of natural origin, HSA is biocompatible and biodegradable [1]. Circular dichroism (CD) is a method which allows to study secondary structure of proteins, including albumin. CD is most commonly used to study changes in secondary structure under the influence of factors, such as drugs however, for the study of drug delivery systems, it is not so common [1,2].

HSA nanoparticles with 5-fluorouracil (HSA 5FU NPs) were prepared based on the desolvation method with glutaraldehyde as a crosslinking factor. 5FU was used in two different concentrations (1 mg/mL and 5 mg/mL) to observe changes in the secondary structure of modified albumin. Circular dichroism (CD) spectra of HSA 5FU NPs and native HSA were recorded using a JASCO J-1500 spectropolarimeter (Hachioji, Tokyo, Japan). The spectra were recorded in the wavelength range of 200 to 250 nm.

The desolvation method allows obtaining nanoparticles with encapsulated 5FU. The process of obtaining nanoparticles causes significant changes in the secondary structure of HSA. A lower concentration of the drug (1 mg/mL) causes greater changes in the secondary structure of the protein and the increase in absorbance compared to drug at concentration five times higher (5 mg/mL). The amount of encapsulated drug affects changes in the secondary structure of the protein.

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Keywords: Albumin, nanoparticles, drug delivery system

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PM-038

Kidney Injury Molecule-1 Detection by a Novel Mass-Based Biosensing System

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Kidney Injury Molecule-1 (KIM-1) is a type I transmembrane glycoprotein [1]. In the case of acute kidney injury in the patient, it is a potential biomarker for detecting kidney damage because there is a change in the level of KIM-1 in the patient's urine [2]. Early detection of acute kidney injury is crucial for effective treatment and prevention of progression to chronic kidney disease. In this study, a mass-based immunosensor was designed to determine the KIM-1 biomarker. Quartz Tuning Fork (QTF) was used as the working electrode for the developed biosensor system. QTF-based sensors are portable, fast and can be integrated into point-of-care devices. The ability to integrate into point-of-care devices makes QTF-based sensors accessible to a wider range of people. The gold-tipped QTF electrode surface was modified with a mercapto compound to immobilise the anti-KIM-1 biomolecule. This study aims to measure the change in the resonance frequency of the QTF by binding to the antibodies on the surface of the QTF electrode when a sample containing KIM-1 is given to the designed immunosensor. The change in frequency is proportional to the concentration of KIM-1 in the sample, which the immunosensor can measure. Optimisation studies of the parameters used in the biosensor design were made. After the optimum conditions were determined, selectivity, reproducibility and repeatability studies were performed. The immunosensor showed good reproducibility and stability. A modified QTF sensor accomplished simple, sensitive and selective mass-sensitive detection of KIM-1.

Keywords: KIM-1, QTF, mass-based biosensor

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PM-039

Production and Characterization of a Hand-Made Disposable Panel Electrode for Use in Biosensor

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Abstract

In this study, the hand-made panel electrodes consisting of

four working electrodes (WE1-4), a reference (RE), and a counter electrode (CE) were fabricated by a screen-printing method. The WE1-4 surfaces of the panel electrodes were modified with gold nanoparticles (AuNP). Morphological, chemical, and electrochemical characterizations of the produced AuNP-modified hand-made panel electrodes were performed. To prepare the panel biosensor, the panel electrodes were modified with 6-mercapto hexanoic acid, EDC-NHS, Anti-AGR2, Anti-FOLR1, Anti-GLY, Anti-SMRP, BSA, AGR2, FOR1, GLY, and SMRP, respectively. The prepared panel biosensor was electrochemically characterized. Morphological, chemical, and electrochemical characterization results show that the hand-made panel electrodes and panel biosensors were successfully prepared.

Keywords: Hand-made panel electrode, panel biosensor, AGR2, FOR1, GLY, SMRP

I. Introduction

In producing sensors and biosensors, it is important to prepare disposable carbon electrodes, which are frequently used, with low cost, good repeatability, and reproducibility (Afonso et al. 2016). Disposable carbon electrodes have been used frequently for the last 20 years to detect cancer biomarkers. Since a single cancer biomarker is not sufficiently specific and sensitive in diagnosing a certain cancer type, developing panel biosensors that allow simultaneous analysis of biomarkers in recent years is important. Anterior gradient-2 protein (AGR2) (ovarian, pancreatic, prostate, and lung cancers) (Edgell et al. 2010), folate binding protein (FOLR 1) (ovarian, lung, breast cancers) (Maurer et al. 2014), glycodelin (GLY) (ovarian and cervical cancers) (Kalyani et al. 2020), and soluble mesothelin-related protein antigens (SMRP) (ovarian cancer) (Fritz-Rdzanek et al. 2012) stand out among the biomarkers for detecting various cancer types. In this work, the hand-made panel electrodes were prepared, characterized, and used to prepare the panel biosensor.

II. Material and method

In this study, the hand-made panel electrodes consisting of four working electrodes (WE1-4), a reference (RE), and a counter electrode (CE) were prepared by a screen printing method using templates with a cutter printer. Carbon ink was used for WE1-4 and CE, and Ag/AgCl ink was used for RE. The WE1-4 surfaces of the panel electrodes were modified with AuNP by cyclic voltammetry (CV) (Bilgi Kamaç et al, 2023). Morphological characterizations of the produced panel electrode were performed by Scanning Electron Microscopy; chemical analyses were carried out with Fourier Transform Infrared Spectroscopy and X-ray Photoelectron Spectroscopy; electrochemical characterizations were performed with differential pulse voltammetry (DPV) and CV. To prepare the AGR2-FOLR1-GLY-SMRP panel biosensor, the panel electrodes were modified with 6-mercapto hexanoic acid, EDC-NHS, Anti-AGR2, Anti-FOLR1, Anti-GLY, Anti-SMRP, BSA, AGR2, FOR1, GLY, and SMRP, respectively. Electrochemical characterizations of the panel biosensors were carried out by CV and DPV techniques.

III. Results and discussion

The fabrication steps of the hand-made panel electrodes and panel biosensors are given in Figure 1. The hand-made panel electrodes were properly fabricated, and AuNP was successfully deposited on the electrode surface, according to its morphological, chemical, and electrochemical characterization results. The AGR2-FOLR1-GLY-SMRP panel biosensors were prepared using the AuNP-modified hand-made panel electrode, and each preparation step of the panel was characterized by CV and DPV. The peak currents in CVs and DPVs of the panel biosensor were found to decrease at each immobilization step due to decreased diffusion.

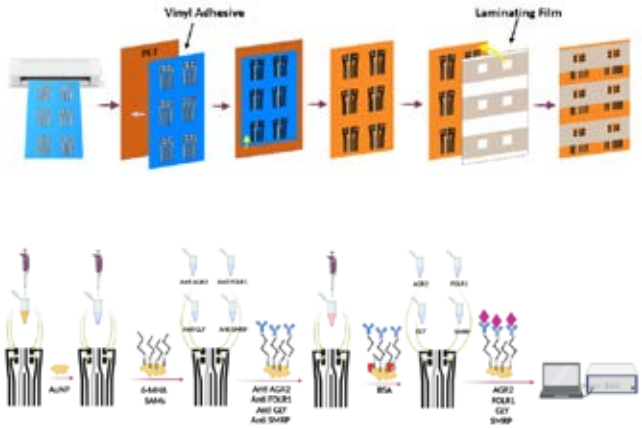

Fig. 1. The preparation steps of hand-made panel electrodes and panel biosensors

IV. Conclusion

In conclusion, the disposable AuNP-modified hand-made panel electrodes and the AGR2-FOLR1-GLY-SMRP panel biosensors were successfully prepared. The fabricated panel biosensors can be used for rapid and practical detection of AGR2-FOLR1-GLY-SMRP.

Acknowledgments

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PM-040

Investigation Of New Potential Bcl-2 Inhibitors via In Silico Approaches

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According to the data of the World Health Organization (WHO), deaths due to cancer cases are increasing day by day in the world. The B-cell lymphoma 2 (Bcl-2) family is one of the main regulators of cellular apoptosis. Therefore, there are many studies on Bcl-2 inhibitors for cancer chemotherapy in the literature. There are many different strains of the Bcl-2 family that are resistant to cancer agents. It is used today as a tool both to make new drug discoveries and to design drugs that are more effective and less toxic than approved drugs, with in silico approaches including Computer-Aided Drug Design (CADD) methods. In addition, in recent scientific studies, it has been understood that saving both time and money via in silico approaches is only possible with CADD methods. Compounds that could be drug candidates can be suggested using computational drug discovery strategies by creating a High-throughput Virtual Screening library from in silico approaches. In this study, all molecules in the ChEMBL Database were scanned virtually based on drugs with Bcl-2 inhibitory properties, one of the receptor tyrosine kinases that are effective in cancer cells. Based on the parameter values of the reference drugs determined during this High Throughput Screening (HTS), the number of compounds was minimized by filtering method out of 2.4 million compounds. As a result of in silico approaches and molecular docking analysis, the ten compounds with the highest binding parameter scores were determined and a model compound that could be a new drug candidate was proposed.

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Keywords: In silico approaches, CADD, Bcl-2, cancer

PM-041

Selenium Nanoparticle Characterization and Determination in B Tc Cells by Single Particle Inductively Coupled Plasma-Mass Spectrometry

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Selenium nanoparticles (SeNP) have been recognized as promising nanomaterials at diabetes treatment due to their low toxicity and high therapeutic properties. Selenium nanoparticles were used both to increase the drug effect and for therapeutic purposes, and its effectiveness was examined (1,2). Current techniques to characterize SeNPs are too expensive and cumbersome to be routinely applicable to biological samples and do not provide all the necessary information individually. These methods provide mostly qualitative information regarding NP detection, size, and/or morphology, and are insufficient to detect, characterize, and quantify SeNPs at low concentrations ($\mu g \cdot kg^{-1}$). Therefore, it is necessary to develop new analytical methods to determine and characterize SeNPs in biological samples. The single particle inductively coupled plasma-mass spectrometry (SP-ICP-MS) technique can be used to detect and characterize nanoparticles in extremely low concentrations in biological samples, with high detection capacity of nanoparticle number and

mass concentration simultaneously.

In this study, naked and liposome-encapsulated SeNPs of two different sizes (70 and 140 nm) were incubated with β TC cells for 24 hours and a SP-ICP-MS method was developed for the size characterization and quantification of SeNPs uptake by cells. It was observed that the size of nanoparticles uptaken into the β TC cells remain same, and the liposome encapsulated nanoparticles were more uptake for 80 nm SeNPs. However, no difference was observed for 140 nm SeNPs with or without liposomes. The size detection limit (LOD_{size}) and the LOD value for SeNPs of the proposed SP-ICP-MS method were calculated as 35.6 nm, and 770 particles mL^{-1}, respectively.

Acknowledgement: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (grant number 123Z013) and Gazi University Scientific Research Fund (grant number TDK-2023-7802)

Keywords: Selenium nanoparticles, SP-ICP-MS, pancreatic β TC cells, liposomal SeNP, nanoparticle characterization

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PM-042

Electrochemical Determination of Enzyme Inhibitor Thiram Based on Bimetallic Nanoparticles Utilizing Green Electroanalytical Nanosensor

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In this study, it is aimed to develop the first bimetallic electrochemical nanosensor for thiram determination. This nanosensor has been modified with AuNPs and green synthesis AgNPs. A newly formed AuNPs@AgNPs/GCE has been successfully applied as a novel electrochemical nanosensor for thiram analysis in commercial preparation and human serum sample. The oxidation behavior of thiram were examined by cyclic voltammetry (CV) and adsorptive stripping differential pulse voltammetry (AdSDPV). The effect of basic parameters such as pH, amount of nanomaterial coated on the surface, deposition time, deposition potential was optimized for the sensitive determination. The peak current of thiram was significantly increased with the modified electrode compared to the bare GCE. The electrochemical performance of the developed electrode can be attributed to the large surface area and high conductivity of AgNPs and AuNPs. The proposed nanosensor has linear response between of 0.2 ppm to 2 ppm with a low detection limit value of 0.0298 ppm, which is lower than the limits set in MRLs (0.1 ppm). High recoveries of 103.6–104.3% in commercial preparation and human serum indicated that the developed method for real sample analysis was successfully applied. To investigate the selectivity of the method, some interfering agents such as KCl, NaCl, glucose, dopamine and ascorbic acid were studied. These compounds were separately included in the phosphate buffer solution (0.2 M, pH 3.0) containing thiram at the same concentration, taking $1x10^{-5}$ M of each. The electrochemical responses of thiram at the AuNPs@AgNPs/GCE revealed that they did not significantly effect thiram analysis in the studied conditions.

Acknowledgments: This study was supported by a grant of TUBITAK (123Z070)

Keywords: Pesticide, thiram, nanosensor, electrochemistry, bimetallic nanoparticles, green synthesis

PM-043

An ITO-based Biosensor for the Detection of Citrullinated Vimentin as an Important Biomarker for Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the joints. In RA, the immune system mistakenly attacks the body's healthy tissues, leading to inflammation in the lining of the joints (synovium), which can cause joint damage, pain, stiffness, and swelling. [1,2] According to the results of a meta-analysis suggesting that various environmental factors and genetics influence the prevalence of

rheumatoid arthritis, which varies by geographic location, the global prevalence of RA was found to be 0.46% between 1980 and 2018. [3] RA is a relatively common condition that can significantly impact a person's quality of life. It is important to seek early diagnosis and treatment to help manage symptoms and slow down the progression of the disease. [4]

Electrochemical immunosensors are important in early diagnosis of diseases because they offer several advantages over traditional diagnostic methods. Besides features of biosensors such as highly sensitive, specific, and rapid therefore biosensors are vital for the early diagnosis of diseases. Moreover, electrochemical immunosensors are relatively simple, low-cost, and require minimal sample preparation, making them highly suitable for use in point-of-care diagnostics. This can be especially beneficial in areas with limited access to healthcare facilities, where rapid and accurate diagnosis is critical. [5]

This study aims to detection of mutated citrulline vimentin (MCV) as a biomarker of RA. It is designed based on an ITO-PET electrode, a single-use, low-cost electrochemical immunosensor for the detection of MCV. For this purpose, a 3-APTES silane agent was used to form a SAM layer on the ITO-PET electrode due to its sensitivity, practicality, and ease of use. EIS and CV measurement techniques were used in the immobilization, optimization, and characterization stages, and real commercial serum samples were tested for the reliability of the immunosensor. As a result of the study, a reproducible, specific, and sensitive immunosensor was obtained.

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Keywords: Immunosensor, ITO-PET electrode, autoimmune disease, rheumatoid arthritis, electrochemical ımpedance spectroscopy

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PM-044

Development of a Novel EGFR-Targeted Peptide for Targeted Delivery of Drugs in Anaplastic Thyroid Cancer

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Abstract

Anaplastic thyroid carcinoma (ATC) represents the most aggressive and deadliest thyroid cancer in human with a high proliferative and metastatic potential. The median survival of patients with ATC is of about 4 months after diagnosis and the mortality rate attains almost 100%. There is no cure for this cancer, which is highly invasive and resistant to conventional therapies. Accordingly, targeted therapies and delivery, such as those studied in the present work, should be considered to improve the prognosis of patients. Our targeted therapy aims to inhibit the PI3K/AKT/mTOR signaling pathway, thereby inducing apoptosis of target cells with a therapeutic peptide (TP) developed in our laboratory. The epidermal growth factor receptor (EGFR) is commonly studied in oncology as it is overexpressed in cancer cells and is actively investigated in the framework of receptor-mediated drug delivery. Therefore, an EGFR-targeted peptide was also developed by our group and coupled to TP in a peptide complex (PC) to enable the specific drug delivery to ATC cells.

Our results show that EGFR is overexpressed and overactivated in ATC. VP is endocytosed independently of the EGF presence and without activating the EGFR. Within cells, VP is colocalized with EGFR, following its trafficking pathway. Moreover, 10 µM of PC induces cell apoptosis after 1h of incubation.

To conclude, our studies confirmed that VP is a good EGFR-targeting candidate to deliver TP to cancer cells. In addition, this VP is able to induce endocytosis of EGFR and thus to deliver TP intracellularly to induce apoptosis.

Keywords: EGFR, anaplastic thyroid carcinoma, targeted delivery, apoptosis, peptides, endocytosis

I. Introduction

Although rare, the anaplastic thyroid carcinoma (ATC) is an

aggressive and invasive type of thyroid cancer (TC), with a dismal prognosis, presenting a median survival rate of 2 - 6 months after diagnosis [1]. The standard treatment consists of combining surgery with ionizing radiation and chemotherapy. However, chemotherapeutic agents are administered systemically producing many undesirable secondary effects, whereas ATC becomes resistant to standard therapies [2]. Therefore, a fundamental change of the therapeutic strategy's conception is required to manage this life-threating oncologic disease.

The dysregulated signaling pathways in ATC mainly concern MAPK, PI3K/AKT/mTOR, and JAK-STAT, which control cancer cell growth, proliferation, and drug resistance. In this context, EGFR and PI3K/Akt/mTOR pathway represent potent targets for improved delivery and pharmacological action of chemotherapeutic agents [2,3]. EGFR is indeed overexpressed in ATC and the PI3KAKT/mTOR pathway is dysregulated.

Engineered peptides can be very helpful in this regard due to their lower toxicity and immunogenicity [4].

In the present work, our ATC-targeted therapy is based on two peptides with different functions: (A) the therapeutic peptide (TP) induces apoptosis by blocking PI3K/Akt/mTOR signaling pathway; (B) the vector peptide (VP) targets EGFR and have the potential to be endocytosed and lead to the TP delivery to cancer cell. The peptide complex (PC) was synthetized by coupling PT and PV via a scaffolding small molecule. EGFR is naturally endocytosed after ligand binding and its intracellular trafficking was lately investigated in the context of receptor-mediated drug delivery [5].

II. Material and method

The experiments were carried out on 8505c and Cal-62 ATC cell lines. Nthy-ori 3-1 healthy cell line was used as control. For endocytosis studies by fluorescent microscopy, VP was coupled to rhodamine (VP-Rhod).

To evaluate the effect of VP on EGFR endocytosis and expression by immunofluorescence (IF) and Western Blot (WB), cells were grouped in four experimental conditions: (1) negative control in culture medium free of FBS, (2) positive control (1 µM EGF), (3) test condition with 40 µM VP or VP-Rhod, (4) test condition with 1 µM EGF and 40 µM VP or VP-Rhod. Condition 2 was pre-incubated with incomplete culture medium and condition 4 was pre-incubated with 40 µM VP for 30 minutes (for EGFR expression studies) or with 1 µM EGF for 2h (for VP-Rhod colocalization with EGFR). The cells were incubated (37°C, 5% CO_2) for 2h (for EGFR expression studies) or for 1h (for VP-EGFR colocalization studies). To corroborate the specific mechanism of endocytosis, VP was preincubated for 30 min with EGFR in solution before adding them to the conditions 3 and 4. Total EGFR ($EGFR_{tot}$) expression was determined with EP38Y antibody, while phosphorylated EGFR ($EGFRpY^{1068}$) was observed with EP774Y antibody (both from Abcam). For WB, the proteins blotted onto nitrocellulose membranes were imaged using a Bioimager Fusion FX (Vilbert, France) and were semi-quantitatively analyzed by densitometry.

The PC effects were investigated by IF (activated caspase 3) observation of apoptotic cells after incubation (30 min, 1h, 2h) with various concentrations (5, 10, 20 µM) of PC.

III. Results and discussion

Our experiments showed that EGFR is overexpressed and overactivated in ATC. Furthermore, our VP is endocytosed into the cells independently of the presence of EGF and without activating the EGFR and thus the downstream signaling pathways. Once in the cell, VP is more than 80% colocalized with EGFR, following either the degradation, recycling, or transport pathway to other cellular compartments (i.e., nucleus).

The preincubation with the EGFR in solution has shown that the VP does not undergo competition in cells not stimulated with EGF and is still as much endocytosed in cells with competition as without competition. On the other hand, there is a phenomenon of competition in cells stimulated with EGF, because the VP is less endocytosed in cells with EGFR competition than without competition.

In cancer cells, the same pattern of EGFR phosphorylation was observed between conditions, meaning that EGF stimulated its activation compared to the negative control. VP alone does not induce receptor activation, being identical to the negative control. On the other hand, VP inhibits EGFR phosphorylation induced by EGF at a level that is in the range of negative control and of P20 alone. P20 could induce this effect when combined with EGF either by enhancing EGFR endocytosis, or by an antagonist effect produced on the receptor itself.

The activation of caspase 3 has been observed by IF on the three cell lines treated with PC. Our previous studies revealed that BAD was activated by dephosphorylation following the inactivation of AKT by PC. We thus hypothesized that apoptosis might be triggered by the mitochondrial pathway. The purpose of this test was therefore to confirm this hypothesis and explore the optimal concentration and treatment duration. Based on these studies, an optimal PC concentration of 10 µM and 1 hour of incubation were identified as inducing the maximal apoptotic level (i.e., 100%).

IV. Conclusion

All these results confirm that the VP is a good candidate for targeting overexpressed and overactivated EGFR in ATC. We can then conclude that the VP: (1) targets EGFR overexpressed by cancer cells to deliver the therapeutic peptide intracellularly; (2) induces EGFR endocytosis without activating it and without interfering with EGF binding; (3) VP is a non-competitive antagonist-type inhibitor of EGFR; (4) VP contributes to the therapeutic effect by decreasing the expression and activation of

EGFR through its lysosomal degradation and, therefore, decreases the activation of the PI3K/AKT/mTOR pathway. All these effects make it possible to bring the TP intracellular to cancer cells while sparing healthy cells as much as possible.

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PM-046

Immuno-Modulatory Role of Microbiota-Derived Postbiotics Against Periodontal Diseases

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Abstract

The multi-factorial etiopathology of periodontal diseases can be summarized by the complex series of links between microorganisms related to dental biofilms and the immuno-inflammatory response of the host. The aim of this study is to determine the immune-modulatory roles of various postbiotics (cell-free supernatant, cell surface-bound exopolysaccharide, release exopolysaccharide, cell lysate, cell surface protein, inactivated cell) of *Lactiplantibacillus plantarum* EIR/IF-1 strain isolated from breastfed infant fecal microbiota, against inflammation in human periodontal ligament fibroblast (hPDLF) cells caused by *Porphyromonas gingivalis* infection. For this purpose, target postbiotics were extracted using different methods and their doses which did not show cytotoxic effects on hPDLF cells were determined by MTT analysis. The effect of selected doses for each postbiotics on *P. gingivalis*-derived LPS-induced inflammation in hPDLF cells was determined by ELISA. When the data were evaluated in terms of IL-8 production, it was determined that LPS of *P. gingivalis* induced IL-8 production, while postbiotics reduced this effect. It has been also shown that the production of IL-10 with anti-inflammatory properties, was dose-dependently induced by postbiotics. However, no significant effect was observed in IFN-gamma production. In conclusion, although the use of postbiotics against periodontal diseases is a relatively new field, it has been shown that postbiotics produced by *L. plantarum* EIR/IF-1 strain, containing biologically active and functional molecules can be used as potential candidates to control periodontal tissue inflammation.

Keywords: Periodontal diseases, *Porphyromonas gingivalis*, inflammation, *Lactiplantibacillus plantarum*, postbiotics

I. Introduction

Periodontal diseases that can damage not only the dental tissue but also the different systems of the human body, are considered a "silent" but important global outbreak with their significant impacts on the huge audiences and socio-economic damage. The accumulation of bacteria in the microbial dental plaque under the gum causes inflammation in the surrounding tissues (gingivitis). If the resulting microbial dental plaque cannot be removed from the environment, the infection may affect other tissues of the periodontium (periodontitis). When successful treatment is not performed, early tooth loss is the inevitable end. Besides, oral pathogens can cross the blood-vascular barrier, enter the bloodstream, then colonize other tissues of the host which can be associated with many diseases. Therefore, inflammation is defined as the key factor for periodontal diseases (Hajishengallis and Chavakis 2021), and new and effective strategies for periodontal inflammation gain attention not only for the treatment of periodontal diseases but also for systemic diseases.

As a new term in the "biotics" field, postbiotics can be regarded as an umbrella term for all of the microbial fermentation end-products. Research to date indicates that postbiotics can have direct possess on anti-bacterial, anti-inflammatory, anti-oxidant, immuno-modulatory and clinically relevant effects and evidence can be found for the use of postbiotics in healthy individuals to improve overall health and to relief symptoms in a range of diseases (Balta et al. 2021, Wegh et al. 2019). The aim of this thesis study is to determine the immune-modulatory effects of postbiotics obtained from *Lactiplantibacillus plantarum* EIR/IF-1.

II. Material and method

To obtain postbiotics, EIR/IF-1 strain was inoculated in 1% MRS broth and incubated at 37°C for 24 hours. Following the incubation, centrifugation was performed for 20 minutes

at 15,000g at 4°C. The cell-free supernatant (CFS) containing extracellular postbiotics was passed through a sterile membrane filter and then lyophilized. Cell surface-bound exopolysaccharides (EPS-b) and release exopolysaccharides (EPS-r) were extracted according to Tallon vd. (2003). Cell lysate (CL) was obtained by sonication, while cell surface proteins (CSP) were extracted by LiCl method and purified using dialysis. 3 hours of incubation at 60°C was used to obtain heat-inactivated cells (HIC).

Human periodontal ligament fibroblast (hPDLF) cells were used for *in vitro* inflammation assays. To determine the non-cytotoxic doses of postbiotics, their different concentrations were treated with hPDLF cells (10.000 cells/well) for 24 hours, and their cell viability was determined using the MTT assay. *Porphyromonas gingivalis* derived lipopolysaccharide (LPS; 1 µg/mL) was used for inducing the inflammation on hPDLF cells. The immuno-modulatory effects of postbiotics which did not show cytotoxic effects on hPDLF were evaluated in terms of cytokine expressions [interleukin (IL)-10, interleukin (IL)-8 and interferon (IFN)-gamma] using ELISA.

All analyzes were performed in triplicate and analyzed using GraphPad (GraphPad Prism v.3.0, GraphPad Software, San Diego, CA, USA). Differences between groups were determined by the Tukey test one-way analysis of variance (ANOVA) and $p<0.05$ was considered significant.

III. Results and discussion

Although the use of postbiotics against periodontal diseases is a relatively new field, current information indicates that postbiotics may be potential candidates for controlling periodontal tissue inflammation (Zhang et al. 2022). Within the scope of this study, the effect of postbiotics (CFS, EPS-b, EPS-r, CL, CSP, HIC) on the inflammatory response of *P. gingivalis*-infected hPDLF cells were investigated. For this purpose, viability of the hPDLFcells treated with different concentrations of postbiotics was analyzed by taking into the absorbance values obtained from the control and experimental groups using the MTT method. According to the result, 1000 µg/mL doses of CFS, EPS-b, EPS-r, and CL,100 µg/mL dose of CSP and 10^{10} cfu/mL dose of HIC exhibited toxic effects on hPDLF cells (Fig. 1).

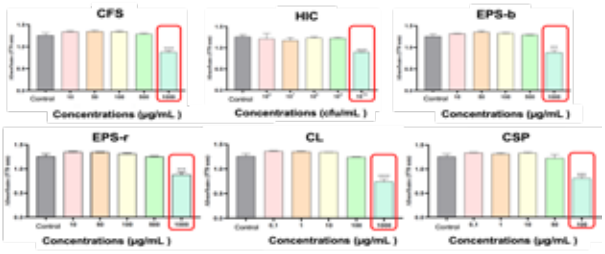

Fig. 1: The effect of postbiotics on hPDLF cell viability

Porphyromonas gingivalis is considered an important oral pathogen that modulates the host's immune-inflammatory responses and disrupts the homeostasis of the normal cell cycle which leads to periodontal tissue destruction. hPDLF cells are recognized as key players in host immune responses and periodontal tissue regeneration. Therefore, to evaluate the immune-modulatory role of postbiotics, hPDLF cells were co-treated with *P. gingivalis*-derived LPS and non-cytotoxic concentrations of each postbiotic, then IL-10, IL-8, and IFN-gamma levels were determined by ELISA. When the data were evaluated in terms of IL-8 production, it was determined that LPS of *P. gingivalis* induced IL-8 production on hPDLF cells, while postbiotics reduced this effect (Fig. 2a). It has been also determined that the production of IL-10 with anti-inflammatory properties, was dose-dependently induced by postbiotics (Fig. 2b). However, no signal was detected in IFN-gamma production.

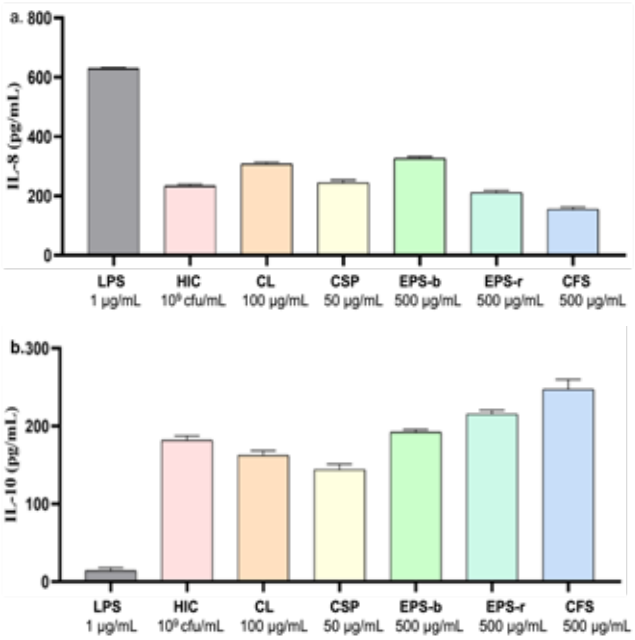

Fig. 2: Effect of postbiotics on IL-8 (a) and IL-10 (b) production of hPDLF cells induced by *P. gingivalis* LPS

IV. Conclusion

Oral diseases, while largely preventable, pose a major health burden for many countries and affect people throughout their lifetime, causing pain, discomfort, disfigurement, and even death. Severe periodontal diseases are estimated to affect around 19% of the global adult population, representing more than 1 billion cases worldwide. The main risk factors for periodontal disease are poor oral hygiene which finally leads to inflammation. Our results showed that postbiotics did not exhibit toxic effects on target cells and reduce the inflammation induced by *P. gingivalis* LPS. In conclusion, although the use of postbiotics against periodontal diseases is a relatively new field, it has been shown that postbiotics produced by *L. plantarum* EIR/IF-1 strain which include biologically active and functional molecules can be used as potential candidates to control periodontal tissue inflammation as a new, natural, and safe strategy.

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PM-047

Weisella spp.-derived Postbiotics: A Novel Therapeutic Strategy for Dental Caries

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Abstract

Oral diseases are a major cause of morbidity and are among the most prevalent diseases worldwide. The most prevalent oral diseases globally are dental caries (tooth decay). *Streptococcus mutans* is the main etiological agent of human dental caries, and lives primarily in biofilms on the tooth surfaces called dental plaque. The main aim of this study is to evaluate the effects of postbiotics against *S. mutans* ATCC 25175, a major cariogenic pathogen. Within this aim *Weisella* spp. isolated from honey bee (*Apis mellifera* L.) pollen microbiota was used as the source of postbiotics. The agar well diffusion assay was used to determine the anti-microbial activity, and the crystal violet staining procedure was used to determine the anti-biofilm activities of postbiotics. According to our results, postbiotics of *W. cibaria* 29.1 displayed anti-microbial activity against *S. mutans* with the inhibition zone of 20 mm. Following the co-incubation, it was observed that sub-MIC values of postbiotics inhibited the cariogenic biofilm formation. In conclusion, postbiotics with their anti-microbial and anti-biofilm activities can be used as a potential strategy for dental caries.

Keywords: Dental plaque, cariogenic biofilm, *Streptococcus mutans*, microbiota, postbiotic

I. Introduction

Dental caries characterized as a biofilm and sugar-induced disease is a major public health problem globally and is the most widespread non-scommunicable disease. *Streptococcus mutans* has been indicated as the main microorganism related to dental caries development. This microorganism can grow in acidic environments and metabolize sugars to produce acid that demineralizes the hard tissues of the teeth (enamel and dentine) as an important factor for caries lesion progression. Moreover, *S. mutans* hydrolyze sucrose from diet and synthesize glucan polymers from the resulting glucose, which contributes to the three-dimensional architecture of dental biofilms (Bertolini et al. 2022). Therefore, the inhibition of important factors related with biofilm production mechanisms, and the effect of these factors on disease progression, also need to be better elucidated. Toothpaste containing stannous fluoride are in use with their anti-caries effects. However, consumers tend to use products with natural ingredients, in recent years. Therefore, biotics has gained recent interest as some present anti-bacterial activity against oral bacteria, and potentially reduce side effects, and therefore could emerge as an adjunct anti-biofilm treatment in the future. Postbiotics known as the microbial fermentation end-products of probiotics can be a potential candidate with their anti-microbial, anti-inflammatory, immunomodulatory, anti-hypertensive, anti-carcinogenic, and anti-oxidant activities. The main aim of this study is to evaluate the anti-microbial and anti-biofilm effects of postbiotics against *S. mutans* ATCC 25175.

II. Material and methods

Weisella spp. isolated from honey bee (*Apis mellifera* L.) pollen microbiota were cultured for 24 hours in order to obtain postbiotics. Following the incubation, centrifugation was performed for 20 minutes at 15,000g at 4°C. The cell-free supernatant containing extracellular postbiotics was passed through a sterile membrane filter and then lyophilized. Antibacterial activity of postbiotics against *S. mutans* ATCC 25175 was determined using the agar well diffusion method. Following the incubation period, the diameters of the inhibition zones around the wells were measured in millimeters. To find out whether a bacteriocin-like compound or organic acids played a role in the antibacterial activity against the pathogens, the inhibitory effect was also analyzed following the treatment of the postbiotics with the 1 mg/mL proteinase K and neutralization (Todorov and Dicks 2005). Minimum Inhibitory Concentration (MIC) of the lyophilized postbiotics against *S. mutans* ATCC 25175 was determined using microtiter plate assay, according to Clinical and Laboratory Standards Institute guidelines. Antibiofilm

activity of various postbiotic concentrations was carried out using the crystal violet staining method (Onbas et al. 2018). All assays were performed with three independent experiments (biological replicates) and each measurement was carried out in triplicate (technical replicates). Data were analyzed using SPSS version 22.0 (IBM, New York, NY, USA), by one-way analysis of variance (ANOVA) followed by Dunnett's test and unpaired t-test (GraphPad Prism v.3.0, GraphPad Software, San Diego, CA, USA). All results were presented as a mean ± standard deviation and $p < .05$ was used to indicate a significant difference.

III. Results and discussion

Weissella spp. isolates from honey bee pollen microbiota were tested for their antibacterial activity against *S. mutans* ATCC 25175. According to our results, all isolates showed an inhibitory effect against cariogenic strain. Among the isolates, *Weisella cibaria* 29.1 exhibited a remarkable inhibitory effect with an inhibition zone of 20 ± 1.7 .mm (Fig.1). The effects of postbiotics were also analyzed in different ways to find out the components, such as organic acids, or bacteriocin-like compounds responsible for the antibacterial activity. Although the inhibitory effect was not affected by proteinase K treatment, the activity disappeared after neutralization (Tab.1), which may be related to their acidity. MIC value of the postbiotics secreted into the culture medium of the isolates was determined as 30 mg/mL and 35 mg/mL as shown in Tab.1 and Fig.2.

The anti-biofilm activity of postbiotics against *S. mutans* ATCC 25175 was evaluated within the co-incubation approach. Following the co-incubation protocol, sub-MIC values of all postbiotics were found sufficient to inhibit the biofilm formed by *S. mutans* ATCC 25175 by more than 90% (Fig.3). Besides, MIC10 value of postbiotics derived from *Weisella cibaria* 29.1 was decreased the biofilm formations as 96.15 ± 0.92%. Additionally, co-treatment with MIC25 values of postbiotics from *Weisella cibaria* 18.3 and *Weisella confusa* 15.1 strains were able to decrease the formation of biofilms, with the eductions of 95.44 ± 0.91% and 85.56 ± 0.94%, respectively (Fig.3).

As Streptococci are important cariogenic microorganisms due to their role in dental plaque formation and dental caries development, they remain at the center of the investigations. Moreover, biofilm-associated infectious diseases are hard to control since bacterial cells become resistant to antimicrobial agents and host immune defense mechanisms (Zhang et. al 2011). Owing to the fact that chemical agents used in commercially available products are not sufficient, clinical trials focused on natural compounds, such as plant-derived phytochemicals, and proved that they are effective in preventing dental caries. However, these powerful compounds also showed toxic effects on human cells as well. Therefore, the development of functional antimicrobial agents including novel and effective natural compounds is still needed. Lately, postbiotics are gaining interest for the prevention of dental caries and they are used in this study because their mode of action is mainly based on the production of bioactive compounds such as antimicrobial peptides, organic acids, and hydrogen peroxide [43]. These results showed that postbiotics have the ability to inhibit the activity of pathogenic bacteria while preventing biofilm formation, interfering with biofilm integrity/quality and finally leading to biofilm eradication.

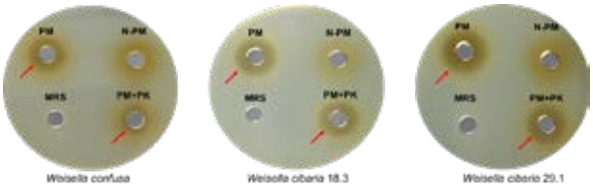

Fig. 1: Inhibition zones of postbiotics on plates against *S. mutans* ATCC 25175 (PM; only postbiotics, N-PM; neutralized, PM+PK; proteinase K treated, MRS; only media. Arrows show the inhibition zones)

Fig. 2: MIC values of postbiotics against *S. mutans* ATCC 25175 obtained by micro-broth dilution assay. (Circles show the MIC values, + is un-treated control)

Tab. 1: Antibacterial activity of postbiotics against *S. mutans* ATCC 25175

Agar well diffusion assay (inhibition zone diameter in mm; - no zone)			
Strain	**PMs**	**PMs+ Proteinase K**	**Neutralized PMs**
Weisella confusa	16 ± 1.5	14 ± 2.4	-
***Weisella cibaria* 18.3**	19 ± 1.2	19 ± 1.8	-
***Weisella cibaria* 29.1**	20 ± 1.7	20 ± 1.2	-
MIC value (mg/mL)			
Weisella confusa	30		
***Weisella cibaria* 18.3**	35		
***Weisella cibaria* 29.1**	35		

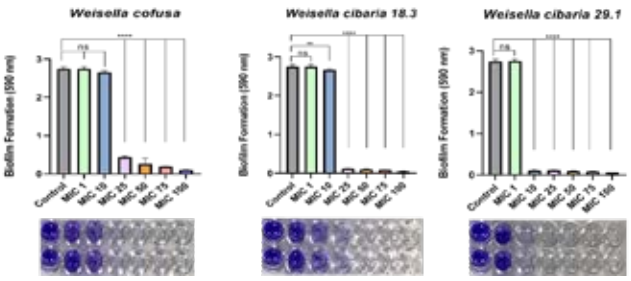

Fig. 3: Anti-biofilm activity of postbiotics against *S. mutans* ATCC 25175 in polystyrene microtiter plates.

IV. Conclusion

The present study demonstrated that the postbiotics as the metabolic by-products secreted by *Weissella* spp. originating from honey bee pollen microbiota, might be used as a promising agent for the prevention of dental caries, with its anti-bacterial and anti-

biofilm activity against cariogenic pathogens. However, as the successful treatment of oral diseases depends on several factors, further studies are needed to prove the efficacy of postbiotics in managing dental caries, *ex vivo* or *in vivo*.

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PM-048

Enhancement of SERS Signal Sensitivity Using Hybrid Nanoparticles

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Surface-enhanced Raman spectroscopy (SERS), which is based on the enhancement of Raman signal on different surfaces, is an important method because it is highly sensitive, time-saving, and widely applicable. However, appropriate placement of the reporter molecule on the metal nanoparticle surface and aggregation are big challenges. Several approaches have been suggested for this purpose and multipurpose functionalized hybrid nanoparticles are very promising for the detection of trace amounts of analyte. In the present work, new hybrid magnetic nanoparticles consisting of core-shell structured Fe_3O_4@ polymer which are suitable for biomolecules immobilization were synthesized in order to develop SERS assay platforms and microfluidic chip systems. The synthesis of magnetic nanoparticles and subsequent modification with different polymers provide magnetic separation and SERS applications. The optimization strategies for avoiding aggregation and the analytical performance of the SERS-based assays will be presented.

Keywords: Surface-enhanced Raman spectroscopy. magnetic nanoparticles. microfluidic chip systems.

PM-049

Highly Selective Molecularly Imprinted Polymer-Based Electrochemical Sensor for The Determination of Pazopanib

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Pazopanib hydrochloride (PZB) is a multiple kinase inhibitor, and it is used for the treatment of advanced renal cell carcinoma and advanced soft tissue sarcoma [1]. This study is targeted high selectivity and sensitivity for determining PZB using a molecularly imprinted polymer (MIP) based electrochemical sensor. MIP sensor was developed by thermal polymerization (TP) directly on a glassy carbon electrode (GCE) using PZB as a template molecule, 4-amino benzoic acid (4-ABA) as a functional monomer, sodium dodecyl sulfate (SDS), tetraethyl ortho silicate (TEOS), and NH_3 solution. The electrochemical behavior of the designed sensor was examined using differential pulse voltammetry (DPV). Morphological and electrochemical characterization of the 4-ABA/PZB@MIP-GCE sensor was applied using scanning electron microscopy (SEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). In addition, the significant parameters involved in MIP (template: monomer ratio, dropping volume, TP time, removal solution and removal time, and rebinding time) were optimized. Under the optimized

experimental conditions, the developed 4-ABA/PZB@MIP-GCE sensor exhibited good analytical results for the determination of PZB with a linear response ranging from 1.0×10^{-13} M to 1.0×10^{-12} M with a limit of detection and quantification of $1.04\text{x}10^{-14}$ M and $3.46\text{x}10^{-14}$ M, respectively. Furthermore, the applicability of the sensor was examined by determining commercial serum samples and tablets, and excellent results and recoveries (ranging from 98.20% to 101.61%) were obtained. The designed sensor demonstrated an excellent electrochemical response for PZB. The results indicated that the MIP could specifically identify PZB compared to structurally similar drugs such as axitinib, erlotinib, nilotinib, granisetron, and vismodegib. Furthermore, the interference agents of substances such as K^+, NO^{3-}, Na^+, SO^{42-}, Mg^{2+}, Cl^-, dopamine (DOP), ascorbic acid (AA), uric acid (UA), and paracetamol (PAR) which are commonly found in biological fluids were investigated. Finally, the sensor's design was approved using a non-imprinted polymer (NIP)-based GCE.

Keywords: Pazopanib; molecularly imprinted polymer; electrochemical sensor; determination

PM-050

ITO-PET Coated Electrode Based Biosensor System for Determination of Kidney Injury Molecule-1

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Kidney Injury Molecule-1 (KIM-1) is a type-1 transmembrane glycoprotein, which functions as an early indicator of kidney damage, including acute kidney injury. The European Medicines Agency (EMA) and the Food and Drug Administration (FDA) have recognized KIM-1 as a highly sensitive and specific urinary biomarker for monitoring drug-induced kidney injury in preclinical research (Bonventre, 2008). Under normal physiological conditions, KIM-1 is absent. However, KIM-1 expression occurs on the apical membrane of proximal tubules following injury (Vaidya, 2009). Indium tin oxide polyethylene terephthalate (ITO-PET) coated electrodes have gained prominence as low-cost transparent electrodes due to their unique combination of electrical conductivity, exceptional optical transparency, and chemical stability (Yadav, 2023). 3-(trimethoxysilyl)-1-propanethiol (3-TMSPE) was utilized for this research. Anti-KIM-1 protein was immobilized, subsequent to 3-TMSPE immobilization. 3-TMSPE concentration optimization, N-Hydroxysuccinimide (NHS) concentration optimization, anti-KIM-1 concentration optimization, anti-KIM-1 incubation time optimization and KIM-1 incubation time optimization were performed throughout this study. Subsequently, impedance levels were measured to examine changes in the ITO-PET coated electrode conductivity. An ITO-PET coated electrode-based biosensor system was developed to detect KIM-1 concentrations ranging from 0.1 fg/mL to 1000 fg/mL. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used as measurement techniques for this research.

Keywords: Kidney Injury Molecule-1 (KIM-1), Indium Tin Oxide Polyethylene Terephtalate Coated (ITO-PET) Electrode, Biosensor

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PM-051

An ITO-PET Based Biosensor System for AFP Early Detection: Process Optimization Studies

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Abstract

Alpha-fetoprotein (AFP) is a major mammalian embryo-specific and tumor-associated protein in small quantities in adults under normal conditions(Terentiev et al.,2013). AFP determination in biological fluids during fetal growth has been done, and it was discovered that abnormal concentrations correlate with fetal defects and malformations (Kal-Koshvandi et

al.,2020). Indium tin oxide (ITO), one of the most widely utilized transparent conductive oxide thin films, is a promising material to develop different technologies such as biosensors, flat-panels and photovoltaics due to its two main properties; good electrical conductivity and optical transparency (Aydın et al.,2017). In this investigation, a biosensor based on ITO-PET (indium tin oxide-polyethylene terephthalate) was devised to detect AFP. First, the hydroxylated ITO-PET surface was modified with mercaptopropyldimethoxysilane (MPDS). The cross-linker, also known as NHS, is then immobilized on the surface of the ITO-PET. The ITO-PET was then incubated in an antibody solution for covalent immobilization of the antibody. In order to avoid unspecific interactions on the ITO-PET surface, bovine serum albumin (BSA) is used as a blocking protein. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) are used for immobilization, optimization and analytic studies. In conclusion, the ITO-PET determination range was between 0.05 fg/mL and 250 fg/mL.

Keywords: Alpha-fetoprotein, ITO-PET, biosensor

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PM-052

Determination of the Therapeutic Efficacy of Atranorin Associated with the Ferroptosis Pathway in Breast Cancer at the Biochemical Assays

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Breast cancer is the most prevalent type of cancer, with 2.3 million new cases and a 6.9% death rate in 2020, according to data from the World Health Organization (WHO). Despite the advances in diagnosis and treatment processes, an effective treatment method has not yet been found with conventional treatment methods for breast cancer. In addition, conventional treatments have serious side effects that damage healthy cells and tissues as well as cancer cells, and this adversely affects the treatment processes. In recent years, research has concentrated on identifying the molecular mechanisms behind cancer formation, developing personalized treatment methods, and discovering novel drug candidate molecules and cell death types in order to treatment of breast cancer. Atranorin (ATR), a lichen secondary metabolite, is known to have a variety of significant biological functions, including anti-oxidant, anti-cancer, and anti-proliferative properties. Ferroptosis, a type of cell death pathway different from other cell death mechanisms, results from the accumulation of iron-dependent lipid peroxides and reactive oxygen species (ROS). The aim of our study is to determine the anti-proliferative effect of atranorin associated with the ferroptosis pathway in breast cancer cells with different subtypes and to examine the ferroptosis activity by enzymatic assays. The drug candidate molecule atranorin (ATR) was obtained commercially and prepared by dissolving it in DMEM medium containing 0.05% DMSO. The anti-proliferative effect of ATR in breast cancer cell lines MDA-MB-231 (ER-, PR-, HER2-), MCF-7 (ER+, PR+, HER2-), SK-BR-3 (ER-, PR-, HER2+), BT-474 (ER+, PR+, HER2+), and the normal breast cell line MCF-12A was determined using the MTT assay and the xCELLigence real-time cell analyzer. Four enzymatic test kits were used to examine the ferroptosis activity of ATR in MDA-MB-231 and BT-474 cells: ferrous (Fe^{+2}) iron assay, T-GSH/GSSG ratio detection, lipid peroxidation (malondialdehyde, MDA) assay and ROS assay kit. All data were obtained in three replicates, and the Graphpad Prism 9.5.1 program was used for data analysis. Our study showed that ATR decreased cell viability and cell proliferation in different sub-types of breast cancer cell lines in a dose- and time-dependent manner without showing cytotoxic effects on normal breast cells. Compared with MCF-7 and SK-BR-3, BT-474 and MDA-MB-231 cells were found to have less potential for anti-proliferative effects on ATR. The inhibition concentration (IC50) of ATR in BT-474 and MDA-MB-231 cells was determined to be 14.7 µM and 19 µM at 48 h, respectively. According to the ferroptosis-related enzymatic assay results, it was found that the intracellular iron (Fe^{+2}) levels, lipid peroxidation (MDA) levels, and ROS levels increased, while the T-GSH/GSSG ratios decreased in BT-474 and MDA-MB-231 cells treated with ATR (IC50) compared to untreated cells. Our study has revealed that ATR has anti-cancer activity by significantly reducing the viability and proliferation of breast cancer cells and also shows its anti-proliferative activity by the ferroptosis pathway in cellular and biochemical assays. In conclusion, the potential of ATR as an innovative drug candidate molecule in the treatment of breast cancer has been determined.

Keywords: Breast cancer, ferroptosis, atranorin.

PM-053

Determination of the Therapeutic Efficacy of the Drug Candidate Molecule Atranorin at the Transcriptomic Level as an Innovative Strategy in the Treatment of Melanoma Cancer

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There are many routine treatment methods for the treatment of melanoma cancer. However, toxicity and resistance to treatment are observed in some treatment methods such as chemotherapy. Apoptosis, the programmed pathway of cell death, is defined as the natural barrier that restricts the survival and spread of cells in the process of cancerization. In this study, the antiproliferative efficacy of the drug candidate small molecule atranorin (*ATR*), which is a secondary metabolite of lichen with many biological activities, in melanoma cancer was examined at the transcriptomic level. Cell viability was determined with 3-(4,5-Dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method after apply to atranorine to human melanoma cancer cell lines A-375, G-361 and MDA-MB-435 and melanocyte normal cells. According to results, no cytotoxic effect of atranorin on the melanocyte cell line was observed. Atranorin was found to have the highest antiproliferative effect on the A-375 melanoma cancer cell line. The antiproliferative effect of atranorin on the A-375 cell line was also examined with xCELLigence Real-Time Cell Analyzer and the IC_{50} value was determined as 12 µM. In addition, the antiproliferative potential of the drug candidate molecule atranorin was also examined at the mRNA level. For qRT-PCR analysis, a total of 88 target genes related with apoptosis and *GAPDH* gene was used as housekeeping. According to the qRT-PCR results, the expression levels of 87 genes from 88 target genes were determined. After apply to atranorin, the *BCL2* gene was suppressed 47.50-fold and the *MCL1* gene was 38.32-fold suppressed among the anti-apoptotic genes. In total, two anti-apoptotic genes (*Cas6, Cas7*) from the Caspase family, four (*TRAF 1, TRAF2, TRAF4, TRAF6*) from the TRAF family, ten (*BAG1, BAG2, BAG3, BAG4, BCL2, BCL2L2, BCL2A1, BCL10, BIK, MCL1*) from the *BCL2* family, two (*CHECK1, CHECK2*) from the Kinase family, and seven (*BRE, BIRC1, BIRC2, BIRC3, BIRC4, BIRC5, BIRC6*) from other apoptosis related genes were suppressed. The increase in the expression levels of pro-apoptotic and apoptotic genes and the suppression of the expression of anti-apoptotic genes have an antiproliferative effect on the drug candidate molecule atranorin melanoma cancer.

Keywords: Melanoma, apoptosis, atranorin.

PM-054

Investigation of Potential Binders to GPR17 Membrane Receptor by Surface Plasmon Resonance and Grating-Coupled İnterferometry

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G-protein coupled receptors (GPCRs) are a large family of membrane proteins which hold great potential as drug targets. In particular, GPR17 is an interesting target in demyelinating diseases since it is involved in modulating the maturation of oligodendrocytes, the cells responsible for myelin production.

Both surface plasmon resonance (SPR) and grating-coupled interferometry (GCI) allow to study the interactions between molecules based on the changes in refractive index occurring at a sensor surface. However, while in SPR a localized evanescent field is created on the sensor chip, in GCI the light runs through the entire length of the sample enhancing the sensitivity and the signal-to-noise ratio.

In a previous study carried out by our research group [1], a new SPR method was developed to investigate the interaction of potential binders to GPR17. The aim of the present work is to transfer the method to a Creoptix WAVE GCI system and, in a second phase, to apply it to new potential more selective and potent GPR17 ligands.

The intrinsic instability of GPCRs outside their natural membrane environment presents an analytical challenge for their extraction, immobilization and use in a SPR/GCI system. Therefore, in an earlier stage of the project [1], a suitable protocol was developed allowing to extract engineered GPR17 from crude membranes, immobilize it on a SPR chip and maintain its stability and binding activity for over 24 h after immobilization. The same procedure was applied in this work and GPR17 was captured from solubilized membrane extracts on the sensor chip through a covalently bound anti-His6 antibody. The maintenance of receptor binding activity was assessed by kinetic analyses of the high affinity antagonist Cangrelor and agonist Asinex 1.

Dissociation constants resulted in agreement with those obtained using a Pioneer AE SPR optical biosensor [1], confirming the successful transfer of the method to the Creoptix WAVE GCI system.

In parallel, docking studies by molecular modeling on a homology model based on the P2Y1 receptor were carried out to screen potential GPR17 ligands (Laboratory of Professor Eberini, University of Milan, Italy). From 130 potential ligands, 4 purine ribonucleotides were selected and synthesized (Laboratory of Professor Speranza, University of Milan, Italy) for SPR and GCI studies.

This study represents the first step towards the integration between SPR/GCI analysis and GPR17 computational assay for the rational identification of candidates to develop new drugs for demyelinating and ischemic diseases, for which no effective therapy is yet available.

Keywords: GPR17 membrane receptor, surface plasmon resonance (SPR), grating-coupled interferometry (GCI)

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PM-055

First Voltammetric Detection of Karbutylate via Different Carbon-based Electrode

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This study covers the investigation of the electrochemical behavior of carbamate pesticide-type karbutylate with boron-doped diamond (BDDE), glassy carbon electrode (GCE), and pencil graphite electrode (PGE) electrode, respectively, and analytical determination in real samples for the first time. Similar to organophosphate insecticides, carbamate pesticides are produced from carbamic acid and kill insects. They are extensively utilized in agriculture, gardens, and residences. Therefore, rapid and simple detection of karbutylate in real samples is of great importance. Carbon-based electrodes are now widely used in electroanalytical chemistry due to their rich surface chemistry, chemical inertness, broad potential window, low background current, and congruency for various demanding applications. Therefore, three different carbon-based electrodes were used in this study. The effect of buffer solutions, scan rate, square wave (SW), and differential pulse voltammetry parameters on the voltammetric response of karbutylate was tested. Using cyclic voltammetry, two irreversible anodic peaks, were observed for glassy carbon in 0.04 M pH 7 Britton Robinson buffer. Under optimum experimental conditions, calibration curves for RUX were obtained as 6.00×10^{-7}-8.00×10^{-5} M, 4.00×10^{-7}-8.00×10^{-5} M, and 8.00×10^{-8}-8.00×10^{-5} M with a limit of detection of 2.18×10^{-7}, 3.71×10^{-8} M and 1.33×10^{-9} M by the BDDE, GCE, and PGE, respectively using SWV. As a result, sensitive determination of Karbutylate has been successfully performed using different carbon-based electrodes.

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Keywords: Pesticides, karbutylate,voltammetry, boron-doped diamond electrode, glassy carbon electrode, pencil graphite electrode

PM-056

Metal Affinity-Based Polymeric Drug Nanocarriers

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Triacetyluridine (TAU) is a uridine type that has a much higher oral bioavailability, is 4-7 times more potent, and thus represents a novel mechanism for delivering exogenous pyrimidines to the brain. Triacetyluridine is a pyrimidine nucleoside that has effects with different mechanisms for prevention of cells from damage, it is heavily used for reducing side effects of chemotherapy, increasing antitumor efficacy, reducing toxicity, improving brain functions and memory, therapeutic supportive effects in nervous system diseases and hereditary diseases.

In the context of this study, polymeric drug nanocarriers were synthesized by the non-surfactant emulsion polymerization method with low cost, high drug loading capacity, high release, and targeting properties. Synthesized p(HEMA-MAC)-Cu+2 nanopolymers were modified for TAU specificity by using

immobilized metal ion affinity and characterized with advanced methods such as SEM, FTIR, Zeta size, and surface area calculations. Different parameters for TAU adsorption were optimized and controlled release studies of TAU were performed. At physiological pH=7.4, with approximately 97,6% of 2 mg/mL TAU loaded nanoparticles were shown to be controlled, efficient release within 90 min.

These developed nanocarriers can be combined with the innovative approaches of nanobiotechnology, many promising developments in the field of medicine will be possible, such as the detection of diseases by biological molecules, accurate and rapid analysis, and the targeted and effective use of these drugs.

Keywords: Triacetyluridine, immobilized metal affinity interactions, controlled drug release systems

PM-057

Preparation and Detailed Characterization Rod-like CuO Nanoparticles/waste Masks Carbon for Voltammetric Detection of Pazopanib

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In this study, the first voltammetric method was developed for the detection of pazopanib, belonging to the tyrosine kinase inhibitor class, using a modified glassy carbon electrode. Rod-like CuO nanoparticles prepared by hydrothermal method and finger-like carbon material obtained from hydrothermal carbonization of waste masks were used as modification materials. The physicochemical properties of the materials prepared in the study were elucidated through SEM-EDX, TEM, XRD, XPS, FTIR, TGA-DTA, and BET analysis techniques, respectively. As a result, the impacts of carbon material and nanoparticles on the electrooxidation behavior of pazopanib were examined and evaluated. The impact of scan rate, pH, and supporting electrolyte on the voltammetric response of pazopanib was analyzed on bare and modified electrodes. In addition, for the first time, the electrochemical oxidation mechanism of pazopanib was elucidated within the scope of this study. The square wave voltammetry and stripping conditions parameters were both optimized. Using adsorptive stripping square wave voltammetry, the calibration curve was linear in the 2.0×10^{-10}-1.0×10^{-6} M range. The proposed method was utilized to accurately and precisely measure the quantity of pazopanib in human serum samples and pharmaceutical dosage forms. At nano-detection levels, the technique demonstrated good analytical performance. Additionally, the nanosensor was assessed while exposed to specific interfering ions and chemicals.

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Keywords: Voltammetry, square wave voltammetry, glassy carbon electrode, pazopanib, CuO nanoparticles, carbon material

PM-058

Aripiprazole Imprinted pHEMA Cryogel Patches for Neurological Treatments

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Neurological diseases have incorporated new terms in line with the understanding of human brain function, and therefore, various ways have been used for their treatment. Since the treatment processes are aimed at eliminating the symptoms rather than ending the disease, it becomes crucial to cope with the side effects of the drugs as well as their functions. Controlled drug release rises as a favorable method when it is aimed to get rid of the side effects by reducing the drug dosage. Thanks to the controlled release applied with biocompatible materials, minimizing drug side effects and using effective treatment methods is one of the latest innovations in the medical field. Cryogels are materials that have proven their effectiveness as versatile biocompatible polymers used in controlled drug release. In this study, transdermal patches were synthesized by imprinting Aripiprazole which is used in a wide range of neurological diseases to poly-HEMA-based cryogels. Controlled cumulative drug release of up to 80% was observed in studies with different crosslinker ratios and drug loadings. In addition, its biocompatibility has been proven by

cytotoxicity studies and its release kinetics has been summarized, showing its potential to be an alternative and strong candidate for use in treatment processes.

Keywords: Aripiprazole, drug release, cryogel, molecular ımprinting,

PM-059

Electrochemical DNA Biosensor Design for Determination of the Interaction between DNA and Favipiravir Drug Used in the Treatment of COVID-19

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The Coronavirus (COVID-19), which emerged at the end of the 2019 and reached Turkey in March of 2020, spread around the world and deeply affected everyone's lives. A number of drugs were used as methods as treatment when people were infected by the virus. One of these drugs called Favipiravir was prescribed to patients for five days. On the other hand, great and rapid progress has been made in various fields for the design of biosensors in the scientific world for about 20 years. Biosensors consist of two subunits, the "sensing part" with biomaterial content and the "transducer part" that translates the detection event into a readable numerical value. These devices are developed for DNA base sequence determination, analysis of compound-DNA interactions, etc. and there are types that can measure multiple DNA, they also carry the infrastructure of microchips (microarrays), which are devices with reduced size.

In this study, we present a nanomaterial-free and nanomaterial integrated pencil graphite electrode (PGE) based electrochemical DNA biosensor for the determination of Favipiravir-DNA interaction for the first time. For this purpose, surface interactions between immobilized herring sperm double-stranded (dsDNA) and Covid 19 effective drug Favipiravir were analyzed using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Here, preliminary results of the determinations performed on the nanomaterial-free PGE surface are presented. According to the results of the measurements repeated at least 3 times, after the Drug-DNA interaction, both guanine and drug signal decreased by approximately 45 percent compared to the pre-interaction peak heights of them. The changes in the experimental conditions such as the concentration of DNA and Favipiravir, scan rate, the interaction time between Favipiravir and DNA were studied. In addition, the reproducibility parameter was also evaluated. With this study, it was determined whether there was a Favipiravir - DNA interaction and contributed to the elucidation of the mechanism of this interaction. In conclusion, obtained results showed that designed DNA biosensor could be used for the rapid, sensitive, and cost effective detection of Favipiravir–DNA interaction.

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Keywords: Electrochemical DNA biosensors, Favipiravir, Drug-DNA interaction, nanomaterials.

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PM-060

Pseudomonas Aeruginosa Imprinted Polydopamine@ Graphene-Coated Pencil Graphite Electrode for Selective Bacterial Detection

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Pseudomonas aeruginosa is a ubiquitous rod-shaped Gram-positive bacterium with the ability to survive under various environmental conditions thanks to the large genome of P. aeruginosa that allows the bacterium to encode large amounts of regulatory enzymes and proteins for metabolism and transportation. *P. aeruginosa* causes nosocomial and fatal infections and is often found in water sources as well as swimming pools and spas. The detection of *P. aeruginosa* is crucially important because of these features. In this study, molecularly imprinted polydopamine films were prepared on graphene oxide-modified

graphite electrodes by chemical oxidation of dopamine in the presence of template molecules of *P. aeruginosa*. The electrodes were electrochemically characterized by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). The electrodes were also chemically characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). According to the results, films were successfully deposited on the electrodes and displayed increased electrical conductivity in combination with graphene oxide. After template removal, selective cavities for P. aeruginosa were exposed. The analytical performances of the electrodes were tested using DPV in the concentration range of 10^2 – 10^8 CFU/mL. Meanwhile, the limit of detection (LOD) and limit of quantification (LOQ) were calculated as 0.26 CFU/mL, and 0.81 CFU/mL, respectively. The sensor was also highly selective against P. aeruginosa in comparison to Escherichia coli, Staphylococcus aureus, and Bacillus subtilis being evaluated as potential interfering competitors.

Keywords: Dopamine, molecular ımprinting, bacteria detection, conductive ınterface, electrochemical detection

PM-061

A Comperative Controlled Release of Lycopene from Three Different Composites

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Electrospinning is a well-known technique in order to synthesize polymeric nanofiber for drug delivery systems. Besides, with the rapid development of nanotechnology, the use of membranes for drug release studies offers a unique top-down approach. The usage of biodegradable polymers such as polycaprolactone (PCL) has been extensively preferred in literature for this purpose. Hydrophobic properties of polyesters cause low-drug loading capacity and so natural silicates can be preferred as the agents which have low-cost and high surface area. In this study, PCL polymeric nanofiber were used as a matrix polymer for loading materials including bentonite, diatomite, and poly(2-hydroxyethyl methacrylate-N-methacryloyl-L-glutamic acid) [poly(HEMA-MAPA)] beads in order to release lycopene, a naturally occurring antioxidant. For this reason, lycopene-containing bentonite, diatomite and poly(HEMA-MAPA) beads incorporated into PCL nanofibers were obtained by electrospinning technique. In addition, lycopene-containing membranes of PCL interacted with bentonite, diatomite and HEMA-MAPA, respectively, were synthesized by solvent casting technique. Lycopene loading efficiency of the composites were evaluated by UV–VIS spectrophotometry. The release profiles of the obtained lycopene-containing nanofibers and membranes were studied at different pH (6-8), temperature (4-37 °C) in 7 days. Morphological structures of the prepared materials were characterized by scanning electron microscopy and, structural-properties were characterized by FTIR-ATR.

Keywords: Lycopene, antioxidant materials, nanofibers, composites

PM-062

Study on Molecular Modeling of Some New Adenosine Monophosphate Activated Protein Kinase (AMPK) Activators

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AMPK acts as a sensor that detects energy levels inside the cell. It consists of three subunits in eukaryotic cells; α is the catalytic subunit, β and γ are the regulatory subunits. The catalytic module contains a typical eukaryotic kinase domain (KD) and β subunit known as the carbohydrate-binding module (CBM) (1,2). Between these two modules there is a site for AMPK activators (3).

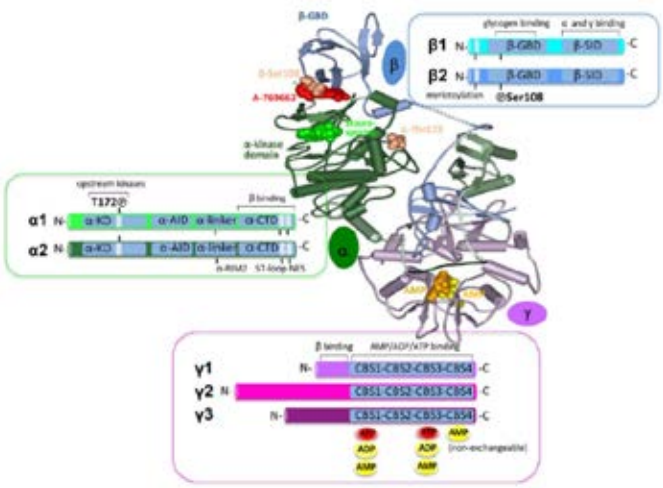

Figure. Structure of AMPK (2)

With AMPK activation, catabolic pathways such as fatty acid oxidation, glucose uptake, glycolysis, autophagy, and mitophagy are activated, anabolic pathways such as protein synthesis, fatty acid synthesis, sterol synthesis, glycogen synthesis, gluconeogenesis are inhibited (4). While AMPK is allosterically activated by AMP, it is also activated in various conditions such as starvation, hypoxia, intoxication, and inhibition of the mitochondrial respiratory chain, where cellular energy is consumed (5).

These important roles in cell metabolism have made AMPK a target for small drug molecules, from metabolic diseases to cancer.

Some molecules have been discovered that will allosterically activate AMPK (1) and also mediate the CBM-KD interaction, thus providing active conformation for KD. Biphenyl ring system draws attention in these compounds (6,7). Inspired by the importance of AMPK in cell metabolism, design and molecular docking studies of new AMPK activators bearing chromone core and biphenyl pharmacophore was carried out in this study.

Acknowledgements: This study was supported by a grant of TUBITAK (220S193)

Keywords: AMPK, chromone, cancer

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PM-063

Evaluation of Dexpanthenol Bilayer Oral Films

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Dexpanthenol has emollient and wound healing efficiency on mucosal membranes, and thus its liquid dosage forms has been investigated against sore throat in post-intubation. Orally disintegrating films are alternative dosage forms that shows the advantages of accurate dosing, waterless application, ease of use for geriatric and pediatric patients, in dysphagia or mental problems and as a carrier for oromucosal drugs. Either they can provide fast or sustained release according to the dosage form design related with the polymer properties (1). There is not any commercial Dexpanthenol oral film in the market, probably due to its relatively high dose for film preparation. However, being in liquid form with high solubility in aqueous media provides an advantage in production of Dexpanthenol films.

Bilayer film production is a newer approach preferred for dose or release profile modification in oral films (2). In this study, Dexpanthenol bilayer films were produced by connecting two separate films with different methods. Each monolayer was designed to provide 50 mg of Dexpanthenol and were produced by solvent casting method. The first monolayer was produced using Pullulan whereas the second was produced from HPMC: Kollicoat IR: Maltodextrin mixture and the total polymer percentage of each layer was 60-63%. These layers were later connected to form a bilayer film by applying either double casting, compression, drop or compression with pasting methods (3). The content uniformity of Dexpanthenol was determined with a validated HPLC analysis method at 210 nm using 0.1% phosphoric acid (pH 3): Methanol buffer (80:20) as mobile phase. SEM analyzes were used to examine the thickness and morphological properties of films. Disintegration and dissolution of films was evaluated using incubator at 37°C, 50 rpm and pH 6.75 PBS buffer as dissolution medium. Released amount of Dexpanthenol was analyzed using HPLC.

The bilayered films were between 287-330 µm in thickness and the first layer was thinner than the second layer. According to SEM studies most promising method was compression with pasting. The individual disintegration times were 3 and 19 minutes for first and second monolayers, respectively. These disintegration times were found suitable for providing a modified bilayer film in the treatment of sore throat, which the second layer was chosen as the bottom of the bilayer system. Dexpanthenol release from the bilayer films continued up to 45 minutes that was attributed to the swelling and erosion property of HPMC used in the bottom layer of the films. As a result, a bilayer film formulation containing approximately 100 mg of Dexpanthenol in a surface area of 8.5 cm^2 was obtained.

Acknowledgement: This study was granted by TUBITAK 1002 with the project number of 220S866.

Keywords: Orally disintegrating films, dexpanthenol, pullulan, hydroxypropyl methyl cellulose, solvent casting

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PM-064

Potential Binding Mechanisms Between Silk Fibroin and Sulfonamide Drugs

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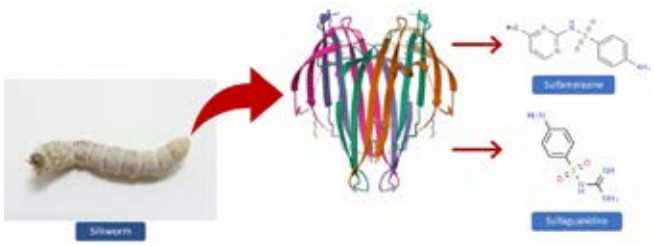
Anticoagulant materials have been widely used in the treatment of thrombosis. Many researchers have attempted to design a new synthetic anticoagulant or antithrombogenic material with a heparin-like structure and activity that mimics heparin. The anticoagulant effect is demonstrated by the presence of ionic functional groups, and anticoagulant materials are mostly composed of ionic polymers containing sulphate, sulphamide and carboxylic acid groups. It has been shown in the literature that the addition of sulphate and sulphonate groups to polymers influences the anticoagulant activity of the polymer. Silk fibroin (SF), derived from the silkworm Bombyx mori, is a fibrous protein widely used as a potential biomaterial in biomedical applications. In addition, SF has good biocompatibility, oxygen permeability, biodegradability, minimal inflammatory effect, morphological elasticity and mechanical properties. Sulfomerazine and sulfaguanidine, also known as antibacterial agents, belong to the sulfonamide class of drugs. These drugs inhibit the growth and reproduction of several types of bacteria that cause wound, respiratory and urinary tract infections. In this study, we investigated potential binding pathways between SF, sulfomerazine and sulfaguanidine using in silico approaches. To complement the in silico studies, experimental investigations were also carried out. For this purpose, regenerated SF solution was synthesized and then mixed with sulfomerazine and sulfaguanidine drugs at defined concentrations. The physical and chemical binding mechanisms were also characterized by viscosity measurements. The overall results showed that molecular and binding interactions can guide further experimental studies to validate the binding and assess the potential of using silk fibroin as a carrier system for sulfomerazine and sulfaguanidine in the design of anticoagulant materials.

Keywords: Silk fibroin, sulfomerazine, sulfaguanidine, molecular docking, anticoagulant materials

Figure 1.



Binding mechanisms between silk fibroin and sulfonamide drugs

PM-065

Development of Lanthanide Probes for Mapping ROS Levels in Brain Tissue

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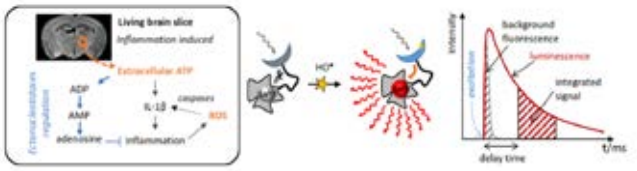
Inflammatory reactions are commonly associated with infections and acute damage to the nervous system, as well as with chronic pathologies. The chronic inflammatory state is known to contribute to the progression of neurodegenerative diseases, and inflammation to be associated with conditions of oxidative stress, i.e. an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense systems. These conditions ultimately lead to the appearance of neuronal damage. However, the contribution of ROS to the inflammatory response is still poorly understood. It is possible to study the inflammatory response by working on slices of brain, kept alive, for which the tissue architecture of the regions of the brain is preserved. The quantification of $HO^{\bullet}$ radicals, in particular, in very low concentrations, can be carried out by time-resolved fluorescence microscopy using a lanthanide luminescent probe. The interest of such probes lies in their specificity and their detection

sensitivity thanks to a time-resolved (TR) measurement which makes it possible to overcome the autofluorescence of biological media[1].



The HO• lanthanide probe is prepared from a macrocyclic-terpyridine ligand[2], by introducing a *p*-aminophenyl group, followed by complexation with the Eu^{3+} and/or Tb^{3+} ions. The response of the probes is evaluated by TR- luminescence measurements in an aqueous phase at pH 7.4, after generation of HO• by a Fenton-like reaction, in the presence of copper ions, ascorbate and hydrogen peroxide. The probe has been fully characterized, before and after reaction with HO•, by Nuclear Magnetic Resonance (NMR), by TR-fluorescence measurements and by Ultra-High Pressure Liquid Chromatography coupled to Mass Spectrometry (UPLC/MS).

Acknowledgement: This work was granted by CNRS-MITI Metallomix program.

Keywords: Lanthanide probe, reactive oxygen species, brain tissue, luminescent

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PM-066

Spectrophotometric Analysis of Folic Acid with Dispersive Liquid-Liquid Microextraction-Deep Eutectic Solvents (DESs) Technique

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Folic acid is the synthetic form of folate, a naturally occurring B vitamin. Folate helps make DNA and other genetic material. It is especially important in prenatal health. Folate, also called vitamin B-9, is a B vitamin found naturally in some foods [1]. Folic acid is the form of folate that manufacturers add to vitamin supplements and fortified foods. For example, it helps the body make healthy new red blood cells. Red blood cells carry oxygen throughout the body. If the body does not produce enough of these, a person can develop anemia, which can cause fatigue, weakness and a pale complexion.

In this study, a spectrophotometric determination method was developed using the dispersive liquid-liquid micro-extraction technique[2] for the easy, accurate and sensitive analysis of trace levels of folic acid in vitamin supplements samples. The absorbance values for folic acid were observed at two different wavelengths, 288 and 365 nm. It was used a phosphate buffer solution at pH 9.0. it was possible to determine the concentration of folic acid in at a λ_{ideal} of 288 nm in a linear range of 250–2500 ng ml−1 and recovery between 94.0 and 108.0 %.

Acknowledge: This study has been supported by TUBITAK as a 2209-A student project with the 1919B012111838 code.

Keywords: Folic acid, UV-VIS, Dispersive liquid-liquid microextraction, vitamin supplements

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PM-067

Amine Functionalized Multi-Walled Carbon Nanotube-based Electrochemical DNA Biosensor for Evaluation of Efavirenz-DNA Interaction

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Efavirenz is a non-nucleoside reverse transcriptase inhibitor primarily used for treating human immunodeficiency virus [1]

and against colorectal, glioblastoma, and pancreatic cancers [2, 3]. Lately, a growing approach has been that approved drugs have been tested for additional purposes.

The construction of DNA nanobiosensor based on amine-functionalized multi-walled carbon nanotubes is fabricated for Efavirenz-DNA interaction. The functionalized multi-walled carbon nanotube is characterized by SEM, FTIR, Raman Spectroscopy and XRD techniques. The experimental conditions are optimized for nanobiosensor such as dropping volume of nanomaterial suspension, activation of nanosensor and concentration of ct-dsDNA. The experimental measurements were performed by differential pulse voltammetry using a glassy carbon electrode. Thanks to the amine-functionalized multi-walled carbon nanotubes, the peak currents of dsDNA were increased and the peak potentials of Efavirenz have shifted to the less positive potential. In addition, the interaction mechanism between dsDNA and Efavirenz was investigated using bare glassy carbon electrodes at different times, various concentrations and different physiological conditions.

Acknowledgement: The authors would like to thank the Scientific Research Projects Coordination Unit (BAP) of Ankara University, Turkiye, for supplying financial support with Project 20B0237004.

Keywords: Efavirenz, biosensor, DNA-drug interaction, amine-functionalization, MWCNTs

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PM-068

Multicomponent (Ugi-4MCR, Passerini-3MCR) reactions for the synthesis of adamantane containing bioactive derivatives

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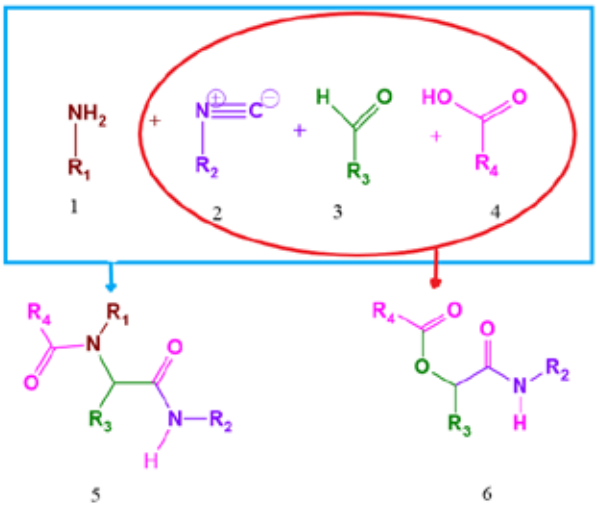
In modern synthetic chemistry, multicomponent reactions (MCR) are considered diversity-oriented syntheses that provide the opportunity to create structurally diverse molecules. Compared to classical methods, multicomponent reactions involve the interaction of three or more reacting components in one flask, where condensation, (re)functionalization or cyclization reactions take place simultaneously and, accordingly, new compounds with increased molecular complexity, interesting structure or properties are obtained.

Among these multicomponent reactions, the Ugi four component reaction and Passerini three-component reaction are worth mentioning, where amine, aldehydes, isonitriles, and carboxylic acids are used as reactive components, which ensure the synthesis of polyfunctionalized α-acyloxyamides (depsipeptides) and dipeptides. The mentioned reactions are distinguished by its flexibility and molecular diversity, and due to the properties of the reactive groups, these reactions provide an opportunity to obtain analogs of natural compounds, the synthesis of which is a long process by known methods or does not proceed at all. Therefore, during the last three decades, advances in the Ugi and Passerini reaction have been impressive for obtaining interesting structures, also heterocycles and peptidomimetics from α-acyloxyamides. The mentioned syntheses turned out to be very interesting for pharmaceutical chemistry, and therefore the heterocycles, peptidomimetics and pseudopeptides obtained in this way have widely gained a place in medicine as drugs.

The conducted research aimed to synthesize adamantane moiety-containing new compounds based on the Ugi and Passerini reaction, investigating their structure-property relationship, and subjecting them to pharmacological screening.

The introduction of the adamantane moiety in the mentioned reactions are inspired by the special properties of adamantine-containing compounds, which caused great attention from scientists since the sixties of the last century. The discovery of amantadine (1960-67) as a drug with antiviral and antiparkinsonian properties laid the foundation for the synthesis of new adamantine-containing compounds and their study on biological activities. Many compounds synthesized based on adamantane are anti-viral (including anti-influenza A, anti-herpes, anti-hepatitis C, and anti-malarial), anti-bacterial, anti-fungal, anti-inflammatory, antiparkinson, and anti-cancer drugs.

In the research, the multicomponent Ugi reaction was performed by interaction of amine (1), isonitrile (2) aldehyde (3) and carboxylic acids in ethanol at 40°C, stirring for 72 hours. The corresponding pseudopeptides (5) are obtained with a yield of 40-55%. The Passerini reaction was conducted by the interaction of isonitrile (2) aldehyde (3) and carboxylic acid (4) in dichloromethane at room temperature, stirring for 24 hours and the corresponding α-acyloxyamides (6) were isolated with a yield of 75-85%.



The structure of the synthesized compounds was determined by IR, NMR spectroscopy, and Mass spectral analysis.

The synthesized compounds (5 and 6) were tested against certain pathogenic bacteria species (Salmonella, Klebsiella, bacillus, Proteus, Streptococcus, Enterococcus, pseudomonas, Staphylococcus, Shigella, Escherichia Coli) using disc diffusion methods. The test results for each compound revealed interesting antimicrobial activities.

Acknowledgment: Financial support for the project was provided by the Shota Rustaveli National Science Foundation under Grant No. YS-21-1340.

Keywords: Multicomponent reaction, ugi reaction, passerini reaction, adamantane

PM-070

Inkjet Printing of 2D/1D MXene/hCNT Nanoflowers Flexible Electrochemical Sensors for the Detection of Caffeine

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MXenes are considered one of the most widely used two-dimensional (2D) materials in electrochemistry owing to their large physical surface area, high electronic conductivity, and highly hydrophilic nature. In the present work, we designed MXene/carbon nanotube-containing inks for the fabrication of flexible electrochemical caffeine sensors. The sensors were printed on flexible substrates using a personnel ink-jet printer and the electrochemical performance against caffeine was evaluated. The results indicated that flexible electrodes with high electronic conductivity and enhanced electrochemical performance can be fabricated by the incorporation of MXene and holey CNTs in the electrode layer.

Keywords: Inkjet printing, flexible sensors, MXene, holey CNT, electrochemical sensor

PM-071

Accurate and Precise Determination of Pregabalin in Pharmaceutical Formulations using HPLC-UV Methodology

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Pregabalin is a widely used antiepileptic drug with anxiolytic and analgesic properties. The accurate and precise determination of pregabalin is of great importance in pharmaceutical formulations, clinical studies and forensic cases. High-performance liquid chromatography (HPLC) is a popular analytical technique for the analysis of pregabalin due to its high sensitivity and specificity but the number of HPLC-UV studies in the literature is very limited.

In this study, an HPLC-UV method was developed and validated for the determination of pregabalin in pharmaceutical dosage form and biological samples. The method involved the use of a monolithic column (Chromolith® RP100 ,18- x 4.6 mm analytic column) with a mobile phase consisting of acetonitrile and 0.1% phosphate buffer (pH 7.0) in a ratio of 5:95 (v/v) at a flow rate of 2 mL/min. The detection was performed at 200 nm using a UV detector.

The method was validated according to ICH guidelines for linearity, precision, accuracy, specificity, and robustness. The

method showed excellent linearity over a concentration range of 1-50 µg/mL with a correlation coefficient of 0.9995. The precision was found to be within the acceptable limits with a %RSD of less than 2%. The accuracy of the method was also within the acceptable limits with a % recovery of 99.5-101.5%.

The method was successfully applied for the determination of pregabalin in pharmaceutical dosage forms. The developed method was found to be simple, accurate, and precise and can be used for the routine analysis of pregabalin in pharmaceutical formulations and clinical studies.

In conclusion, the HPLC method developed in this study can be used as a reliable tool for the analysis of pregabalin in pharmaceutical formulations and biological samples.

Keywords: Lyrica, HPLC-UV, Method Development, Pregabalin, Validation, Forensic Toxicology

PM-073

Development of an Electrochemical Biosensor for the Determination of Lactate Dehydrogenase

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Lactate dehydrogenase (LDH) is a cytoplasmic enzyme in the glycolytic anaerobic pathway. It catalyzes the reversible conversion of lactate to pyruvate while reducing nicotinamide adenine dinucleotide (NAD) to dihydro nicotinamide adenine dinucleotide (NADH). In normal cells, the pyruvate is converted to acetyl coenzyme A through an oxidative phosphorylation process in the mitochondria to produce adenosine triphosphate (ATP). However, tumor cells exhibit a phenomenon known as the Warburg effect, where they produce large amounts of lactic acid even in the presence of sufficient oxygen. This is due to the increased glycolysis rate in tumor cells, which converts pyruvate to lactate instead of entering the mitochondria. LDH plays a crucial role in this process. LDH catalyzes the conversion of pyruvate to lactate, contributing to the elevated levels of lactate in tumor cells. As a result, LDH levels are often elevated in cancer patients, and it has become a consensus biomarker for tumors. For these reasons, it has become important to develop rapid and sensitive methods for LDH detection [1, 2].

In this work, we proposed an electrochemical biosensor for the detection of LDH. Prior to the detection of LDH, we modified the SPCEs (Dropsens 220AT) with AuNPs (Au@SPCE) for enhancing the conductivity. Then we modified Au@SPCE with CoHCF film for its electrocatalytic activity to enzymatic reaction. As the first step, we optimized the experimental conditions including scan rates and cycles for the deposition of AuNPs, the procedure of CoHCF film modification, electrolyte solutions, the concentrations of lactate, and NAD. Under the optimized conditions, we modified the SPCEs with AuNPs by electrodeposition using the CV technique in a solution of 0.01M $HAuCl_4$ (prepared in 0.5 M H_2SO_4 and 0.1 M KCl). The electrochemical behavior of Au@SPCEs was performed with CV using 5.0 mM $[Fe(CN)_6]^{3-\ 4-}$. Then CoHCF film was electrochemically deposited on the Au@SPCEs using 1.0 mM $CoCl_2.6H_2O$ and 2.0 mM $K_3Fe(CN)_6$ solutions.

Finally, to demonstrate the applicability of the electrochemical sensor for sensitive quantification of LDH, we performed amperometry with various concentrations of LDH solutions under the optimized conditions. Hence, 0.1 M KCl, 2.5 mM NAD (prepared in 0.1 M PBS pH 8.4), and 10.0 mM lactate (prepared in 0.1 M PBS pH 8.4) were dropped onto the working electrode, respectively. Then the enzymatic reaction was started by adding different concentrations of LDH solution. The measurements were performed at a constant potential of + 0.6 V for 180 s. The change in current with time was recorded due to the production of NADH. The linear relationship between varying LDH concentrations and current is observed in the range of 0.03 U/mL to 30 U/mL, with a limit of detection (LOD) at 0.01 U/mL. The corresponding linear equation was $y = 0.0029x + 0.0228$ with a correlation coefficient (R^2) of 0.9981.

Acknowledgement: This research was supported by the Scientific and Technological Research Council of Turkey (TUBITAK) (22AG008).

Keywords: Lactate dehydrogenase, electrochemical detection, optimization, amperometry

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PM-074

Electrochemical Behavior of Enzalutamide In The Presence of Human Serum Albumin

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The interaction between Enzalutamide and human serum albumin (HSA) was investigated using the cyclic voltammetric (CV), differential pulse (DPV) and electrochemical impedance spectroscopy (EIS) techniques. The results demonstrates that the addition of the drug to the $K_3Fe(CN)6/K_4Fe(CN)_6$ electrolyte solution led to a slight decrease in the redox peak currents. However, in the presence of HSA, the decrease in peak currents and the negative shift in peak potentials were highly significant. Furthermore, the subsequent addition of Enzalutamide in the presence of HSA resulted in further reduction in redox peak currents and negative shift of peak potentials (curve d), indicating an interaction between HSA and Enzalutamide that decreased the electron transfer rate constant of $K_3Fe(CN)_6/K_4Fe(CN)_6$.CV was employed to analyze a fixed concentration of HSA (1 µM) in $(K_3Fe(CN)_6/K_4Fe(CN)_6$ (pH 7±0.01), while the concentration of ENZ in the solution was varied from 9^{-10}×1 M to 8^{-10}×1 M.

Keywords: Enzulatamide, interaction, albumin, electrochemistry

PM-075

Investigation of Electrochemical Interaction Between Olaparib and Albumin

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Olaparib is a poly(ADP-ribose) polymerase (PARP) inhibitor used in cancer treatment, particularly for ovarian and breast cancers with BRCA mutations. Albumin is the most abundant protein in human plasma and plays a crucial role in drug transport and distribution in the bloodstream.The investigation of the electrochemical interaction between olaparib and albumin can provide insights into their binding affinity and potential influence on drug efficacy and distribution in the body. Electrochemical techniques, such as cyclic voltammetry and impedance spectroscopy, can be employed to study the interaction between olaparib and albumin. These techniques can provide information about redox reactions, charge transfer processes, and changes in electrical properties that occur during their interaction. By comparing the electrochemical signals obtained in the absence and presence of albumin. The binding of drugs to albumin can have implications for their pharmacokinetics, including distribution, elimination, and therapeutic efficacy. Albumin has multiple binding sites, and it can bind various drugs, including small molecules like olaparib. Such interactions can affect the free concentration of the drug in the bloodstream and its ability to reach the target site. In this study, olaparib and human serum albumin interaction was investigated by electrochemical techniques cyclic voltammetry, differential pulse voltammetry and eelctrochemical impedance spectroscopy. The ferri-ferro redox couple was used with 0.1 M NaCl at pH 7.4. The binding constant between albumin and olaparib was investigated.

Keywords: Olaparib, electrochemistry, albumin

PM-076

Development and Validation of Analytical Method for Quantitative Analysis of Plumbagin: Application to Proliposomal Formulation

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Cancer is a significant health issue due to its high occurrence and fatality in recent times. Existing chemotherapy methods used in cancer treatment lack tumor specificity, often exhibit toxicity in healthy tissues, and face challenges of drug resistance, resulting in limited effectiveness. The role of oxidative stress in cancer development, increased tumor aggressiveness, and resistance to treatment are well-known. Nuclear factor E2-related factor (NRF2) plays a role in redox metabolism and antioxidant defense. Recent studies have demonstrated frequent activation of NRF2

in various types of cancer, making NRF2 inhibition a promising strategy for cancer treatment [1]. Plumbagin has been shown to suppress NRF2-mediated defense mechanisms and promote tumor suppression in different cancer type [2]. However, formulations of plumbagin with high efficacy and specificity for clinical applications have not yet been discovered and developed. Our research interest focuses on the preparation and in vitro characterization of proliposomal formulation of plumbagin. The current study involves the development and validation of the spectrophotometric analytical method and its application for the formulation development of proliposome based plumbagin formulations.

The spectrophotometric analysis of plumbagin was carried out using Agilent Carry 60 UV-Vis (Santa Clara, USA). The standard solutions of plumbagin in ethanol were analyzed at a λmax of 416 nm to obtain calibration curve was obtained and proposed methods was further validated according to ICH Q2 Validation of analytical procedures in terms of precision, accuracy, linearity and range. The limit of detection and quantification values were also calculated. The linearity range of the method was 0.005-0.045 mg/ml (r^2:0.9994) and the precision of the method was demonstrated. The developed method was used to determine the amount of Plumbagin loaded in proliposomal formulations. Proliposomes were prepared by slurry method using soy phosphatidylcholine, cholesterol, and maltodextrin [3]. The quality tests including production yield and consolidation properties were conducted on drug loaded proliposomal powders. Proliposomes were hydrated to obtain liposomes and the encapsulated drug percent was 53.7±3.4% as estimated by the validated method. The liposomes were nanosized (669±12.22 nm) and their zeta potential was -70.9 ±2.7 mV.

Proliposomes encapsulating plumbagin were prepared and well characterized. The encapsulation efficiency was estimated using the validated spectrophotometric method. The analytical validation results revealed that the developed method is accurate and can be used for plumbagin assay.

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Keywords: Analytical validation, spectrophotometric analysis, plumbagin, proliposomes

PM-077

Electrochemical, Spectrofluorimetric & Molecular Docking Approaches to Evaluate the Interaction of Cinacalcet HCL with Calf-Thymus dsDNA

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The ability of drugs to bind to DNA is crucial for discovering novel therapeutics and creating more effective pharmaceuticals. In this work, the interaction and mechanism of binding of cinacalcet (CIN), a calcimimetic medication that mimics the effects of calcium on tissues, with calf thymus double-stranded deoxyribonucleic acid (ct-dsDNA) were examined. Using the differential pulse voltammetry (DPV) method, the interaction of CIN with ct-dsDNA was investigated by observing the decline in electrochemical oxidation signals to deoxyguanosine and adenosine. To investigate the drug's interaction with ct-dsDNA by fluorescence spectroscopy, a competitive assay on the indicator methylene blue was carried out. Studies of interactions have suggested that groove-binding may be the mechanism through which CIN interacts with ct-dsDNA. The values of the binding constants, as determined by the cyclic voltammetry (CV) and spectroscopic approaches, were found to be 6.30×10^4 M^{-1} and 3.16×10^5 M^{-1}, respectively, at 25 °C. Through molecular docking studies, potential interactions between CIN and dsDNA were investigated. Through H-bonding and π-π stacking interaction with CIN, the docked structure showed that CIN could fit into the minor groove of the DNA with effortlessly

Keywords: Calf thymus double-stranded deoxyribonucleic acid, cinacalcet, electrochemistry, fluorescence spectroscopy, molecular docking

PT-001

New Developments in Coated Blade Spray-Mass Spectrometry for High-Throughput and Rapid Analysis

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Coated blade spray coupled with mass spectrometry (CBS-MS) combines solid-phase microextraction's (SPME) efficient sample clean-up and enrichment and ambient MS's fast analysis and has proven to be an appealing alternative tool for the fast screening of target analytes in complex matrices. Herein, we focus on two important technical developments regarding CBS-MS including: 1) Analysis in both positive/negative modes using single blade; 2) Improving negative electrospray ionization (ESI) in CBS-MS by using a blade with barrier design.

High-throughput screening and quantitation of large numbers of compounds, especially in omics analysis, requires the use of both positive and negative modes in ESI-MS. For realizing the polarity switching using single blade, three different strategies were proposed including: 1) One side of the blade for positive mode, and the other for negative mode; 2) Separating the ESI to two segment and applying negative and positive mode in sequential; 3) Real time polarity switching on the ESI source. From the results, all the above three strategies showed ideal reproducibility and have different features and advantages. Among them, the first strategy showed the best overall sensitivity as the desorption solvents for positive mode and negative mode can be optimized and applied separately on different sides of the blades. By using this strategy, high-throughput SPME was coupled with CBS-MS for the rapid analysis of 20 drugs of abuse in saliva samples in both positive and negative mode. The proposed method provided LODs between 0.005-10 ng/mL, with $R^2 \geq 0.9925$, accuracy between 72% and 126%, and RSD% < 15% for all three validation concentration levels.

Substrate-based electrospray ionization (ESI) techniques like paper, wooden tip, plastic tip, and metal-needle-based spray suffer from corona discharge, high background noise, and unstable spray in negative ionization mode, especially for the analysis of complex biological matrices, such as blood and urine. We developed a new CBS design that features a barrier at the far end of the ESI tip. The findings of this work show that the addition of this simple barrier enabled the total RSD% to be reduced to less than 10% for sample preparation, ionization, and the MS detection of several drugs of abuse in negative mode, without compensation using internal standards. The improved stability of ESI in negative mode was investigated by observing the ESI process with a micro-camera and testing via CBS-MS. The new design was applied for the analysis of three drugs of abuse in urine, with the calibration curve correlation coefficient ($R^2 \geq 0.9997$) being calculated without the use of internal standards. The overall RSD% of the peak area for one compound in 42 samples was 6.9%, which highlights the method's incredible reproducibility compared to other ambient MS techniques for analyzing real samples. The CBS device with a barrier was also applied for the on-blade sampling of 14 drugs of abuse in 20 µL of plasma spot in positive ionization mode. The results of these tests yielded a calibration curve correlation coefficient of $R^2 \geq 0.9883$ and limits of quantification (LOQ) between 0.25-25 ng/mL.

Keywords: Coated blade spray, Mass spectrometry, Solid-phase microextraction, High-throughput, Automation

PT-002

Vertical İntroducing Bio-Compatible Solid-Phase Microextraction Pin to Mass Spectrometry Using Probe Electrospray Ionization Interface for High Sensitivity and Low Matrix Effect Analysis

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Probe ionization mass spectrometry (PESI) using an ultrafine needle to sample extremely small volume of sample on its surface and subsequently applying high voltage to initiate ESI, forming nano droplets with reduced matrix effect. The sensitivity of PESI-MS can be an issue because the amount of ions generated is limited. Herein, SPME pin-PESI device with large diameter and bio-compatible SPME coating on the surface was proposed for increasing the sensitivity of PESI-MS by increasing the picked-up volume and enrichment effect of SPME, while minimizing matrix effect because selectivity of the biocompatible coating. The new design was applied for the analysis of 8 drugs of abuse in urine samples with the good linearity ($R^2 \geq 0.9997$), high sensitivity with limits of detection (LODs) between 0.003 to 0.03 ng/mL and good reproducibility with RSD% ≤ 6%.

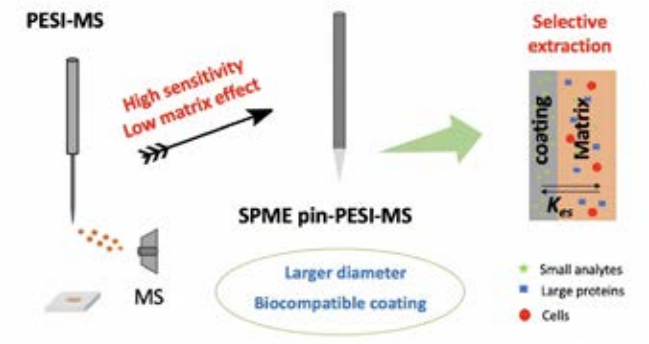
A stainless-steel pin device with diameter of 1.5 mm and cone length of 5 mm was coated with a thin layer of biocompatible SPME coating on the cone part. The coating consisting of hydrophilic-lipophilic balanced (HLB) particles embedded in

polyacrylonitrile (PAN) binder. After extraction for 30 min by direct immersion in the sample with vortex and brief washing with water for 5 s, the SPME-pin device was directly insert into the Shimadzu DPiMS source for automated desorption and ionization by pick-and-spray strategy.

The pin device is compatible with the Shimadzu DPiMS source and doesn't need any modification of the MS instrumentation. Without coating material, the new pin design showed much higher sensitivity than the original PESI device when analysis the same concentration of the target drugs in solvents, as the pin device which has larger diameter can pick up more sample after dipping. After coating, a thin bio-compatible coating material (HLB/PAN) can be immobilized on the pin device. The SPME pin can be directly used in biological samples, as the PAN acts as both a binder and a matrix-compatible barrier, thus enabling the enrichment of small molecules while eliminating interferences associated with presence of interfering macromolecules. For the extraction of 8 drugs in the same PBS solutions, the extracted amount of the new SPME pin device was 22-82 times higher when compared with the previous SPME probe-PESI device because of the larger volume of coating material. Both of the above two factors, including more pick-up volume and higher extraction efficiency, made the SPME pin-PESI-MS show significant improvement of sensitivity when compared with the PESI-MS and the coated original SPME-PESI device reported previously. Finally, the SPME pin-PESI-MS method was used for the quantitative analysis of 8 drugs in urine samples, this new method showed 166-1666 times lower LODs when compared with the PESI-MS method, which demonstrated its ultra-high sensitivity. The SPME pin-PESI-MS method also showed good linearities with $R^2 > 0.9997$. In addition, the vertical arrangement of the SPME pin-PESI-MS system facilitates future fully automated and high-throughput analysis by incorporating with CTC autosampler system.

Keywords: Solid-phase microextraction pin, probe ionization, mass spectrometry, bio-compatible coating, matrix effect

Direct coupling of biocompatible SPME pin and MS using PESI



The new SPME pin device for the SPME-PESI-MS analysis with high sensitivity and low matrix effect

PT-003

Validated LC-MS/MS Method for Simultaneous Quantification of KRASG12C Inhibitor Sotorasib and Its Major Circulating Metabolite (M24) in Mouse Matrices and Its Application in a Mouse Study

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Sotorasib (LumakrasTM; LumykrasTM) is the first approved KRASG12C inhibitor by both US Food and Drug Administration (US FDA) and European Medicines Agency (EMA). M24 (des[methylpiperazinylpropenone (MPPO)]-sotorasib dione) was one of the major circulating metabolites of sotorasib across species. Using a reversed-phase liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS), a bioanalytical method to quantify sotorasib and its major circulating metabolite (M24) in mouse plasma and seven tissue-related mouse homogenates was developed. We utilized a fast and efficient protein precipitation method in a 96-well plate format to extract both analytes from biological matrices. Erlotinib was selected as the internal standard in this assay. Gradient elution using methanol and 0.1% formic acid in water (v/v) was applied on an Acquity UPLC BEH C18 column to separate all analytes. Sotorasib, M24, and erlotinib were detected with a triple quadrupole mass spectrometer in positive electrospray ionization in multiple reaction monitoring mode. During the validation and sample quantification, a linear calibration range was observed for both sotorasib and M24, in a range of 4 – 4,000 nM and 1 – 1,000 nM, respectively. The %bias and %CV (intra- and inter-day) for all tested levels in all investigated matrices were lower than 15%, as required by the guidelines. Sotorasib has a rather short room temperature stability in mouse plasma for up to 8h compared to M24, which is stable up to 16h at room temperature. The validated method was successfully applied to measure sotorasib, and M24 from a mouse study consisting of three different mouse strains, *i.e.*, wild-type (FVB/NRj), Cyp3a-/- (Cyp3a knocked

out), and CYP3XAV (human CYP3A4 overexpression on the liver and small intestines) mouse. Based on our data, we conclude that the plasma exposure of sotorasib in mice is limited via human CYP3A4- and mouse Cyp3a-mediated metabolism of sotorasib into M24.

Keywords: Sotorasib, M24, KRASG12C inhibitor, major circulating metabolite, LC-MS/MS

PT-004

Modelling the Enantiorecognition of Structurally Diverse Pharmaceuticals on O-substituted Polysaccharide-based Stationary Phases

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More than 60% of the newly commercialized drugs possess chiral properties. Since one enantiomer in a racemic mixture might be ineffective or toxic, strict guidelines are defined for the development of a chiral drug. The separation of chiral drug molecules, mostly by means of chromatography, is therefore an intensively studied domain. While the separation of enantiomers is important in many fields of industrial and pharmaceutical analysis, the selection of a suitable chromatographic system (chiral selector and mobile phase) is not trivial. As a consequence, chiral method development requires considerable experimentation and is often highly demanding with respect to time, material and labour.

This study aims to develop models to predict the retention, enantioseparation and elution sequence of structurally diverse enantiomers. More specifically, Quantitative Structure Retention Relationship (QSRR) models are built that describe the relationship between molecular descriptors and retention.

Eighteen structurally diverse chiral molecules were analyzed on two polysaccharide chiral stationary phases, Chiralcel OD-RH (cellulose tris(3,5-dimethylphenylcarbamate) selector) and Lux amylose-2 (amylose tris(5-chloro-2-methylphenylcarbamate) selector), using a basic and an acidic mobile phase, and their retention times and elution sequences were determined. Both achiral and in-house developed chiral descriptors were used as independent variables to build the models. Linear regression techniques, such as multiple linear regression (MLR) and partial least squares (PLS) regression, were applied to model the retention or separation as a function of the descriptors. In a first step, models were built with only achiral descriptors to model the global retention of the molecules. Subsequently, models were built with only chiral descriptors to predict the enantioseparation and finally models were constructed with both chiral and achiral descriptors to predict the retention, separation and elution sequence of enantiomers.

The general retention was predicted well by MLR models containing only achiral descriptors. The models built with only chiral descriptors were not able to predict the enantioseparation or elution sequence. Finally, the models containing both chiral and achiral descriptors allowed predicting the retention well, but the predictions of elution sequence and separation showed varying degrees of success.

Although the results of the latter models are promising concerning the prediction of the enantiomeric retention, the number of well-separated compounds in the test set should be increased in order to obtain a more representative set of molecules.

Keywords: Quantitative structure - (enantioselective) retention relationships, chiral descriptors, polysaccharide chiral selectors, reversed-phase liquid chromatography

PT-005

FPSE-HPLC-UV/Vis Method for the Favipiravir Quantification in Biological Matrices

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In the present project, an easy, robust and fast method in FPSE-HPLC-UV/Vis for the quantification of favipiravir (FVP) in human plasma and breast milk has been developed and validated.

In that method, a polycaprolactone-block-polydimethylsiloxane-block-polycaprolactone sol-gel (sol-gel PCAP-PDMS-PCAP) coated on 100% cellulose cotton fabric was selected as the most efficient membrane for FPSE in human plasma samples and breast milk, while the HPLC-UV/Vis analysis

was performed using a RP C18 column under isocratic conditions. Following method development in biological matrices, the overall chromatographic analysis time was only 5 minutes without encountering matrix interference. The validation process made it possible to highlight the wide range of linearity (0.2–50 µg/mL and 0.5–25 µg/mL for plasma and breast milk, respectively). The sensitivities of the method in terms of limit of detection (LOD) and limit of quantification (LOQ), were respectively equal to 0.06 and 0.2 µg/mL for plasma and 0.15 and 0.5 µg/mL for the milk. Intraday and interday precision and trueness were lower than 3.61% for both matrices.

The present method was finally applied and tested on real samples for therapeutic drug monitoring (TDM). To our knowledge, this is the first validated FPSE-HPLC-UV/Vis method in human plasma and breast milk for TDM purposes applied on real samples. The validated method provides a rapid, simple, cost-effective and sensitive assay for direct quantification of favipiravir in real biological matrices, also applying a well-known robust and cost-effective instrument setup. Furthermore, by providing for an elution in isocratic conditions, it allows bypassing the problems associated with the transferability of the method from one instrument to another, allowing the chromatographic performances to be maintained unaltered.

Keywords: HPLC, Favipiravir, Fabric phase sorptive extraction, Plasma, Breast milk, TDM

PT-006

FPSE-HPLC-PDA Quantification of Seven Antidepressant Drugs in Post–Mortem Samples

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In the present work, the FPSE technique was applied for the first time for the simultaneous, simple, and rapid analysis of seven common antidepressant drugs (AD, venlafaxine, citalopram, paroxetine, fluoxetine, sertraline, amitriptyline, and clomipramine) in post-mortem samples, especially whole blood and cerebrospinal fluid collected during autopsies. This technique, very advantageous as it allows minimal manipulation of the sample and therefore a reduction of possible losses of analytical information has made it possible to obtain a sample preparation that complies with the principles of Green Analytical Chemistry (GAC) and Green Sample preparation (GSP).

Among all the FPSE membranes tested in the present project, the Carbowax 20 M sol-gel coating (20 M CW sol-gel) on cellulose substrate showed optimal extraction efficiency for the considered ADs. The selected drugs were analyzed and detected by the reversed phase high performance liquid chromatography (RP-HPLC) method coupled to the photodiode detector (PDA). An isocratic elution was applied which allows complete separation of all analytes in just 20 minutes using ammonium acetate buffer as the aqueous mobile phase and acetonitrile (AcN) as the organic modifier. The limit of detection (LOD) ranged from 0.04 to 0.06 µg/mL and the limit of quantification (LOQ) was 0.1 µg/mL for all analytes except venlafaxine, which was 0.2 µg /mL.

This method, fully validated in accordance with international guidelines, has been tested on real samples to evaluate its applicability in the forensic field. All the real samples resulted negative, as was also confirmed by the confirmatory analyzes from the accredited laboratory of Pharmatoxicology - Hospital "Santo Spirito". Furthermore, by providing for an elution in isocratic conditions, it allows bypassing the problems associated with the transferability of the method from one instrument to another, allowing the chromatographic performances to be maintained unaltered.

Keywords: FPSE, Microextraction, post–mortem samples, forensic sample preparation, antidepressant drugs, GAC

PT-007

Adulteration Detection of Corn Oil, Rapeseed Oil, Peanut Oil, and Sunflower Oil in Argan Oil by FT-NIR and FT-MIR Spectroscopy Coupled to Chemometrics

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One of the rarest and purest vegetable oils in the world is Argan oil. For centuries, it has been used to prevent several diseases, showing anti-inflammatory, cardioprotective, and antidiabetic activities. Consequently, there is an increasing worldwide demand. This has led to adulteration practices with unhealthy, synthetic, or less expensive oils that may have negative health effects on the customers. Therefore, there is a high need for rapid, low-cost and accurate analytical methods able to identify and quantify the respective adulteration.

In the present study, Fourier Transform Near-Infrared (FT-NIR) and Mid-Infrared (FT-MIR) spectra were subjected to chemometric techniques (Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS)) to distinguish Moroccan Argan oil from other vegetable oils and to predict the percentage of its adulteration.

Argan oil was adulterated with one of four vegetable oils (sunflower, corn, rapeseed and peanut oil). Oils with different percentages of the adulterant, ranging from 0% to 40% w/w, were prepared and analyzed. The raw FT-NIR and FT-MIR spectra were acquired and preprocessed with different techniques to select the best preprocessing. PCA was used to visualize clustering trends and extract relevant information from the spectral data. As result, no clusters were identified on the FT-NIR data. Nonetheless, four clusters were clearly discernible on the FT-MIR data, dividing between samples of Argan oil adulterated with the four adulterants, each with a trend based on percentages. PLSR models using either FT-NIR or FT-MIR spectral data allowed the prediction of the percentage of the adulterant in Argan oil with a determination coefficient (R^2) higher than 0.97 and 0.96 for FT-MIR and FT-NIR, respectively; a root mean square error of calibration (RMSEC) and of prediction (RMSEP) lower than 0.063 and 0.147, respectively. Therefore, the spectroscopic tools applied in our work, associated with chemometric approaches, showed good performance in the detection and prediction of Argan oil adulterations. They may have an important impact to ensure the authenticity and quality of food.

Keywords: Argan oil, adulteration, spectroscopic techniques, chemometrics

PT-008

Analytical Niche Techniques in Phytochemistry – a Comparison of SFC and CE

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Different techniques have been employed for natural products analysis in the past, however mostly being based on well-established GC or LC. Nonetheless, there are interesting alternatives, especially when trying to combine the demands for environmentally friendly operation and high separation efficiency. Such methodologies like supercritical fluid chromatography (SFC) or capillary electrophoresis (CE) are increasingly used, yet they are still considered as niche approaches. To indicate that both are indeed equivalent and reliable alternatives, we compared them for the qualitative and quantitative determination of five dihydrochalcones (DHCs) in apple leaves. These natural products are of high pharmacological relevance as they have shown potent immunomodulatory, antioxidative, antitumor and antihyperglycemic effects as well as neuro- and hepatoprotective properties [1, 2].

Both SFC, which utilizes a CO_2-based mobile phase in supercritical state [3], as well as CE, where analytes are resolved depending on their size and charge state [4], were well suitable for the separation of five standard compounds, namely phloretin, 3-hydroxyphloretin, phloridzin, trilobatin and sieboldin in the crude methanolic extracts of apple leaves within short separation time (5 min SFC, 9 min CE). In SFC this was enabled using a Torus Diol column and methanol as a modifier. For this application the rather uncommon additive oxalic acid proved to be most suitable and was used in a concentration of 2.5 mM. In CE only borate buffers permitted the separation of the target analytes. A pH of 8.25 and the addition of isopropanol (2.5%) were found to be optimal. Method validation following ICH guidelines was performed, whereby both assays met the respective requirements for linearity

(correlation coefficients over 0.9993), accuracy and precision in a broad linear range (3.91 to 1000 µg/mL). With the developed methods a selection of different Malus sp. was analyzed for their DHC content, with results revealing a significant quantitative and qualitative variability; e.g. the total concentrations ranged from 5.47% to 17.24%.

Overall, it could be shown that for the selected application SFC and CE not only were comparable to more established techniques in terms of reproducibility and user-friendliness, but could even surpass them concerning separation efficiency and time as well as greenness. The here presented study is therefore an encouraging example to consider also less common methodologies for the analysis of natural products.

Keywords: Supercritical fluid chromatography, SFC, capillary electrophoresis, CE, dihydrochalcones

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PT-009

A Rapid Chiral HPLC Method for Determination of Enantiomeric Impurity of Lifitegrast

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Lifitegrast is an anti-inflammatory drug utilized to treat dry eye disease (DED). DED is complicated and poorly understood, but it is found that T-cell inflammation plays a significant role in the disease (Abidi et.al., 2016). Lifitegrast is a novel small molecule integrin antagonist that inhibits a specific T cell-mediated inflammatory pathway involved in the pathogenesis of DED. Based on the current understanding of its mechanism of action, lifitegrast blocks the recruitment and activation of T-cells to the ocular surface, thus lessening overall inflammatory responses. Since it protects corneal surfaces and prevents symptoms of dry eye syndrome with a rapid onset of action and an acceptable tolerability profile, this drug has been shown to be safe in the clinic as well as at systemic levels. Lifitegrast is the first treatment approved by the US Food and Drug Administration (FDA) indicated to treat both signs and symptoms of DED. The ophthalmic solution (5%) of lifitegrast is used for the treatment (Hussar & Cheeseman, 2017).

The IUPAC name of lifitegrast is "(*S*)-2-(2-(benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline -6-carboxamido)-3-(3-(methylsulfonyl)phenyl) propanoic acid" and *S*-enantiomer of lifitegrast is used to treat DED. However, *R*-enantiomer of lifitegrast "(*R*)-2-(2-benzofuran-6-carbonyl)-5,7-dichloro-1,2,3,4-tetrahydroisoquinoline-6-carboxamido)-3-(3-(methylsulfonyl)phenyl) propanoic acid" shows no effect for DED (Perez et al., 2016). Enantiomeric impurities should be controlled under the specification limits followed by the International Council for Harmonisation (ICH) rules. Our literature serch showed that there is no any chiral purity method published for determination of *R*-lifitegrast in the drug substance *S*-lifitegrast.

In this study, a chiral HPLC method was investigated for the determination of *R*-lifitegrast in the drug substance *S*-lifitegrast and its application to the ophthalmic solution containing 5% lifitegrast. Enantiomers are well separated under optimized conditions selected in line with ICH guidelines. The system suitability was conducted and evaluated throughout the validation study by injections of system suitability solution and relative standard deviation of the average areas obtained from six replicate injections of standard solution. System suitability was also controlled by resolution, tailing factor, capacity factor, separation factor parameters in the chromatogram of system suitability solution. All validation parameters were fulfilled with optimized conditions. As a result, the validated lifitegrast chiral purity method was specific, sensitive, linear, precise, accurate and robust.

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Keywords: Lifitegrast, HPLC, validation, enantiomeric separation

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PT-010

Development and Validation of an HPLC Method For Determination of an Eltrombopag Precursor and Its Eleven Related Substances

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Immune thrombocytopenic purpura (ITP) is a blood disease which occurs with a temporary or permanent decrease in platelet count. Depending on the severity of the disease, there is an increased risk of bleeding (Cooper et. al., 2006). Eltrombopag, (Z)-3'-(2-(1-(3,4-dimethylphenyl)-3-methyl-5-oxo-1H-pyrazol-4(5H)-ylidene)hydrazinyl)-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid, is a trombopoietin receptor agonist (TPO-RA) approved in 2015 by the U.S. Food and Drug Administration (FDA) and has been commonly used for the treatment of ITP (Serebruany et. al., 2010). Unlike the natural TPO that binds to the extracellular domain of TPO-RA, eltrombopag interacts with the transmembrane domain (H499) of TPO-RA. Thus, autoantibody formation will not be detected and there will be an increase in platelet production (Kim et. al., 2018; Gonzalez-Porras et. al., 2018).

There can be several pathways with different precursors to synthesize eltrombopag which can lead to the formation of different process-related impurities, due to different manufacturing processes, starting materials and intermediates. In this study, one of the common eltrombopag precursor "3'-amino-2'-hydroxy-[1,1'-biphenyl]-3-carboxylic acid (ELTRO-1)" has been investigated. ELTRO-1 has eleven potential impurities (characterized by using NMR, mass and IR spectroscopy), which form during the synthesis of ELTRO-1 and should be monitored via a well-developed method, as the limit of these impurities are in the range of 0.15 – 1.0%. To date no analytical method for the determination of ELTRO-1 and its impurities has been reported.

The scope of this study includes the development and validation of a specific HPLC-related substances analytical method for the eltrombopag precursor ELTRO-1 and its eleven impurities. For this analytical method, optimum wavelength, column temperature, sample unit temperature, column type, elution program, mobile phases, flow rate, and injection volume parameters were determined. The system suitability criteria were determined. The relative standard deviation (RSD) of six replicates of the standard solution of ELTRO-1 peak areas must be less than 5.0%. After development of ELTRO-1 related substances method, it was validated according to the ICH Q2 guideline. The determined specification limit of each impurity is ≤ 0.15%, apart from one impurity limit was specified as ≤ 1.0%. According to the validation studies, the developed analytical HPLC method was found specific, sensitive, linear, precise, accurate, and robust.

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Keywords: Eltrombopag, HPLC, related stubstances.

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PT-011

Development and Validation of Stability Indicating Analytical Methods for Determination of Enzalutamide and Related Substances

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Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells that can result in death if not treated. Prostate cancer is the second most common type of cancer in men (after lung cancer) worldwide. Enzalutamide [4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide] is a novel androgen receptor inhibitor approved in 2012 by the US-FDA

and the most frequently used drug for the treatment of patients with metastatic castration-resistant prostate cancer (mCRPC) (CHMP, 2013). Enzalutamide therapy is mostly initiated in male patients who don't respond well to hormone therapy. It is recommended to be taken before or after chemotherapy. Enzalutamide aims to reduce prostate cancer cell proliferation and reduce prostate-specific antigen (PSA) levels in the serum. Enzalutamide is found as 40 mg of capsule form and should be taken orally at a dosage of 160 mg/ per day (Semenas, et al., 2013). The most used analytical techniques for the determination of enzalutamide in biological samples and/or pharmaceutical dosage forms are LC with UV–VIS and MS detection (Zhou, A. et al., 2018). A molecularly imprinted electrochemical sensor for the detection of enzalutamide was also reported (Kaya et al., 2022).

Eleven potential related substances of enzalutamide (characterized by using NMR, mass and IR spectroscopy) which include process-related and degradation impurities, were used during development and validation studies of new stability indicating RP-UPLC related substances and assay methods. Furthermore, stability studies were conducted under stress, accelerated and long term stability conditions in line with ICH guidelines (ICH Q3A (R2), 2008; ICH Q2 (R1), 2005; ICH Q1A (R2), 2003)

Requirements for each stage of the validation studies were fulfilled and the developed related substances method for determination of eleven impurities in enzalutamide drug substance and assay method for enzalutamide was found specific, sensitive, linear, precise, accurate and robust.

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Keywords: Enzalutamide, impurities, validation, stability

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PT-012

Determination of Hyaluronic Acid Concentration by Using HPLC-RID

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Abstract

Hyaluronic acid (HA) is a frequently used chemical especially in pharmaceutical products. The amount of HA should be determined and tested before the product release on the market. HPLC is a commonly used validated technique for this purpose. Unfortunately, usage of UV detector is not possible due to very low absorption coefficient of HA. One of the most promising detector alternative to UV detector is refractive index detector (RID). In this study, RID is used to quantify HA in samples. In order to calculate concentration of HA, a calibration curve is constructed using data obtained from the standard HA solutions. The limit of detection (LOD) and the limit of quantification (LOQ) values are calculated as 2.8 **µg**/mL and 8.5 **µg**/mL, respectively. The regression constant is calculated as 0.9945 which is very promising value for the developed method.

Keywords: Hyaluronic acid · HPLC · refractive index detector

I. Introduction

Hyaluronic acid (HA) is a linear polysaccharide which is formed by linking disaccharide repeats of D-glucuronic acid and N-acetylflucosamine joined together via glycosidic bonds. The molecular weights of HA ranging from 10^4 to 10^7 Da depending on the polysaccharide chain length. HA is found as the salt hyaluronate form in the human body, especially in the skin and umbilical cord [1, 2]. The commercial importance of HA increases due to the unique properties such as the distinctive moisturizing ability due to hygroscopic nature, viscoelasticity, lack of immunogenicity and toxicity. These unique properties make HA an ideal candidate for numerous applications in the pharmaceutical, cosmetic and food industries [3].

Analysis of HA with liquid chromatography is a challenging issue due to unavailability of size exclusion columns for high molecular masses. This problem can be solved by digestion of HA to unsaturated disaccharides which makes the analysis possible by reverse-phase high-performance liquid chromatography (HPLC) [4, 5]. Reverse-phase HPLC is a very promising analytical technique due to its presence in most of the quality control laboratories to analyze HA in biological samples [4]. However, commonly used UV detector is not suitable for HA quantification due to very low absorption coefficient of HA. Refractive index

detector (RID) is a promising detector alternative to UV detector used in the HPLC system.

II. Material and method

Standard stock solutions of 1.5 mg/mL for HA was prepared by accurately weighing 1.5 mg of HA into 10 mL test tube followed by sonication for 10 min. The solution in test tube is dried at 100°C in an oven. The solid residue was dissolved in 0.25 mL concentrated sulphuric acid. The pH of the solution was adjusted to pH 5.0 by adding required amount of 1.0 M NaOH. This stock solution is used for the preparation of the desired HA standard solutions. Finally, 1.0 mL of the prepared solution was transferred into a vial with the help of a micropipette.

1 package of sample (2.26 g) was weighed and dissolved in 240 mL deionized water by the help of 1 min vortex mixing followed by 15 min ultrasonic bath. 1 mL is taken from above solution and the same procedure which was applied to standart solutions was used. Then the solution was filtered by using filters having 0.45-micron size by using vacuum filter. Finally, 1.0 mL of the prepared solution was transferred into a vial with the help of a micropipette.

Water and methanol were mixed in the ratio of 96:4 (v/v) and used as mobile phase. The mobile phase flow rate was adjusted to 0.8 mL/min.

III. Results and discussion

Different ratios of mobile phase were tried for optimization of the developed method to get better and faster results. Finally, water: methanol in the ratio of 96:04, v/v was determined as optimum values which gave high resolution peaks in the sample analysis. A Thermo BDS HYPERSIL C18 (250×4.6 mm, 5 **µm**) column was used and 0,8 mL/min flow rate was fixed as optimized condition due to better separation in the sample analysis. 20 **µL** injection volume and 30°C column oven temperature was used during HPLC measurements. These optimized conditions gave a retention time of 2.216 min for HA.

All the values were in the limits of the ICH which proves the reliability of the developed method for the HA quantification. The degraded peaks from analyte which shows the stability was in the range of 0.07% to 3.34% under all conditions. This result showed the stability of the selected pharmaceutical product. The RSD % values were less than 2.

The optimized results for the developed HPLC-RID method are given in Table 1. The system suitability parameters which are shown in Table 2 were within the acceptance criteria.

Tab. 1: Optimized chromatographic conditions

Parameter	Condition
Column	Thermo BDS HYPERSIL C18 (250×4.6 mm, 5 **µm**)
Mobile phase	Water : methanol (96:04, v/v)
Diluent	Mobile phase
Column temperature	30°C
Flow rate	0.8 mL/min
Run time	5 minute
Injection volume	20 **µL**

Tab. 2: Summary of system suitability parameters

Parameter	HA	Acceptance criteria
Tailing factor	1.18	≤2
Retention time	2.216	≥2
Theoretical plates	2719	≥2000
RSD % of area	1.10	≤2

HPLC chromatogram of standard solutions is shown in Figure 1. The quantification is linear in the concentration range of 25-250 **µg**/mL for HA and the correlation coefficient is calculated as 0.9945 (Figure 2). The results for linearity are shown in Table 3. The calculated LOD and LOQ values are shown in Table 3. The recovery values are in the range of 98.90-99.99% which are in acceptance criteria.

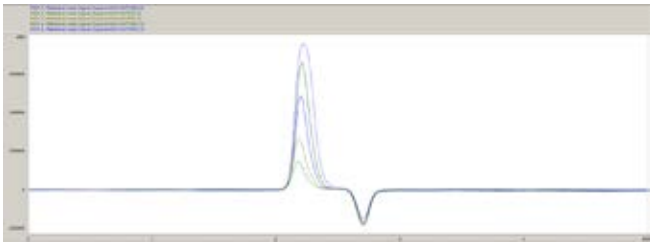

Fig. 1: HPLC chromatogram of HA standard solutions

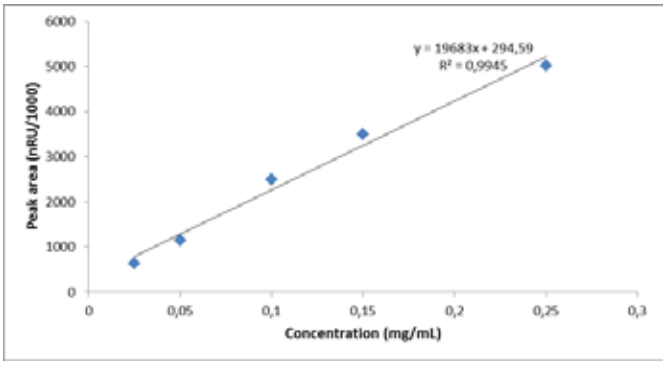

Fig. 2: Obtained calibration curve of HA

Tab 3: Linearity values

Parameter	HA
Linearity range (**µg**/mL)	25-250
Regression coefficient ± SD	0.9945±0.0007
LOD (**µg**/mL)	2.8
LOQ (**µg**/mL)	8.5

A sample chromatogram of prepared sample solution is shown in Figure 3. The peak is not disturbed by any interfering peaks at

the retention time which proves the specificity of the developed method in the analyzed sample (Figure 3). The standard solutions and the marketed formulation solutions are found to be stable for 24 h autosampler stability.

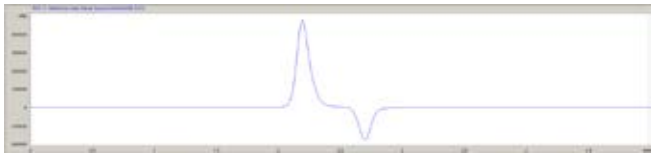

Fig. 3: HPLC chromatogram of analyzed sample solution

IV. Conclusion

The developed method is reliable, rapid, sensitive and economical by considering the discussed results and data. The LOD and LOQ values are quite similar with the other studies with the better short runtime. In conclusion, the developed HPLC-RID method can be used as an alternative method for routine HA determination in bulk and pharmaceutical dosage form.

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PT-013

On-site Determination of Methadone in Saliva by Ambient Ionization Mass Spectrometry Using a Polyamide-Cotton Composite Immobilized into a Hypodermic Needle

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Bioanalysis is a challenging task that requires demanding sensitivity and selectivity levels. In this context, mass spectrometry (MS) is strongly recommended when analyzing biological fluids (e.g., blood, plasma, urine, saliva) to reach the cut-off values and to minimize the presence of interferences provided by the matrix. MS is usually combined with chromatographic techniques that can fulfill these requirements at the expense of the sample throughput. Combining sample preparation with ambient ionization mass spectrometry (AIMS) speeds up the analytical process by circumventing the separation step. However, the resulting ion suppression of the target compounds may imply a lack of reproducibility that can be solved by using an internal standard of the analyte.

Substrate spray is a modality of AIMS that consists of a sharp solid support where the analytes had been deposited or extracted. Then, an organic solvent is added to elute the analytes. When applying a high voltage to the solid substrate (or ESI emitter), an electrospray is formed in the tip ionizing and transferring the analytes to the MS inlet. Different materials have been used as ESI emitters, including paper, wooden tips, coated blades, SPME fibers, threads, probes, and needles, among others.

In this work, we present a composite based on polyamide-coated cotton fibers (N6-cel) immobilized in the hub of a hypodermic needle (HN) to isolate methadone from oral fluid samples. The cotton fibers are modified with nylon-6 to reinforce the interactions with the analytes by H-bonds and dispersive forces. Moreover, the porosity of the composite allows the flow of the sample through the sorbent, maximizing the sorbent-analyte interaction. The role of HNs is double: i) HNs act as sorbent holders to immobilize 2 mg of the sorbent in the Luer connector, ii) HNs act as ESI emitters providing disposable AIMS interfaces.

The variables affecting the extraction of methadone were

studied using a univariate approach: quantity of nylon-6 in the sorbent, ionic strength of the sample, number of strokes, sample dilution, and flow rate of the eluent/ionizing agent in AIMS. Finally, the proposed analytical method was validated using matrix-matched calibration curves in terms of limit of detection (0.3 µg/L), linear range (0.9-300 µg/L), and linearity (R^2>0.993). Trueness and precision were evaluated using a different pool of saliva samples at three different concentration levels. Trueness, expressed as relative recovery, ranged from 90 to 109%, while the precision, expressed as relative standard deviation, was lower than 9.3%.

Keywords: Ambient ionization mass spectrometry, bioanalysis, hypodermic needles, on-site, polyamide-cotton composite

PT-014

Voltammetric Quantification Studies of the DNA Binding of the Antineoplastic Drug Azacitidine from Injection Suspension

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In this study, the interaction mechanism between Azacitidine and double-stranded DNA (dsDNA) has ben enlightened with the aim of analytical and theoretical methods. The binding constant (Kb) has been determined quantitatively by using voltammetric techniques and it was found to be $2.04\pm0.30\times10^5$ M^{-1} for deoxyguanosine (dGuO) and $1.23\pm0.30\times10^5$ M^{-1} for deoxyadenosine (dAdo). The UV-Vis and fluorescence spectra has also been used to estimate the Kb value. The obtained values are in a very good agreement with the voltammetrically obtained results and found to be $4.13\pm0.23\times105$ M^{-1}, $1.67\pm0.24\times10^5$ M^{-1}, respectively. Competitive displacement studies were performed with ethidium bromide, an intercalator and Hoechst-33258, a groove binder which are commonly used as DNA markers in fluorescence studies. In thermal denaturation studies, the Tm value of Azacitidine and dsDNA mixture was found to be 8.60 °C. Experimental results revealed that the dsDNA binding mode of the Azacitidine is intercalation. DFT calculations were confirmed the intercalation by showing the disappearance of the H-bonds between the guanine and adenine nucleotides in the IR spectra.

We developed a voltammetric method for sensitive determination of Azacitidine in pH 4.80 acetate buffer medium. The peak current reductions in the oxidation signals of the dGuo upon the interaction of Azacitidine with dsDNA, showed linear dependence to DNA concentration in the range of 2-20 µM Azacitidine, with a detection limit of 0.62 µM.

Keywords: Azacitidine, dsDNA, spectroscopy, voltammetry, spectroscopy, DFT

PT-015

NMR-based Metabolomics Studies to Investigate TNAP (Tissue-Nonspecific Alkaline Phosphatase) Functions in Brain and Liver

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TNAP (Tissue Non-specific Alkaline Phosphatase) is an enzyme that belongs to the family of alkaline phosphatases. TNAP is expressed in various tissues including bone, kidney, brain and liver. Dysregulation of TNAP activity leads to hypophosphatasia, a rare genetic human disorder associated with altered bone formation and neurodevelopmental disorders. In bone, TNAP is involved in mineralization processes through PPi regulation. In the brain, TNAP plays a crucial role in neuronal development and functioning through the metabolism of pyridoxal phosphate, the active form of vitamin B6, and the control of extracellular nucleotide level. In contrast, the functions of TNAP in the liver remain obscure. It is thought to be involved in bile acid metabolism and excretion, contributing to the digestion and absorption of dietary fats and fat-soluble vitamins.

Previous studies conducted by our consortium support the role of TNAP in brain [1] and hepatic functions [2]. This poster summarizes NMR-based metabolomics studies we have recently designed to gain a deeper understanding of the functions of TNAP in the brain and liver. The following models were used: neuronal cultures (SK-N-SH neuroblastoma cell line), in vitro mouse brain slices, and tissues from C57BL/6 mice (brain and liver) collected following in vivo manipulations. The role of TNAP in inflammation in the liver and brain was explored using post-prandial models and LPS injection. TNAP activity was inhibited

using either MLS-0038949 (in vitro) or SBI-425 (in vivo).

The metabolome was investigated using a global approach through 1H NMR profiling. Tissues or cell cultures were subjected to a biphasic extraction using the methanol-chloroform-water system, following Beckonert's procedure. 1H NMR spectroscopy analysis was performed on the aqueous fraction. Depending on the experiment, spectra recording was conducted in deuterated buffers at pH 10 or pH 7.4.

The purpose of this poster is to provide a brief overview of experiments and results and to discuss methodological challenges and limitations of the NMR-based metabolomics approach within the context of biological studies, particularly when the number of samples is limited.

Keywords: NMR, metabolomics, brain, liver, alkaline phosphatase

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PT-016

A Simple and Rapid LC-MS/MS Method Development for the Quantification of Octreotide in Cancer Patients Plasma Samples

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Octreotide is a synthetic octapeptide derivative that has pharmacological effects similar to natural somatostatin but with a much longer duration of action. Octreotide is widely utilized in clinical practice for patients with neuroendocrine cancers. In the last decade, an increasing incidence of neuroendocrine cancers has been reported. In this current study, a new rapid method for the analysis of octreotide from plasma by LC/MS-MS was developed. The analyses of octreotide and lanreotide (internal standard) were performed in positive ionization. The separations were performed on a C18 analytical column with a gradient mobile phase consisting of a mixture of 0.1% formic acid in water and 0.1% formic acid in acetonitrile at a flow rate of 0.3 mL/min. The total run time was 5 min. The injection volume was 10 µL and the column temperature was maintained at 55°C. Plasma sample preparation was based on organic solvent precipitation of proteins. The method was linear in the range of 1-100 ng/mL for octreotide in plasma. Finally, the plasma samples from cancer patients treated with octreotide were quantified with the developed LC-MS/MS method.

Keywords: LC-MS/MS, octreotide, method development, cancer

PT-017

Bioanalytical Method Development and Validation of Nifuratel in Human Plasma by LC-MS/MS Detection and Its Application to a Pharmacokinetic Study

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Abstract

Nifuratel, an antimicrobial agent, shows a broad spectrum of activity in *in vitro* and *in vivo* studies against microorganisms causing infections of the genitourinary system and moreover, it has antiprotozoal and antifungal activity. This study presents the development and validation of a bioanalytical method for the quantification of nifuratel in human plasma using liquid chromatography-tandem mass spectrometry detection. The method development involved optimizing the sample preparation technique, employing liquid-liquid extraction with diethyl ether for extraction of nifuratel and nifuratel 13C d3 (internal standard) from human plasma samples. Chromatographic separation was achieved on an ACQUITY UPLC BEH C18 column using a gradient elution process. The method had a

total run time of 3 min. The method was validated following the guidelines set by the US Food and Drug Administration (FDA) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The validation results demonstrated excellent accuracy, with percent relative errors ranging from −3.04% to 7.09%, and high precision, with percent relative standard deviations below 7.64%. The lower limit of quantification (LLOQ) for nifuratel was determined to be 0.25 ng/mL. Stability studies confirmed the robustness of the method under various storage conditions, including autosampler, short-term, freeze/thaw, and long-term stability. The validated bioanalytical method was successfully applied to a pharmacokinetic study of nifuratel, allowing for the accurate determination of nifuratel concentrations in human plasma samples. This method offers a reliable and efficient tool to assess the pharmacokinetic profile of nifuratel for bioavailability/bioequivalence studies.

Keywords: Nifuratel, LC-MS/MS, pharmacokinetic, bioanalytical method validation

I.Introduction

Nifuratel is a nitrofuran derivative with the chemical name [5-([methylthio] methyl)-3-([5-nitrofurfurylidene] amino)-2-oxazolidinone an antimicrobial agent, shows a broad spectrum of activity in in vitro and in vivo studies against microorganisms causing infections of the genitourinary system and moreover, it has antiprotozoal and antifungal activity. Nifuratel exerts an intense and efficacious action against the bacterial, protozoan, and fungal species that attack the female genital tract. Nifuratel is mainly eliminated through renal excretion and has a strong antibacterial action in the urinary tract. It is prescribed for the treatment of urethritis, cystitis, pyelitis, and urinary septic retentions of various etiological and preparatory stages of surgical operations on the urinary tract [1].

A few analytical techniques have been employed for the quantitative determination of nifuratel in human plasma, with High-Performance Liquid Chromatography (HPLC) coupled with ultraviolet (UV) detection being the most commonly reported method [2,3]. Although a limited number of HPLC studies are available for the analysis of nifuratel, there is currently no existing literature on the implementation of LC-MS/MS for this purpose. We have developed the first LC-MS/MS method for accurately determining nifuratel in human plasma. This LC-MS/MS method not only enables multiple sample injections within a short timeframe, leading to improved efficiency and throughput but also exhibits high sensitivity for accurate determination of nifuratel in human plasma.

II. Material and method

Chemicals and Materials

Nifuratel (100%) was supplied by Chongqing Southwest No.2 Pharmaceutical Factory Co, Ltd. (Chongqing, China). Nifuratel 13CD3 (internal standard, IS) was purchased from TLC Pharmaceutical Standards Ltd. (Ontario, Canada). Acetonitrile, diethyl ether, and formic acid were purchased from Merck (Darmstadt, Germany). K_2EDTA blank human plasma was obtained from Bioivt (UK) and Gaziantep University Farmagen GCP Centre (Turkey). Ultrapure (Type 1) water was obtained from Milli-Q plus water purification system (USA).

Instrumentation

The LC-MS/MS system (Waters, UK) consisted of an Acquity I-Class UPLC system and Xevo TQ-S microTandem Mass Spectrometer. Separations were carried out on ACQUITY UPLC BEH C18 (1.7µm, 2.1 x 50 mm). The mobile phase consisted of acetonitrile, water, and formic acid (0.01%). The chromatographic run was performed under gradient elution at the flow rate of 0.6 mL/min and the runtime was 3 min. A 4µL sample was injected into the sampling system, and the autosampler was conditioned at 10◦C. The multiple reaction monitoring (MRM) transitions were performed at m/z 286.1 > 241.97 for nifuratel and m/z 290.1 > 246.04 for nifuratel 13Cd3. Mass spectrometric detection was performed using an ESI ion source in the positive ionization mode. Detailed MS parameters were given in Table 1. Waters MassLynx V4.2 SCN1040 was used for data acquisition and evaluation of chromatographic data.

Tab. 1 MS parameters for nifuratel(NIF) and Nifuratel ^{13}C D3(IS)

Compound	Precursor	Product	Dwell time(s)	Cone (V)	Collision energy(V)
NIF	286.1	241.97	0.06	55	9
IS	290.1	246.04	0.06	55	9

Stock solutions, calibration standards, and QCs

Nifuratel stock solutions (1 mg/mL) in acetonitrile were diluted to prepare working solutions (0.005-3 µg/mL). The internal standard working solution was prepared at a concentration of 100 ng/mL. Stock solutions of nifuratel and IS were stored at -20◦C. Calibration standards (0.25-150 ng/mL) and QC samples (0.25, 0.75, 6, 60, and 120 ng/mL) were prepared in human blank plasma. All calibration standards and QC samples were stored at -70◦C until analysis.

Sample preparation

Aliquots of 200µL plasma samples and 50µL of IS (100ng/mL) were added into a 10 mL centrifuge tube and vortexed for 5 s. Nifuratel was extracted from plasma using diethyl ether in a liquid-liquid extraction under sodium lamps. The supernatants were transferred to clean tubes, evaporated under nitrogen, and reconstituted with 200µL reconstitution solution before injection into the LC-MS/MS system.

Pharmacokinetic study

28 healthy volunteers were enrolled in a pharmacokinetic study which has been reviewed and approved by the ethics committee. Written informed consent was collected from all participants. Blood samples were taken by a short intravenous catheter and were collected into tubes using K_2EDTA as an anti-coagulating agent. Blood samples were collected before medication and at 0.25, 0.50, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00 h after drug administration. After centrifugation at 3000 rpm for 10 min, the separated plasma from each sample was transferred into transparent, polypropylene tubes and stored−70◦C until analysis.

III. Results and discussion

Method validation

The method was validated following both US-FDA Bioanalytical Method Validation guidance [4] and EMA-ICH guideline M10 on bioanalytical method validation and study sample analysis [5], covering selectivity, linearity, calibration curve, precision, accuracy, recovery, matrix effect, and stabilities.

Selectivity

The selectivity of the method in K_2EDTA human plasma was tested by spiking ten sources, including lipemic and haemolysed samples. Peak responses in blank lots were compared against the response of spiked LLOQ and no interferences were observed at the retention times of analyte and IS. The selectivity of the method was demonstrated with the chromatograms of blank plasma and LLOQ samples (Figure 1).

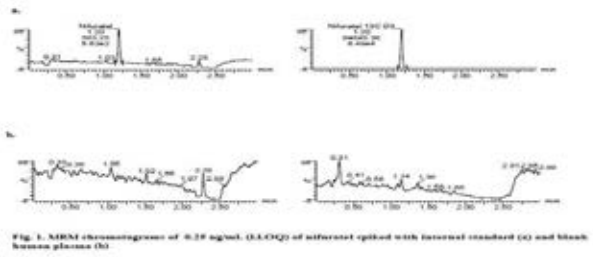

Fig. 1 MRM chromatograms of 0.25 ng/mL (LLOQ) of nifuratel spiked with internal standard (a) and blank human plasma (b)

Linearity

A calibration curve was constructed from the blank, zero, and eight non-zero samples. The range was 0.25-150 ng/mL for nifuratel including the LLOQ. The linearity of each calibration curve was determined by plotting the peak-area ratio (y) of the analyte to IS versus the nominal concentration (x) of the analyte. Calibration curves were linear with a coefficient of correlation (r2) values more than 0.9983. The r2 values, slopes, and intercepts were calculated using weighted (1/C2) linear regression analysis with five intra and inter-day calibration curves. The standard concentration must be within 15% deviation from the nominal value except at LLOQ, for which the maximum acceptable deviation was set as 20%. At least 75% of eight non-zero samples must be met exception criteria including acceptable LLOQ and ULOQ [4,5].

Accuracy and precision

The within-batch precision and accuracy were evaluated by analyzing QC samples at five different concentration levels (0.25 ng/mL (LLOQ), 0.75 ng/mL (QC Low), 6 ng/mL (QC Medium), 60 ng/mL (QC High) and 120 ng/mL (ULLOQ)) with six replicates in a batch. The between-batch precision and accuracy were determined by analyzing three different batches. The within-batch and between-batch values did not exceed 15% for QC samples, as expected for LLOQ which did not exceed 20%. The data on the method's within- and between-batch precision and accuracy were summarized in Table 2a and Table 2b.

Tab. 2a Within-batch precision and accuracy of the method for determining nifuratel in plasma samples

	Batch No:1 (n=6)			Batch No:2 (n=6)			Batch No:3 (n=6)		
Nominal Conc. (ng/mL)	Conc. Found (mean ± SD;ng/mL)	RD (%)	CV (%)	Conc. Found (mean ± SD;ng/mL)	RD (%)	CV (%)	Conc. Found (mean ± SD;ng/mL)	RD (%)	CV (%)
0.25 (QC1)	0.25±0.01	1.27	3.32	0.24±0.01	-2.73	5.31	0.24±0.01	-4.53	3.05
0.75 (QC2)	0.83±0.06	10.04	7.64	0.79±0.02	5.67	3.03	0.79±0.01	5.56	1.84
6 (QC31)	6.17±0.07	2.83	1.21	6.17±0.13	2.78	2.13	6.08±0.22	1.41	3.54
60 (QC32)	60.47±1.17	0.78	1.93	58.73±1.09	-2.11	1.86	58.37±1.36	-2.72	2.33
120(QC4)	115.12±2.02	-4.07	1.75	114.95±3.04	-4.21	2.64	119.00±3.24	-0.83	2.72

Tab. 2b Between-batch precision and accuracy of the method for determining nifuratel in plasma samples

	Batch No:1-3 (n=18)		
Nominal Conc. (ng/mL)	Conc. Found(mean ± SD;ng/mL)	RD(%)	CV (%)
0.25	0.25±0.01	-2.0	4.55
0.75	0.80±0.04	7.09	5.08
6	6.14±0.15	2.34	2.42
60	59.19±1.48	-1.35	2.50
120	116.36±3.27	-3.04	2.81

Conc: Concentration, n: Replicates at each concentrations., RD: Relative Deviation, CV: Coefficient of Variation, SD: Standard Deviation

Matrix effect

The matrix effect was investigated by extracting blank plasma samples from six different sources, including one hemolytic and one lipemic plasma. Experiments were performed at low (QC2) and high (QC4) quality control levels in six replicates using the ratios of peak areas of the blank plasma samples spiked after extraction to those of pure standard solutions containing analyte at the same concentrations. The precision (%CV) of QC2 and QC4 was 3.01% and 2.96%, respectively. The matrix effect results were summarized in Table 3a and Table 3b. The acceptable precision (%CV) should be ≤15% [4,5].

Tab. 3a Results of matrix effects for QC2 level (n=6)

Plasma QC2	Mean Peak Area	Matrix Factor	Mean Peak Area IS (n=6)	IS Matrix Factor	IS Normalized Matrix Factor
Pure Solution	2162.99	-	284112.47	-	-
Matrix 1	2170.46	1	286685.14	1.01	0.99
Matrix 2	2134.3	0.99	289030.24	1.02	0.97
Matrix 3	2138.99	0.99	291402.12	1.03	0.96
Matrix 4	2137.27	0.99	291863.27	1.03	0.96
Matrix 5	2174.57	1.01	298926.35	1.05	0.96
Matrix 6	2254.78	1.04	286578.65	1.01	1.03
				Mean IS Normalized Matrix Factor	0.98
				CV (%)	3.01

Tab. 3b Results of matrix effects for QC4 level (n=6)

Plasma QC4	Mean Peak Area	Matrix Factor	Mean Peak Area IS (n=6)	IS Matrix Factor	IS Normalized Matrix Factor
Pure Solution	328503.65	-	284112.47	-	-
Matrix 1	314502.68	0.96	286685.14	1.01	0.95
Matrix 2	312939.71	0.95	289030.24	1.02	0.94
Matrix 3	319746.72	0.97	291402.12	1.03	0.95
Matrix 4	321938.20	0.98	291863.27	1.03	0.95
Matrix 5	324876.91	0.99	298926.35	1.05	0.94
Matrix 6	335695.55	1.02	286578.65	1.01	1.01
				Mean IS Normalized Matrix Factor	0.96
				CV (%)	2.96

Recovery

Recovery of nifuratel was evaluated by comparison of analyte responses of six extracted samples of low, medium, and high-quality control concentrations (0.75, 60, 120 ng/mL) with those of six appropriately diluted standard solutions. The mean overall recovery of nifuratel was 98.90%. The mean recovery of internal standard was 99.24%.

Stability

Stability evaluations in the matrix were made using freshly spiked calibration standards. Analytes were stable up to 5 h on the bench top and over 4 freeze-thaw cycles. The processed samples were stable for up to 24 h in an autosampler at 10◦C. During validation, long-term plasma stabilities at both −20◦C and−70◦C were evaluated for 11 days. The stability results were summarized in Table 4.

Tab. 4 Results of stability of nifuratel in human plasma under different storage conditions (n=6)

Storage Condition	Nominal Conc. (ng/mL)	Conc. Found (mean ± SD;ng/mL)	CV(%)	RD (%)
Autosampler stability[a]	0.75	0.79±0.02	3.05	5.91
	120	116.987±1.59	1.36	-2.52
Short-term plasma stability[b]	0.75	0.80±0.02	2.86	7.18
	120	118.11±-1.57	-1.57	2.46
Validation long-term plasma stability[c]	0.75	0.81±0.02	2.49	8.27
	120	117.85±1.84	1.56	-1.80
Validation long-term plasma stability[d]	0.75	0.80±0.02	2.58	6.58
	120	120.52±1.42	1.18	0.44
Freeze-thaw stability[d]	0.75	0.79±0.03	3.51	5.00
	120	120.70±2.42	2.01	0.58

RD: Relative Deviation (Accuracy), CV: Coefficient of Variation (Precision), SD: Standard Deviation

[a] Kept at autosampler temperature, 10^0C.

[b] Stored at room temperature.

[c] Stored at -20^0C.

[d] Stored at -70^0C.

Application to a bioequivalence study

The validated method was successfully applied to a bioequivalence study. 28 subjects were planned and randomized. There has been one drop-out. As a result, 27 subjects completed the clinical phase of the study. The plasma samples obtained from 27 healthy human volunteers following oral administration of the nifuratel 200 mg test and reference formulations were analyzed. In this single-dose, two-period, cross-over, randomized study, nifuratel film-coated tablets were administered after standardized high-fat and high-calorie breakfast. Serial blood samples were collected throughout 24 h. The mean±sd of maximum plasma concentration (C_{max}) for the test and reference product was found 33.215±12.523 ng/mL and 30.864±9.477 ng/mL, respectively. The median (min-max) times to reach peak plasma concentration (T_{max}) for the test and reference product were found 2.660 (1.000-6.000) hr and 3.500 (1.000-5.000) hr, respectively. The mean±sd of the area under the plasma concentration-time curve from zero to last measurable concentration ($AUC_{0\text{-}tlast}$) for the test and reference product was found 93.940±34.763 hr.ng/mL and 96.564±30.711 hr. ng/mL, respectively. The mean±sd of elimination half-life (t½) for the test and reference product was found 1.150±0.289 hr and 1.261±0.444 hr, respectively.

IV. Conclusion

An LC-MS/MS method was developed for the determination of nifuratel in human plasma. The method was successfully validated for selectivity, sensitivity, linearity, accuracy, precision, matrix effect, recovery, and stability in accordance with FDA and EMA-ICH guidelines [4,5]. Furthermore, the method was successfully applied to a bioequivalence study to assess the pharmacokinetics of nifuratel after an oral administration of nifuratel.

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PT-018

Mechanism Elucidation Studies of Wound Healing Potential and Determination of HPLC Profile of Lysimachia verticillaris and Lysimachia vulgaris

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Lysimachia species belong to the Primulaceae family and are popularly called "kargaotu" or "altın kamış" (1). *Lysimachia* species contain abundant secondary metabolites such as flavonoids, triterpenes, phenolic acids. *Lysimachia* species, which have important biological activities thanks to their rich content, are used as wound healing, antipyretic, analgesic and antitussive among the public (2, 3).

In a previous study by us with *L. verticillaris* and *L. vulgaris*, the wound healing potential of the species was found to be quite effective (4). In this study, it was aimed to carry out cell culture studies to elucidate the mechanism of wound healing potential of water extracts of the species. In cell culture studies, the hem oxygenase-1 (HO-1) activity, cyclooxygenase-2 (COX-2) inhibitory effect and nitric oxide (NO) content of cells (L929, RAW-247 cell line) exposed to the extracts were carried out. In addition, HPLC studies were determined the phenolic content of the species.

By mechanism elucidation studies, it has been determined that the species realize their wound healing potential by increasing HO concentration in cells, inhibiting COX-2 enzyme and NO. When the phenolic content of the plants was examined by HPLC studies, it was determined that the main components were quercetin, luteolin and sinapic acid. It is thought that quercetin, luteolin and sinapic acid contribute to the healing of wounds with their known effects such as antioxidant, antiinflammatory and antibacterial (4-6).

Keywords: Cell culture, HPLC, *Lysimachia*, secondary metabolites

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PT-019

Combination of the Targeted Metabolomic Profiling and Bioinformatics Approach for the Diagnostics of Cardiovascular Diseases ("MetaboScan" System)

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Abstract

Today, active development of metabolomic tools gradually leads to the possibility of its introduction into clinical practice. The prototype of the 'Metaboscan' system developed in our laboratory is aimed at screening diagnostics of cardiovascular pathologies through the combination of bioinformatics and targeted metabolomic profiling of patient's blood samples. The proposed metabolomic screening approach is performed using single UPLC-MS/MS analysis of more than 100 metabolites and their ratios. The panel of analytes includes mainly amino acids, acylcarnitines, tryptophan related metabolites, dimethlarginines. Most of these metabolites are mainly responsible for energy metabolism, oxidative stress and inflammation. This assay allows for the identification of up to 100 metabolites and ratios within one 5 min run. The presented approach was applied for diagnostics of cardiovascular diseases (CVD). Our finding revealed that some acylcarnitines, amino acids, methylarginines and methabolites involved in kynurenic and indolic tryptophan conversion pathways showed a significant correlation with progression of CVD. Moreover, application of the machine learning methods gave an opportunity to build an accurate diagnostic model of different stages of CVD.

Keywords: Cardiovascular diseases, targeted metabolomics, machine learning

I. Introduction

Cardiovascular diseases (CVDs) account for approximately 18 million deaths annually, making them the primary cause of death worldwide. Even asymptomatic metabolic disturbances may lead to sever cardiometabolic alterations. Omics technologies, such as metabolomics profiling, have the potential to identify subtle changes in the metabolome that occur before the manifestation of typical disease symptoms. It gives an opportunity to detect and potentially prevent CVDs. Previous metabolomics research has suggested that alterations in plasma amino acid concentrations, acylcarnitines, and tryptophan biodegradation products are associated with cardiometabolic changes. However, the underlying mechanisms and biomarkers for different stages of CVDs are not yet fully understood.

At the same time, due to the nonlinear nature of metabolomic data, predicting clinical outcomes based on metabolomic profiles is usually challenging. However, combination of the metabolomic analysis with modern supervised machine learning (ML) tools may serve for the development of more accurate and sensitive diagnostic models. The main principle of this approach is based on the possibility of the ML-derived models to the identify the most informative metabolic panels that better classify patients according to the considered disease. As the result, it assesses the likehood of the individual to have the disorder based on his metabolomic profile.

II. Material and method

To develop the diagnostic CVD model based on the metabolomic profiling the cross-sectional study was conducted with 109 patients diagnosed with CVD (CVD group): 61 had HTA (HTA subgroup), and 48 were patients with CAD (CAD subgroup). A control group comprised 27 participants without clinical signs of CVD (non-CVD group).

Aminoacids, acylcarnitines, dimethylarginines, tryptophan related metabolites were determined via HPLC-MS/MS method. The presented methods were validated in accordance with the US FDA and EMA guidelines for bioanalytical method validation [1,2].

The Stats package in Python software was utilized for statistical analysis. ANOVA or the nonparametric Kruskal-Wallis test (with a p value<0.05) was employed for the analysis of variance. The Benjamini-Hochberg false discovery rate (BH-FDR) method was used to correct the p values, which provided q values. The diagnostic accuracy of selected metabolites was assessed by calculating the areas under the curve (AUCs) obtained from receiver operating characteristic curve analyses. The AUCs were compared between the non-CVD group and the combined HTA and CAD patients (CVD group), as well as between the non-CVD group and the HTA group. Spearman correlations were used to evaluate the relationships between cardiometabolic risk factors and metabolomics profiling, and heatmap correlation matrices were produced using the Seaborn package in Python. For machine learning methods, six different approaches were utilized for binary classification: logistic regression, random forest classifier, multilayer perceptron classifier, gradient boosting classifier, support vector classifier, and bagging classifier. Hyperparameter optimization of the developed models was performed using the sklearn GridSearchCV tool in Python.

III. Results and discussion

Overall, the prototype of the 'Metaboscan' system developed in our laboratory is aimed at screening diagnostics of cardiovascular and oncological pathologies through the combination of bioinformatics and targeted metabolomic profiling of patient's blood samples. Probability assessment of the disease development is based on the application of the modern supervised machine learning classification models, which were preliminarily trained and validated on experimentally obtained metabolomic data of patients with the considered diseases. The main principle enclosed in the presented approach is based on the fact that first of all, the system establishes the probability of the presence / absence of a disease, in general, and then, in case of its presence, performs differentiation within the pathology.

Thus, our objective was to develop an accurate and validated assay for targeted metabolomic analysis using supervised machine learning algorithms, with the aim of its practical implementation in the field of medicine on the example of the CVD diagnostics. The proposed assay includes targeted quantification of up to 100 metabolites and their ratios across different chemical classes including amino acids and acylcarnitines, biogenic amines, vitamines, nucleosides, indole derivatives, tryptophan and kynurenine related compounds. In addition, several biomedically related metabolite sums and ratios may be calculated from concentrations of these targeted metabolites. The panel is composed of compounds belonging to different biochemical cycles, such as the NO-cycle, fatty acid metabolism, organic acid, tryptophan catabolism, and allows for a comprehensive assessment of health status.

The developed within 'Metaboscan' system HPLC-MS analysis takes 5 minutes, which, combined with simple sample preparation, makes the proposed method suitable for screening analyses. The presented analytical approaches were validated in accordance with the US FDA and EMA guidelines for bioanalytical method validation. The accuracy of QC standards at 3 different concentration levels were in the range of 80% to 120% with satisfactory precision values of less than 20%.

Our findings revealed that the majority of acylcarnitines, amino acids, methylarginines, and metabolites involved in kynurenic and indolic tryptophan conversion pathways showed a significant increase ($p<0.05$) in concentration levels during the progression of CVD. These results suggest a potential link between inflammation, mitochondrial imbalance, and oxidative stress with early stages of CVD.

Although, prediction of clinical outcomes based on metabolomic profiles is challenging due to the nonlinear nature of metabolomic data. However, supervised machine learning (ML) classification algorithms may serve for early prediction of disease development and progression. By using ML-derived models, it is possible to perform quantitative predictions and identify informative features, potentially leading to the discovery of meaningful biomarkers for preliminary hypothesis-driven research. Concerning CVD diagnostics, the developed model using the random forest classifier demonstrated the relitevly higher predictive power among the considered ML-algorithms for multiclass and binary classification of patients with CAD, HTA, and non-CVD individuals, as well as between CVD and non-CVD individuals globally (with accuracy rates of 0.80 and 0.91, respectively).

Thus, the development and implementation of the MetaboScan diagnostic system will allow timely detection of human disorders, which will improve the quality of diagnostics, as well as reduce material costs for healthcare. In future, it is planned to expand the diagnostic system to all key socially significant pathologies and introduce a module for the effectiveness of therapeutic treatment.

IV. Conclusion

In conclusion the presented study overviewed the prototype of the novel diagnostic system based on the metabolomic profiling. We propose that posible development and application of this system will improve the quality of diagnostics, as well as reduce material costs for healthcare.

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PT-020

Metabolomics Investigations of Anti-Virulence Drug-Induced Toxicity

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Abstract

Anti-virulence therapy offers a powerful tool to combat drug-resistant bacteria by targeting bacterial virulence factors to limit bacterial toxic impact on the host. It allows reducing disease

severity, preventing the evolvement of drug resistance as well as improving patients' immune system to overcome the infection. An anti-virulence drug Fluorothiazinon was studied to inhibit Chlamydia trachomatis, Salmonella enterica, drug-resistant Pseudomonas aeruginosa, Escherichia coli and Acinetobacter baumannii. Key stages in drug development are pharmacokinetic and toxicological assessments. In this study, rabbits received repeated oral dose of Fluorothiazinon once a day for 30 days. Blood samples were taken throughout the experiment to evaluate pharmacokinetics and metabolomics. At the end of the observation period hematological, biochemical and urine tests were performed and histological evaluations were conducted. Alterations in tryptophan, amino acids and acylcarnitines metabolism may explain drug-induced blood and organ toxicity seen in rabbits. Such interdisciplinary approach facilitates metabolomics as a powerful tool to gain insights in toxicological impact of anti-virulence therapy and can be used for adverse effects monitoring.

Keywords: Metabolomics, pharmacokinetics, drug-induced toxicity, anti-virulence

I. Introduction

In response to emerging bacterial antibiotic resistance, an anti-virulence therapy offers a powerful perspective to combat drug-resistant bacteria by targeting bacterial virulence factors to limit bacterial toxic impact on the host. It allows reducing disease severity, preventing the evolvement of drug resistance bacteria as well as improving patients' immune system to overcome the infection. The anti-virulence drug Fluorothiazinon was studied to inhibit Chlamydia trachomatis, Salmonella enterica, drug-resistant Pseudomonas aeruginosa, Escherichia coli and Acinetobacter baumannii in vitro and in vivo (Bondareva et al., 2022; Koroleva et al., 2023; Nesterenko et al., 2016; Sheremet et al., 2018). Apart from drug pharmacodynamics, other key stages in drug development include pharmacokinetics and toxicological assessments. Investigation towards drug-induced toxicity is an important step to provide patients with safer medications. The most common manifestations of drug-induced toxicity are liver and kidney damage, gastrointestinal and cardiovascular complications. However, these alterations are being present at terminal stages when well-being of patients is disrupted. Therefore, there is a strong need to uncover these alterations before they are clinically present. To address this issue, metabolomics can be utilized as a promising tool to evaluate drug-induced toxicity. Assessment of alterations in compounds related to essential metabolisms could provide insights into mechanisms behind toxic effects of treatment, making it possible to identify current or possible toxicities at much earlier stages. Therefore, the aim of this study is to assess Fluorothiazinon-induced toxicity by clinical hematological, biochemical and urine testing and histological evaluations supported by semi-untarget metabolomics profiling of blood of rabbits received the drug once a day for 30 days orally.

II. Material and method

Study design. Eight male and eight female Chinchilla rabbits were allocated to receive 100 mg/kg dose of Fluorothiazinon once a day for 30 days intragastrically. Blood was sampled for pharmacokinetics on the 1st, 10th, 20th and 30th days of the experiment. Animals were weighted and monitored throughout the experiment. At the 30th day blood and urine were sampled from four male and four female rabbits to study any possible alterations. Heart and respiratory functions were also estimated. The rest of the treated animals were euthanized to perform histological evaluations. After a recovery period of 30 days, remaining animals were tested similarly to assess reversibility of the alterations.

HPLC-MS/MS analysis. Drug and metabolites were determined via HPLC-MS/MS system using electrospray ionization source. Concentrations were assessed using internal standards. Calibration curve for Fluorothiazinon exhibited linear fit in 0.1 – 1000 ng/mL range.

Pharmacokinetics and statistics. Non-compartmental analysis was performed by PKSolver add-in for Excel to achieve following pharmacokinetic parameters: Cmax, AUC, Cmin, Cτ, Cavg, AUCτ, Fluctuation, Accumulation ratio.

Toxicity assessments included clinical observations, hematological, biochemical tests of blood, urine tests, heart rate and ECG monitoring and histological evaluations of various organs.

III. Results and discussion

Pharmacokinetics. Repeated oral administration of 100 mg/kg dose of Fluorothiazinon to rabbits once a day for 30 days resulted in steady state pharmacokinetics with average drug concentrations (Cavg) 45.45 ± 3.19 and 40.07 ± 6.98 ng/mL for male and female rabbits at the 30th day, respectively. In addition, accumulation ratios were 8.34 ± 1.99 in male rabbits and 11.26 ± 2.72 in female rabbits, implying a significant amount of residual drug between dosing intervals.

However, a few drug-induced alterations in blood and tissues were detected, despite the fact that no symptoms were noticed during clinical observations. Treated male and female rabbits had significantly reduced levels of white blood cells, middle cells, red blood cells, mean corpuscular hemoglobin concentration as well as significantly increased levels of granulocytes and platelets among others. In addition, LDH level was significantly increased in male rabbits. Female rabbits had significantly increased levels of creatinine, cholesterol and protein. These changes lay within normal boundaries. Histological alterations in brain were also observed in the treated group. However, recovery period of 30 days proved a reversibility of these features, as results retrieved from post-recovery rabbits showed no toxicity.

Metabolomics. Metabolites related to metabolism of tryptophan exhibited significant changes between sampling days

in both male and female rabbits. Alterated metabolites are majorly attributed to kynurenine pathway. In addition, amino acids and acycarnitines-associated metabolites were significantly altered between the rabbits. Since kynurenine pathway metabolites are commonly associated with immune activation, these compounds might be involved in mediating drug-induced toxicity [ref]. Moreover, a number of significant metabolites, for example, kynurenic acid, were upregulated at earlier stages of dosing. Tracing such alteration might be beneficial in order to predict any adverse effects of the treatment.

IV. Conclusion

Toxicokinetics and metabolomics of anti-virulence drug Fluorothiazinon were characterized. Continuous daily administration of the drug per os in 100 mg/kg dose to rabbits allowed steady state pharmacokinetics to be developed. Comprehensive assessments of rabbits' health, including clinical observations, blood, urine and physiological tests, made it possible to detect toxicity of the novel drug entity. Several reversible hematological, biochemical and histological drug-induced alterations were observed. Metabolomics approach revealed key metabolites responsible for the development of these toxicities; hence uncovering these metabolites can be useful monitor safety of treatment. In conclusion, interdisciplinary approach of combining pharmacokinetics and metabolomics facilitates a powerful tool to gain insights in toxicological impact of anti-virulence therapy against antibiotics-resistant bacteria.

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PT-021

Possible Effects of Gene Polymorphisms on the Development of Sjogren Syndrome

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Sjögren's syndrome is a chronic autoimmune disease characterized by lymphocytic infiltration in exocrine glands such as salivary and lacrimal glands. Although the etiopathology of the disease is not known completely, it is thought that genetic factors have a triggering effect on the development of the disease. The association of HLA and non-HLA genes with the disease has been previously reported.

For our study; 115 patients with Sjögren's syndrome and 40 healthy volunteers were included. Firstly, blood samples of the individuals were taken and then DNA samples were isolated. Then, HLA-II, STAT4, BAFF and TINIP1 gene variations in both groups were performed via Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. We evaluated and then compared the effects of possible gene polymorphisms between controls and patients. The results were interpreted by statistical methods.

Keywords: Sjogren syndrome, gene polymorphisms, PCR-RFLP technique

PT-023

Analysis of Residue Solvent and Alcohol in Herbal Extracts by Headspace Gas Chromatography-Flame Ionization Detector (HS-GC-FID)

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Abstract

Herbal extracts are concentrated forms of the active ingredients in plants. They have been used for their medicinal

properties for centuries, and today many consumers prefer them for natural and alternative health solutions [1]. However, they may contain significant levels of ethanol arising from its use as an extraction solvent in liquid extracts and tinctures or when added as a diluent to liquid herbal preparations. The use of ethanol is necessary for extraction of some constituents that are important for efficacy. Ethanol is metabolically active, therefore formulations without ethanol or with the lowest achievable level should be selected to avoid systemic exposure [2]. When the level of ethanol in the blood is greater than 50–100 mg/dl, symptoms of ethanol poisoning such as hypoglycemia, coma and hyperthermia occur. The possibility of these complications in people with lower glycogen stores is also possible at lower doses of ethanol. Although ethanol level in distillates and its toxicity is less than methanol, its determination is necessary because of its frequent use especially in children [3].

Head space - gas chromatography- flame ionization dedector (HS-GC-FID) is an effective technique for measuring volatile species, such as ethanol, in samples with complex matrices. The method is based on sampling the equilibrated vapor phase (headspace) above the liquid or solid sample in a closed vial, and then measuring the volatile species in the headspace by GC [4].

In this work, isopropanol (IPA) was preferred as an internal standard, and triton X-100 (TX-100) was used as a diluting solution. Total volume of solution (2 ml), amount of TX-100 (2%, v/v) and extraction temperature (80 °C) were optimized. Calibration curve was drawn between the concentration of %1 to 7.5% by volume (n= 5) (y= 1,7078x - 0,7515, R^2 = 0,9949, y; ratio of peak area- EtOH/IPA, x; EtOH% by volume) The slopes of the standart addition and external calibration curve were statistically same. The method was validated.

Keywords: Headspace, gas chromatography, herbal extract, ethanol

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PT-024

Automated Optofluidic Platform for Cell Counting

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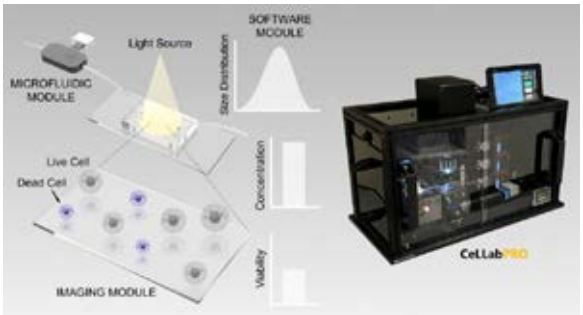
Cell counting is essential in life sciences, medicine, and pharmacology. Traditional approaches e.g., hemocytometry, which require counting cells under a microscope manually. Although hemocytometry is cost-effective, it is also time-consuming and limited by operator experience. To overcome these disadvantages automated cell-counting technologies have been developed to improve cell counting application in terms of both accuracy and efficiency by capturing and processing cell images. Since counting results of these technologies rely on only 100-200 cells, they need further improvement. Additionally, these devices require specialized equipment such as counting chamber and solutions that are compatible with their hardware settings. Here, we developed an optofluidic cell-counting device that addresses these problems, which significantly increases test accuracy by scanning more than 2000 cells. Proposed method has error rate < 1% for cell viability and < 5% for cell concentration results. The platform could provide the count results under a minute, including sample loading, autofocusing, image recording and processing. Presented platform also has a built-in fluidic component which eliminates the need for an external counting chamber enables completely automated sample loading and self-cleaning capabilities that are compatible with any solutions commonly used for cell-counting assays. Proposed optofluidic platform could be valuable asset for accurate and low-cost cell counting application by providing an easy-to-use and rapid feature from sample loading to image analyses.

Keywords: Cell counting, optofluidic, image processing

CeLLABPRO: Automated optofluidic cell counting platform



PT-025

A Dispersive Liquid-Liquid Microextraction Method for Therapeutic Drug Monitoring of Four Antidepressants Drug in Plasma by GC-MS

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Depression is a potentially life-threatening illness that can occur at any age, from early childhood to old age (1). The World Health Organization estimates that more than 300 million people suffer from this disorder (2). There are many antidepressants available for the treatment of depression. These included selective reuptake inhibitors such as fluoxetine and escitalopram and the norepinephrine serotonin reuptake inhibitors like duloxetine and venlafaxine (3). Studies have shown that for antidepressant drugs, plasma concentrations at a given dose can change more than 40-fold. Therefore, therapeutic drug monitoring is important for these drugs (4). For this reason, in this study, an easily applicable, fast, selective, accurate, reliable, and economical GC-MS method was developed for the determination of fluoxetine, duloxetine, escitalopram, venlafaxine and its metabolite o-desmethyl venlafaxine in human plasma for use in therapeutic drug monitoring and also method was validated according to European Medicines Agency (EMA) Bioanalytical method validation guidelines (5).

In the developed method, analytes and internal standard were extracted from plasma by dispersive liquid-liquid microextraction (DLLME) technique and after that injected to the GC system. The limits of quantification of fluoxetine, duloxetine, escitalopram, venlafaxine, and o-desmethyl venlafaxine were determined as 120, 30, 15, and 100 ng/mL respectively. The calibration curves were validated between 120-1000 ng/mL for fluoxetine, 30-240 ng/mL for duloxetine, 15-160 ng/mL for escitalopram, 100-800 ng/mL for venlafaxine and o-desmethyl venlafaxine, with correlation coefficients >0.99. In addition, the developed method was used to determine drug concentration levels in the plasma of real patients.

Keywords: GC-MS, antidepressants, therapeutic drug monitoring, plasma, DLLME

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PT-026

A New Method for Therapeutic Drug Monitoring of Anxiolytic Drugs in Plasma by LC-MS/MS

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Anxiety disorders are a common, serious, and growing health problem worldwide (1). Anxiolytic drugs such as buspirone, alprazolam, clonazepam, diazepam, and lorazepam are used to treat this disorder (2). These drugs are candidates for therapeutic drug monitoring. Measurement of drug plasma concentrations is important as many patients are treated with multiple drugs (3). Therefore, in this study, we developed a rapid, easily applicable, economical, and accurate LC-MS/MS method for the determination of buspirone, alprazolam, clonazepam, diazepam, and lorazepam from human plasma and validated according to the European Medicines Agency (EMA) Bioanalytical method validation guidelines (4).

In the developed method, analytes and internal standard were extracted from plasma by salt-assisted liquid–liquid microextraction (SALLME) technique and after that injected to the GC system. The limits of quantification of buspirone alprazolam, clonazepam, diazepam, and lorazepam were determined as 1, 20, 4, 100, and 30 ng/mL, respectively. The calibration curves were validated between 1–30 ng/mL for buspirone, 20-100 ng/mL for alprazolam, 4-100 ng/mL for clonazepam, 100-3000 ng/mL for diazepam, and 30–300 ng/mL for lorazepam with correlation coefficients >0.99. In addition, the developed method was used to determine drug concentration levels in the plasma of real patients.

Keywords: LC-MS/MS, anxiolytic drugs, therapeutic drug monitoring, plasma, SALLME

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PT-027

Development and Validation of a Fast and Simple LC- MS/MS Method for Quantitative Analysis of Organophosphate Flame Retardants in Human Urine

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Ways of prevention of fire have been an issue and subject of various studies for a very long time. Flame Retardants (FRs) can be defined as compounds which can be used to reduce flammability and stop fires from starting and spreading (Yao et al.,2021). FRs have a wide range of usage including furniture, electrical devices, construction materials and components in transportation vehicles (Shaw et al.,2014). However some FRs have a critical disadvantage which is their quality to leach from the treated materials (Yao et al.,2021). Exposure to these chemicals in every period of human life has become inevitable in our current living conditions. Accumulating scientific evidence shows that FRs are important members of endocrine disruptors and the increasing amount of toxicity information about the exposure due to the use of these substances has become a general concern.

It is seen that most of the studies examined the presence of FRs by liquid chromatography-tandem mass spectrometer (LC-MS/MS) in wastewater, air, dust and materials in daily use. Today LC-MS/MS analysis which is one of the analysis methods used for the determination of FRs exposure in human, requires very complex studies, simple and fast analysis studies are not commonly found in literature. The biological materials that are used in these limited number of studies include urine, whole blood, breast milk (Bastiaensen et al., 2018; Giroud et al.,2021; Kakimoto et al.,2008). In our study we tried to determine FRs exposure in urine samples as biological material and analysis was conducted with LC-MS/MS. When studies investigating the urinary exposures in different societies are evaluated, it is seen that most of these biomonitoring studies include an extraction phase which is mainly solid phase extraction (Bastiaensen et al., 2018). However in our study analysis was conducted with a dilution process instead of solid phase extraction. In this study our effort was to develop an accurate, fast and sensitive LC-MS/MS method in order to determine various organophosphate flame retardants(OPFRs) concentrations in human urine and validate this specific method. Within the scope of this study levels of Tris (1,3-dichloro isopropyl) phosphate (TDCPP), Tris (2-chloroethyl) phosphate (TCEP), Triphenyl phosphate (TPHP), tris(2-butoxyethyl)phosphate (TBOEP) were quantitatively determined by LC / ESI-MS / MS in negative mode, using Reverse phase C-18 column. Confirmed method was successfully conducted on analysis of spot urine samples of randomly selected healthy children. An effort was made to determine very low levels of TDCPP, TCEP, TPHP and TBOEP in human urine by this validated LC-ESI MS / MS method. As a result LOD and LOQ values of analyzed FRs were set.

Keywords: LC-MS/MS, OPFRs, flame retardants

PT-028

Box–Behnken Experimental Design for Optimizing the HPLC Method to Determine Venetoclax in Human Plasma and Bioanalytical Method Validation ICH M10 Guideline

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Abstract

Venetoclax is a specific B-cell leukaemia/lymphoma-2 inhibitor. Patients with chronic lymphocytic leukaemia have

shown venetoclax to have high response rates. This study developed a rapid, accurate and selective method with a low limit of detection and quantification for determining venetoclax in biological samples based on HPLC. The method optimization was carried out using the Box-Behnken design. The chromatographic separation was performed with an Ascentis Express 90 Å C8 column (2.7 µm, 4.6 mm × 10cm). The mobile phase was acetonitrile:water at gradient elution. LOD and LOQ were determined to be 0.34 µg/mL and 1.03 µg/mL, respectively. The results indicated more than 95% recovery of VEN in all cases. The developed method was successfully applied to human plasma samples.

Keywords: Box-Behnken design, HPLC-DAD, human plasma, venetoclax.

I. Introduction

Venetoclax (VEN) is a specific B-cell leukaemia/lymphoma-2 inhibitor. Patients with chronic lymphocytic leukaemia or previously untreated acute myeloid leukaemia (AML) were shown VEN to have high response rates, whether used alone or in conjunction with other medications such as osaconazol or cytarabine. However, these combination therapies are associated with significant rates of side effects in patients with newly diagnosed AML, particularly neutropenia and thrombocytopenia. VEN was mainly metabolized by cytochrome P450 (CYP) 3A [1]. Prophylactic usage of an antimicrobial agent is frequently required due to these side effects' correlation with elevated infection risks, and the co-administration of broad-spectrum triazole antifungal medications, such as osaconazole or fluconazole, decreases mortality in AML patients. [1,2]. Three methods were reported for determining venetoclax in plasma samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [3–5]. In this study, a new method for determining VEN was developed based on the approach of quality by design (QbD) using HPLC. Optimization conditions were determined using Box-Behnken design. The validation parameters of the developed method were performed on the human plasma sample using ICH guideline M10.

II. Material and method

The analysis was performed on a DGU-20A5R chromatography system with an SPD-M20A DAD detector. Chromatographic separation was performed with an Ascentis Express 90 Å C8 column (2.7 µm, 4.6 mm × 10cm). The mobile phase was acetonitrile:water at gradient elution. The pH of the mobile phase was adjusted with acetic acid. The injection volume was 1 µL, the flow rate was 0.8 mL/min at ambient temperature, and sample detection was carried out at 290 nm wavelength. The used chemicals are listed in Tab 1.

Tab 1. Chemicals used in the study.

Chemical	Brand
Venetoclax	Toronto Research Chemicals (Canada)
Agomelatine	Sigma-Aldrich Chemie GmbH (Seelze, Germany)
Acetonitrile	J. T. Baker (USA),
Acetic acid	Fisher Chemicals (USA)
Sodium hydroxide	Sigma-Aldrich (USA)
Human plasma	Sigma-Aldrich (USA)

Blank plasma was prepared by adding 2 mL of acetonitrile to 1 mL of plasma. The resultant mixture was vortexed, followed by centrifugation to precipitate and separate the plasma proteins. The supernatant is filtered and collected. A standard stock solution of VEN was prepared by adding 0.7 mg of VEN to 2 mL of plasma and vortexed then, 2 mL of acetonitrile was added and vortexed again. The resultant mixture was centrifuged, the supernatant was collected, and filtrated volume was made up to 25 mL with acetonitrile. Agomelatine (83 µg/mL) was used as the internal standard (IS).

The method was validated based on ICH M10 guideline on bioanalytical method validation and study sample analysis including selectivity and carry-over, precision and accuracy linearity, matrix effect, and stability.

III. Results and discussion

The method's optimization was carried out utilizing the Box-Behnken design. pH, percentage of the organic phase, temperature, and flow rate are all independent variables. The tailing factor, resolution, capacity factor, peak asymmetry, and theoretical plate number are all analyzed for system suitability. The gradient elution program with a flow rate of 0.8 mL/min, a temperature of 30 °C, a pH of 3, and a wavelength of 290 nm was chosen as optimization parameters. The chromatogram of IS and VEN mixture in plasma is shown in Fig. 1. The retention times of IS and VEN were found to be 2.9 and 6.5 min, respectively.

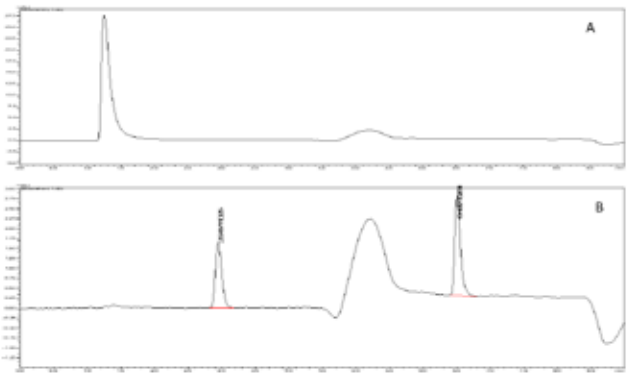

Fig 1. The obtained chromatogram of blank plasma sample (A), and spiked plasma with 5 µg/mL VEN and 24.9 µg/mL IS (B).

The calibration curve for VEN prepared in human plasma, indicated excellent linearity R2 = 0.9979. To evaluate the matrix effect, the LLOQ (1.67 µg/mL) and HQC (10 µg/mL) samples were injected five times for each, and the %RSD and percentage recovery were calculated. The percentage recovery was more than 95%, therefore no interference from other unidentified components in the sample. As a result, it was concluded that there was no matrix effect of the analyte with the plasma matrix. The results are within acceptable limits for bioanalytical method development and validation. The accuracy is determined using the VEN complete recovery in plasma. VEN recovery in plasma was more significant than 95%, indicating that the method developed is accurate. It is significant to be notice that each instance demonstrated repeatable outcomes. LLOQ, LQC, MQC, and HQC (quality control solutions) samples (1.67, 5, 6.25, and 10 µg/mL, respectively) were used in precision studies to assess the consistency of results over numerous samples of the same concentration. The intra-day and inter-day precisions for QC samples were evaluated by calculating SD and RSD. The values of SD were lower than 0.09 and RSD values were less than 5, that shows that the proposed method has a high precision and accurate. Based on the linear regression equation's SD of intercept and slope, the LOD and LOQ for VEN spiked in plasma were determined. LOD and LOQ were determined to be 0.34 µg/mL and 1.03 µg/mL, respectively. These showed that the technique was sensitive for drug identification at lower concentrations. In the current investigation, carry-over is assessed by following each injection of HQC with a blank injection. Whatever does not indicate any peak neither IS nor analyte in the chromatogram. The system suitability study was evaluated by injecting an LLOQ sample five times. System suitability parameters were calculated and within acceptable limits. The short-term stability studies of plasma spiked with VEN were determined at room temperature after 6, 12, 24, and 48 h. The vials containing quality control solutions were kept at room temperature. IS was added for all these quality control samples to get the concentration of 24.9 µg/mL. The mean, SD, and %RSD were calculated for each concentration. Freeze-thaw stability studies were conducted for all quality control samples at three freeze–thaw cycles. The results indicated more than 95% recovery of VEN in all cases.

IV.Conclusion

We developed a simple, accurate and specific method to quantify VEN concentrations based on protein precipitation from small volumes of plasma and subsequent HPLC based analysis. This method was successfully applied to determine VEN concentrations in the plasma sample.

Acknowledgements: This research was funded by Anadolu University Scientific Research Projects Commission (Grant number: 2207S092).

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PT-029

A Novel HPLC Method for the Quantification of Selexipag in Pharmaceuticals Using Box-Behnken Design

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Abstract

Selexipag (SLP) is an oral drug called prostacyclin IP receptor agonist, indicated for the treatment of PAH, which delays the progression of pulmonary arterial hypertension (PAH) and reduces hospitalizations in patients with World Health Organization (WHO) Functional Class II and III. The study aims to develop and validate an inexpensive, fast and reliable HPLC method for determining SLP from a pharmaceutical preparation and to demonstrate its applicability to actual samples. The method developed for this purpose has been validated according to the ICH Q2(R1) guideline. Montelukast sodium (MLS) was used as an internal strand. Supelco Ascentis express phenyl hexyl 10 cm

4.6 mm, 2.7 µm Mobile phase is acetonitrile (0.1% formic acid, pH:3.2): water (68.5:31.5, *v/v*). Recovery values within 98–102% and relative standard deviation values lower than 2% indicate the precision and accuracy of the method. The method was applied to SLP tablets with no interference.

Keywords: selexipag, hplc-dad, internal standard, ICH Q2(R1), pharmaceutical formulation

I.Introduction

Unlike other drugs that act on the SLP prostacyclin pathway, it acts as a selective non-prostanoid prostacyclin IP receptor agonist. Randomized controlled studies have shown that SLP used in combination treatments does not significantly reduce PAH mortality but has therapeutic effects on the frequency of hospitalization and worsening of the disease. Significant side effects are headache, nausea, vomiting and dizziness. There are studies mainly with high-performance liquid chromatography techniques (Damireddy, Pravalika, Praveen, Sathish, & Anusha, 2017; Youssef, Mahrouse, & Mostafa, 2023) and mass detectors (Bhadru, Rao, & Vidhyadhara, 2019; Rao, babu, Koganti, Palakeeti, & Srinivas, 2021; Satheshkumar & Muruganantham, 2021) for SLP analysis. In this study, a fast, reliable, accessible, high accuracy and reproducibility HPLC method was developed for the analysis of pharmaceutical formulations of SLP in the market. All method parameters were thoroughly optimized and fully validated according to the ICH Q2(R1) guideline.

II. Material and method

Nexera-i LC-2040C 3D device belonging to Shimadzu (Japan) company was used for HPLC analysis. The system's software is LabSolutions 5.81, software belonging to Shimadzu (Japan). Since the maximum absorbance of SLP was detected at 212 nm and the spectra were monitored in the detector between 190 and 380 nm at a data sampling frequency of 1.5625 Hz and a time constant of 0.640 sec. Chromatographic separation was performed with an Ascentis Express 90 Å phenyl hexyl column (2.7 µm, 4.6 mm × 10cm) and the mobile phase comprises 0.1% formic acid in water, acetonitrile (31.5:68.5, *v/v*), and pH 3.2. Flow rate 0.8 mL/min and injection volume 1µL. The column temperature was adjusted to 30±2 °C. The developed method was fully validated according to ICH Q2(R1).

III. Results and discussion

Liquid chromatography is a recommended method for analyzing drug analyses with high accuracy and reproducibility in the pharmaceutical industry. The box-Behnken design has been used as a response surface design because it avoids excessive factors and requires less analysis than central composite designs (Hibbert, 2012). The percentage of organic components of the mobile phase and pH, column temperature and flow rate were chosen as factors for this study because of their high effects on chromatographic separation. (Fig. 1).

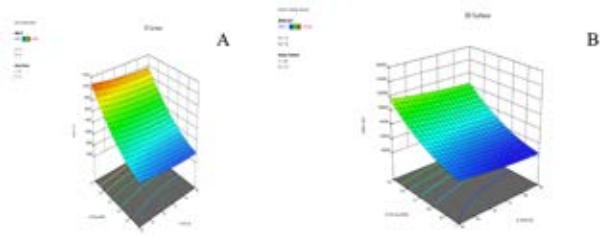

Fig. 1 Surface plot graphics for MLS (A) and SLP (B) (A= acetonitrile; B = flow rate, C= column temperature and D=pH)

The system suitability parameters obtained under optimum conditions are calculated as tailing factor 1.07, capacity factor 1.9 and RSD 0.17 for retention time.

Montelukast sodium was chosen as the internal standard in the study and eluted from the column after SLP. Calibration calculations in the study were made according to the peak normalization method and the results were given Table 1. In addition, the purity of the peak in the detector was calculated as 0.987771, and it was proved again that the current peaks belong only to SLP.

Table 1. Statistical data for the linearity of SLP

Parameters	Obtained Data
Linearity range (µg/mL)	5-50
Slope (intraday, n=7)	1,5437
Intercept (intraday, n=7)	-0.0766
Regression coefficient (intraday, n=7)	0.9954
SD of Slope	1201.24
SD of Intercept	0.0502
LOD (µg/mL)	0.8
LOQ (µg/mL)	2.0

Accuracy studies were carried out after calculating the linearity and precision data of the HPLC method optimized for SLP analysis. For the recovery experiments, SLP was added to the hand-prepared Uptravi® solution by the standard addition method. The study was performed with seven independent analyzes at three different concentrations. When the results are sifted through, it is seen that the effects of processes such as dilution and filtration on the method accuracy are within acceptable limits. In this study, by adopting this understanding, Uptravi®, the commercial pharmaceutical tablet formulation of SLP, was prepared and analyzed for recovery studies and sample applications.

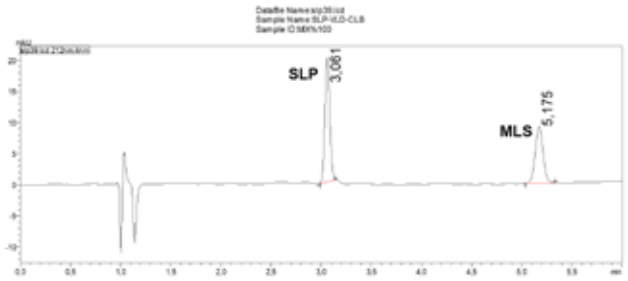

Fig 3. The obtained chromatogram of Uptravi® solution

IV. Conclusion

In the analyses carried out within this study's scope, a column with a brand phenyl hexyl functional group was used. The quality-based design approach was adopted to optimize the management, and the Box-Bhenken design was used. With the developed method, the analysis was performed with high efficiency and the analysis was completed in 6 minutes. Compared to other studies, this study is less costly and can give reliable results. Short-term methods provide significant savings in time and analysis costs. For example, since the time used in the analysis will be shortened, less solvent usage, more energy savings, and less waste will be provided. It will show a more environmentally friendly approach.

Acknowledgment: This research was funded by Anadolu University Scientific Research Projects Fund Commission grant number1606S5567.

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PT-030

Exploring the Performance of Cellulose Tris-3,5-Dichlorophenylcarbamate (CDCPC) as a Stationary Phase for the Chiral Electro-chromatographic Separation of Azole Antifungals

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Miniaturized separation techniques, both pressure-driven and electrophoretic, represent a current trend in analytical chemistry because they meet sustainability requirements through reduced solvent wastes, short analysis times, small amounts of stationary phases, and high chromatographic efficiency. Both nano liquid chromatography (nano-LC) and capillary electrochromatography (CEC) are excellent tools for studying new stationary phases which, often expensive and available in small quantities being synthetic products, can be packed into laboratory-made capillary columns by using just a few milligrams. Moreover, the short conditioning time of capillary columns allows one to study and test new stationary phases on different classes of molecules rapidly. Of special interest are chiral contaminants (i.e. drugs and pesticides) that are often administered as racemic mixtures, although the active form is represented by a specific enantiomer (eutomer).

In this work, we investigated the chiral recognition and separation performance of cellulose tris-3,5-dichlorophenylcarbamate (CDCPC) as a selector for enantioseparations. The CDCPC was chemically bound to porous silica particles of 5 µm in diameter, packed into fused silica capillaries (250 mm x 75 µm i.d.) by the "slurry technique," and applied in CEC coupled to UV detection to separate fourteen selected azole compounds. To study chiral recognition, the composition of the mobile phase was modified in

terms of organic phase, aqueous phase content, type of pH buffer and its concentration. Each change resulted in the enhancement of specific interactions, at the expense of others, leading to chiral recognition. Their effects on retention factors, enantiomeric resolution, and peak broadening were critically discussed together with the effects of the sample dilution solvent and the injection mode (electrokinetic or pneumatic) on the chromatographic profile in order to improve enatioseparation and sensitivity. The developed methodology was used as an appropriate test bench to evaluate the stability of the stationary phase under different mobile phase conditions, demonstrating a remarkable enantioselective capacity even after several tens of hours of continuous use. The optimized CEC-UV method proved to be very effective in the chiral separation of seven of fourteen azole fungicides, of seven chiral azole antifungals, totaling fourteen baseline separated peaks in a single 35 min run analysis.

Keywords: Cellulose Tris-3,5-Dichlorophenylcarbamate, chirality, azole compounds

PT-032

Determination of Zofenopril and Hydrochlorothiazide by Capillary Electrophoresis and High Performance Liquid Chromatography in Pharmaceutical Preparations

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Abstract: ZOF is an antihypertensive drug belonging to the family of the angiotensin-converting enzyme inhibitors, characterized by high lipophilicity, sustained cardiac ACE inhibition, and antioxidant and tissue protective activities Hydrochlorothiazide, is a diuretic of the class of benzothiazines widely used in antihypertensive pharmaceutical formulations, alone or in combination with other drugs, which decreases the active sodium reabsorption and reduces peripheral vascular resistance. These two drugs are successfully used in association with the treatment of hypertension (1).

In this study, a capillary electrophoresis (KE) method was developed for the determination of zofenopril (ZOF) and hydrochlorothiazide (HCT) from pharmaceutical preparations and for comparison high performance liquid chromatography (HPLC) method is used. Telmisartan (TEL) was used as internal standard (IS) and the detection was achieved by photo diode array (DAD) detector in the methods. In the CE method, the separation was performed by bubble cell capillary with an inner diameter of 75 µm and an effective length of 40 cm. Optimum conditions were obtained by applying 10 mM borate buffer containing 15% methanol (pH 9,00) and 25 kV potential. Under these conditions, the migration times of ZOF, HCT and IS were observed as 4,67 min, 3,38 min and 4,25 min. In the HPLC method, for separation C8 column (3,0×150,0 mm, 3,5 µm i.d.) as mobile phase; 10% acetonitrile containing 1% phosphoric acid (H3PO4) and acetonitrile containing 1% H3PO4 solutions were used in gradient system at a flow rate of 1 mL/min. In this method, the retention times of ZOF, HCT and IS were observed as 9,05 min, 1,89 min and 6,53 min, respectively. Validation of the developed methods were examined by linearity, precision, accuracy, selectivity, sensitivity and stability parameters. The limit of quantification (LOQ) values of ZOF and HCT were 8,28×10-8 M and 6,31×10-8 M in CE and 6,93×10-8 M ve 4,40×10-8 M in HPLC, respectively. Both of the developed methods were successfully applied for the determination of ZOF and HCT from tablets. It was applied to tablets containing 30 mg ZOF and 30 mg/12.5 mg ZOF/HTC. The contents of the pharmaceutical preparations were found to be in the limits of USP39 (2). This method is proposed for the routine analysis of ZOF and HTC.

Acknowledgements: This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1505S428.

Keywords: Zofenopril, hydrochlorothiazide, capillary chromatography, high performance liquid chromatography

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PT-033

Quantitative Analysis of Urinary Nucleosides and Deoxynucleosides from Bladder Cancer Patients Before and After Tumor Resection

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The approach most often applied in metabolomics studies is searching of potential disease biomarkers when biological samples are collected at one particular time point. Our study presents a concept of a longitudinal monitoring study of selected potential targets of bladder cancer disease before tumor resection and at different time points up to 12 months after the surgery.

We determined eleven nucleosides and deoxynucleosides which are well known as tentative cancer indicators and oxidative stress markers, respectively. Although their known clinical importance they are rarely determined together which is related with difficulties of their common effective isolation from biological samples. To eliminate this obstacle, the urine samples were only precipitated and centrifuged. For quantitative determination we developed and validated the LC-MS/MS method which revealed to be rapid, selective, robust and offering high sensitivity and simplicity in usage. The method was successfully applied to determine urine metabolites in 133 samples. Next, advanced statistical approach involving linear mixed-effect model that takes into account the nested structure of the measurements and various levels of variations of the data, was applied. The analysis revealed that 2-methylthioadenosine concentration level was decreased while for inosine it was increased 24 h after tumor resection in comparison to the preoperative state. We anticipate that this analytical contribution will support future metabolomic longitudinal studies of bladder cancer recurrence potential indicators.

Keywords: modified nucleosides, modified deoxynucleosides, targeted metabolomics, method validation, bladder cancer, linear mixed-effect model

PT-034

Senstive HPLC-DAD Analysis of Phenobarbital by Fabric Phase Sorptive Extraction in Urine Sample

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Epilepsy is a neurological disease that maintains its importance as a clinical problem today. It is characterized by seizures that cannot be predicted when it starts. The annual prevalence (frequency) of epilepsy is 0.5-1%. It is one of the common serious brain disorders affecting more than 70 million people worldwide (Nazarov,2022) . Phenobarbital is an antiepileptic drug in the barbiturate category that is widely used worldwide due to its antiepileptic effect (Khiltash et al.,2022). With the proposed project study, antiepileptic drugs; A Fabric Phase Sorptive Extraction (FPSE) based chromatographic determination method, which has been widely used in the literature in recent years and offers practicality, will be developed for the monitoring of Phenobarbital active substances that suppress the functioning of the central nervous system. With this method, the determination of Phenobarbital after direct sampling with a simple pre-treatment procedure could be done accurately and sensitively with a conventional HPLC device. The phenobarbital molecule was analyzed at trace level in the proposed method via a part of fabric phase including PEG-PPG-PEG. The target molecules were absorbed onto surface of fabric phase in the presence of pH: 4.0 Britton Robinson buffer medium and desorbed by a smaller volume with Methanol again before chromatographic determinations. Before the analysis, the samples were transferred to HPLC vials by filtering with a 0.45 µm PTFE filter.

Extraction parameters such as pH, type and amount of extracting solvents, adsorption and desorption time, chromatographic determination variables such as column selection, mobile phase composition and sampling amount were optimized in detail. In the developed method; Phenobarbital as antiepileptic drug active ingredient was determined in HPLC device at 228 nm wavelengths using DAD detector with isocratic elution of pH 3.0 (phosphate buffer): Acetonitrile (65:35)

mixture. The detection limit obtained for the phenobarbital molecule under optimized conditions is 12.25 ng mL^{-1}. Relative standart deviations (RSD%) was calculated lower than 7.70 % for 5 replicate measurments by using model solutions including 100 ng mL^{-1} of phenobarbital. Finally, the developed method was applied to urine samples.

Acknowledgements: This study has been supported by TUBITAK as a 2209-A student project with the 1919B012216827 code.

Keywords: HPLC, fabric phase sorptive extraction, phenobarbital, urine samples

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PT-035

Development HPLC Method For Determination Of Naproxen Sodium Drug in Urine Samples By Solid Phase Microextraction Techniques

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This study focus on the naproxen sodium active ingredient which is one of the non-steroidal anti-inflammatory drugs Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used medications. Naproxen sodium (NPS) is a nonsteroidal anti-inflammatory drug commonly used for fever, inflammation, the reduction of moderate-to-severe pain. The main mechanism of NPS action, inhibition of prostaglandin synthesis, makes the drug effective in combating pain and inflammation. (Bhole et al.2015). Accurately and sensitively, independent of interference, a magnetic solid phase extraction-based chromatographic method for separation and enrichment approaches has been developed in order to analyze this molecule. Extraction parameters such as pH of the working medium, adsorption time and desorption time, selection and amount of suitable solvent for desorption were optimized step by step.

In the developed method, Naproxen Sodium molecule was analyzed by HPLC-DAD via isocratic elution of 50 % Acetonitrile, 40% Phosphate Buffer (PBS) adjusted to pH 3 and 10% Methanol mixture. Wavelengths of 231 nm and 272 nm were used for naproxen sodium were analyzed in HPLC device with DAD detector. The limit of detection (LOD) was calculated for the Naproxen sodium molecule under optimized conditions is 4.85 ng mL^{-1} by considering ICH guidelines. Accuracy of method was tested by recovery tests in urine samples and recovery values were obtained in the range 94.6-105.8 %.

Acknowledge: This study has been supported by TUBITAK as a 2209-A student project with the 1919B012216827 code.

Keywords: Naproxen sodium, HPLC, magnetic nanoparticles, urine samples

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PT-036

Development of Sensitive and Accurate Analysis Method based on HPLC-DAD after Magnetic Solid Phase Extraction for Bisphenol A Molecule in Beverage Samples

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Bisphenol A (BFA) is a chemical that is the monomer of

polycarbonate plastics and epoxy resins used as raw materials in food and beverage packaging materials. Bisphenol A is considered an endocrine disruptor chemical because it affects the human hormonal system (Guimarães,2022). It is found in the structure of feeding bottles, storage containers, baby foods, water bottles and bottle caps, eyeglasses and electronic materials, which are used in many areas of daily life.BPA is a toxic product resulting in neurodegeneration, and metabolic, reproductive and kidney disorders and it is important of analysis at trace level (BayatlooandNojavan,2022). In this study, a Magnetic Solid Phase Extraction(MSPE) based chromatographic determination method, which is frequently used in the literature in recent years and provides easy of application, has been developed for the monitoring of the endocrine disruptor bisphenol A. In this way, direct sampling could be done with a simple pre-process, and then the determination of Bisphenol A could be done accurately and sensitively with a conventional HPLC device. In the proposed study, the determination of Bisphenol A molecules enriched with the micro-extraction technique to be developed was analyzed by HPLC-DAD system in beverage samples. Extraction parameters such as pH, type and amount of magnetic solid phases, suitable solvent selection for desorption processes, and chromatographic variables such as column selection, mobile phase composition and sampling amount were optimized step by step.

In optimized method; Bisphenol A molecule was determined by using DAD detector at 226 nm with gradient elution of Acetonitril: Mehanol: pH 3.0 Buffer mixture. The detection limit for the Bisphenol A molecule under optimized conditions is 6.56 ng $mL^{-1}$and linearity was found in the range of 20-800 ng mL^{-1}. Finally, the developed method was successively applied to determination of Bisphenol A in beverage samples while relative standard deviation (RSD%) was lower than 5.0 % for 250 ng mL^{-1} concentration level.

Acknowledgements: This study has been supported by TUBITAK as a 2209-A student Project with the 1919B012216827 code.

Keywords:, Bisphenol A, magnetic solid phase extraction, HPLC-DAD, beverage samples

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PT-037

Application of LC-MS and GC-MS Techniques for Determination of Metabolic Changes in Serum and Urine Samples from Women with Polycystic Ovary Syndrome

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Despite of many research, pathogenesis of polycystic ovary syndrome (PCOS) is not clearly understand. PCOS is a common, endocrine disorder and one of the main causes of female infertility. It is connected with ovulatory dysfunction, presence of polycystic ovaries and hyperandrogenism. Apart from endocrine dysfunction, it may lead to the development of other metabolic abnormalities.

Application of the metabolomic approach allows to identify specific metabolites and biochemical pathways affected during the development of PCOS. The aim of our study was to determine metabolic profiles of serum and urine samples obtained from 35 PCOS women and 35 healthy controls and identify the most discriminating metabolites. Untargeted metabolomic analyses were conducted with the use of two complementary analytical techniques LC-TOF/MS and GC-QqQ/MS. Selection of statistically significant metabolites was performed with the application of univariate and multivariate statistical analysis. Results

of our research indicate few biochemical pathways disturbed in PCOS women such as: elevated steroid hormones synthesis, alteration in fatty acids, sphingo- and phospholipids metabolism as well as alteration in the citric acid cycle, vitamin B metabolism, γ-glutamyl cycle and amino acids like alanine, phenylalanine, histidine, and tryptophan.

Most of identified metabolites can be associated with altered insulin action, which could be a contributing factor to the

development of PCOS.

Keywords: polycystic ovary syndrome, untargeted metabolomics, mass spectrometry, potential biomarkers

PT-038

A plant Metabolomics Approach: Evaluation of the Momordica Charantia Seed and Fruit Extracts

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Abstract

Momordica charantia is also known as karela, bitter gourd, bitter melon, balsam pear. The seeds of *Momordica charantia* is used antihyperglycemic, antibacterial, antiviral, antitumor, immunomodulation, antioxidant, antidiabetic, anthelmintic, antimutagenic, antiulcer, antilipolytic, antifertility, hepatoprotective, anticancer and anti-inflammatory and wound healing. In this study, we aimed to elucidate the untargeted metabolites of 70% methanol extracts of Momordica charantia seeds and fruit in clinical metabolomics. Q-TOF LC/MS-based analysis of the extracts for both seed and fruit was performed using a C18 column (AGT-695775-702 Poroshell HPH-C18, 2.1x100mm, 2.7um). Differences were observed in seed and fruit extracts and these differences were visualized using principal component analysis (PCA) plots and as a result LysoPC(O-18:0), glucosylceramide were high in Fruit, whereas (R)-salsolinol, pantetheine, coumarin, tryptamine, presqualene diphosphate, pyroglutamic acid were high in seed.

Keywords: *Momordica charantia*, LC-MS, metabolomics, anti-diabetic therapy, wound healing

I. Introduction

Plant metabolites are in chemical diversity, as each plant has its own set of complex metabolites (Oksman-Caldentey 2005). The characterization of plant metabolites, which have important plants for human health, is an important application of plant metabolomics (Guy 2005). *Momordica charantia* (MC) belongs to family Cucurbitaceae. It is known commonly known as karela, bitter gourd, bitter melon, balsam pear. *Momordica charantia* (MC) has antihyperglycemic, antibacterial, antiviral, antitumor, immunomodulation, antioxidant, antidiabetic, anthelmintic, antimutagenic, antiulcer, antilipolytic, antifertility, hepatoprotective, anticancer and antiinflammatory activities (Jia 2017). In this study, 6545 Quadrupole Time-of-Flight Liquid Chromatography Mass Spectrometry (Q-TOF LC/MS) based metabolomics approach is applied to compare the metabolic profile of the seeds and fruit of this plant. The methodology of adapting the clinical non-targeted metabolomics approach to a plant metabolomics study was applied.

II. Material and method

Native plant *Momordica charantia* (MC) fruits were collected from Nilüfer district of Bursa province in September 2022 and the color of the fruits was orange and the seeds were red when collected. They were dried at room temperature in the shade. Then, before extraction, the seeds were roughly ground in the grinder and the fruits were cut into small pieces. The materials were individually applied to continuous extraction with methanol was repeated 3 times using fresh solvent each time. The repeated extracts were combined together and concentrated to dryness in vacuum. The concentrated extracts of fruits and seeds were lyophilized separately (Fig 1.).

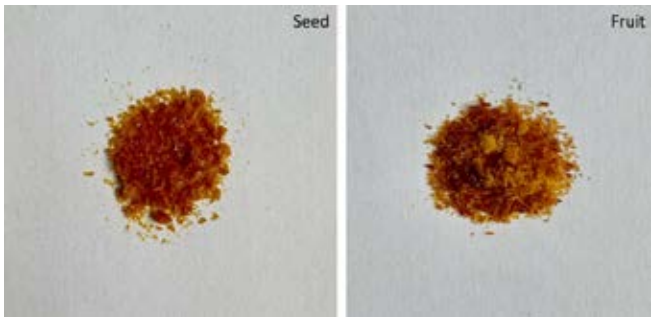

Fig. 1: Aqueous extracts of *Momordica charantia evaporated* to dryness and lyophilized for seed and fruit.

Methanol extracts of seeds and fruits were dissolved in 10 mL of acetonitrile: methanol 50:50 (v/v) mixture. Then, the solutions were sonicated in an ultrasonic bath and diluted with water. C18 column (AGT-695775-702 Poroshell HPH-C18, 2.1x100mm, 2.7um) was used as the chromatography column. The gradient elution mobile phase composition is acetonitrile and water including 0.1% formic acid. Flow (0.30 mL min^{-1}) started with 10% of acetonitrile and increased linearly to 45% acetonitrile till the 5th min. Thereafter, the acetonitrile rate was linearly increased to 90% by the 15th minute and decreased to 10% by the 18th minute. It was kept constant at 10% until the 25th minute. All the samples (n=6) were injected into the system in two replications in random order Mass spectrometry analysis was performed on an Agilent 6530 LC/MS Q-TOF instrument (Agilent Technologies, 184 Santa Clara, CA). The total analysis time was set at 25 minutes. The scanning range for the MS device is set to 100-1700 m/z. The column temperature was set to 35 °C, the drying gas temperature

to 350 °C, and the capillary voltage to 4000 V. The MS device was operated in positive ion mode. The raw chromatograms were processed using XCMS. Isotopologue Parameter Optimization (IPO) was employed to optimize XCMS parameters. Principal Component Analysis (PCA) was employed to understand group similarities and differences. The identification was performed using MetaboAnalyst 5.0.

III. Results and discussion

Base peak chromatograms for Q-TOF LC/MS injections are given for seeds and fruits, respectively (Fig 2.). In fruit and seed extracts, there was a direct separation in the PCA plots. The PCA plot shows the differences in metabolome levels for seeds and fruits (Fig 3.). 618 peaks were found that were at least 2-fold different ($p<0.05$, $FC>2$) at the 95% confidence interval. According to the literature review, 8 of these metabolites differed considerably. The Cucurbitaceae plant family does not have any metabolite equivalents registered in the KEGG databases. In Metaboanalyst 5.0, KEGG codes are found for the plant species Oryza sativa japonica (Japanese rice), Arabidopsis thaliana (thale cress). There are quite a few metabolites in HMDB, but undifferentiated for plant metabolites. With this study, this is the first non-targeted comparison of seed and plant metabolites for the genus Momordica. As a result, 8 metabolites including (R)-Salsolinol, Pantetheine, Coumarin, Tryptamine, LysoPC(O-18:0), Glucosilceramide, Presqualene diphosphate, pyroglutamic acid were found and these metabolites were confirmed by literature research.

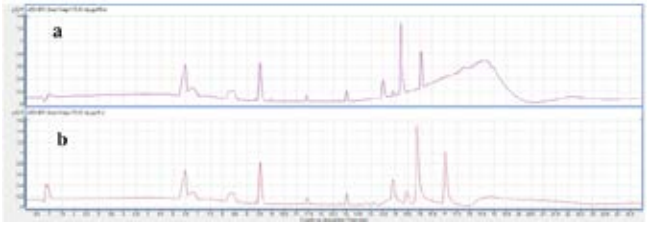

Fig. 2: Base peak chromatograms of the extracts for fruit (a) and seed (b).

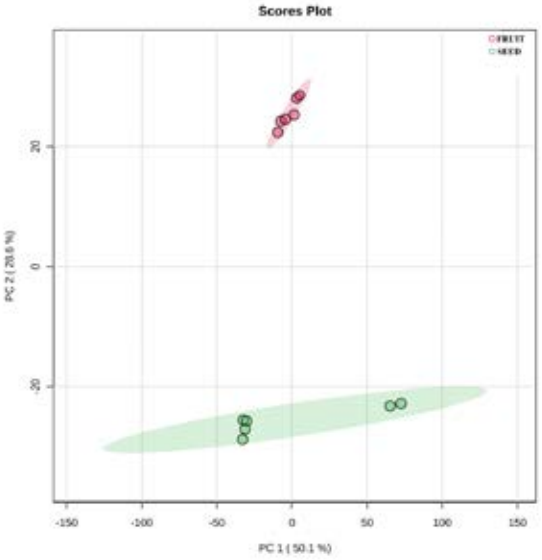

Fig. 3: PCA graph to show the statistical difference of seed and fruit in metabolome level.

IV. Conclusion

Many of the drugs available in the market are used as natural remedies. MC is a well-known medicinal plant with many uses such as antibacterial, antivital, antihyperglycemic, antidiabetic. Our study results clearly show that the plant metabolite profile for the seeds and fruits of MC is statistically different from the PCA graphs. As a result of the analysis of the seed and fruit of Momordica charantia by Q-TOF LC/MS, while the amount of LysoPC(O-18:0), glucosylceramide was high in the fruit, the amount of (R)-salsolinol, pantetheine, coumarin, tryptamine, presqualene diphosphate and pyroglutamic acid in the seed was high in the seed.

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PT-039

Profiling of Carnitine and Acylcarnitines in Gliomas Using Solid Phase Microextraction (SPME) – Extended Studies

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Gliomas are brain tumors with one of the highest mortality rates, which is mainly due to their high histological and molecular diversity. The search for compounds differentiating these tumors is still an extremely important topic. Carnitine and acylcarnitine levels have been reported to be elevated in tumor tissues compared to healthy ones. Previous studies related to gliomas and the level of carnitines and their esters in tumor tissue, using chemical biopsy, were performed on a small study group (by Bogusiewicz et al.). Therefore, it was decided to extend the research and check if the trend would be confirmed in a larger cohort of patients. Herein, carnitine and its esters profiling was carried out in brain tumors (gliomas) with regard to their grade and the presence of IDH mutation.

Brain tumors (from 92 patients) were sampled on-site, directly after removal of the lesion, using C18 solid phase microextraction (SPME) fibers. Subsequently, probes were desorbed into isopropanol:methanol, 1:1 v/v solution, and analyzed with the use of liquid chromatography coupled with Q Exactive Focus. Full scan analysis followed by the MS/MS confirmation of tentative acylcarnitines was performed. Identification was based on fragmentation patterns matched with spectra libraries with < 3 ppm mass accuracy. Peaks area for lipidomic data were normalized on the total peak area for all lipids and these values were used for detailed statistical analysis.

The results showed higher carnitines and acylcarnitines levels in high-grade gliomas, which could be explained by activated proliferation and the higher metabolism rates in malignant lesions. A similar pattern was observed in regard to lesions without IDH mutation in comparison with mutant samples. This observation could be connected with alterations in the tricarboxylic acid cycle (TCA) cycle. Alterations in the TCA cycle influence fatty acids oxidation (FAO) which leads to decreased levels of acylcarnitines in samples with present IDH mutation. The obtained results were consistent with the conclusions of the pilot study performed beforehand (1). Apart from the profiling of acylcarnitines on the extended study group, the possibility of application of rapid analysis such as coated blade spray–mass spectrometry (CBS-MS), the method which combines the features of microextraction and fast ionization methods, was tested. For this proof of concept study, the experiment was performed on a small group of patients, showing that carnitine and its esters were detectable in CBS analysis.

In conclusion, it was confirmed that carnitine and acylcarnitines could be useful in the characterization of gliomas phenotypes, especially in terms of grade as well as the presence of IDH mutations. In addition, CBS could be applied for acylcarnitines analysis however, the protocol should be thoughtfully optimized and validated. This approach can shorten the analysis as much as possible and enable intra- surgical diagnostics in the future.

Keywords: SPME, CBS-MS, brain tumors, gliomas, lipidomics, metabolomics

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PT-040

XCMS And MS-Dial for Q-TOF LC/MS Based Untargeted Metabolomics: Comparison of Data Processing Tools

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Metabolomics is the large-scale analysis of the metabolome in a biological system and reveals the quantitative assessment of low molecular weight biomolecules (<1500 Da). Non-targeted metabolomics studies based on LC-MS include

sample preparation, instrumental analysis, and data analysis (Vinayavekhin 2010).

In this study a dataset obtained from our previous clinical studies was used as a model and two different data processing software, XCMS and MS-DIAL were employed to process the data. Data analysis was performed by applying the identical data processing parameters and statistical tests were performed to compare the final results.

In the Excel table containing the raw data obtained XCMS results, correlation analysis was performed on the prepared consequtive dilution series, and the peaks having areas not in a correlation with dilution factor ($R<0.9$) were excluded. On the other hand, the peaks detected at least 50.0% after processing using MS-DIAL were accepted as "reliable" peaks for MS-DIAL data processing. Both of the final peaks obtained using XCMS and MS-DIAL were compared with the 't-statistics test' to determine the peaks with $p<0.05$ confidence interval between groups. The peak numbers determined are given in Table 1. The processed XCMS results were uploaded into MetaboAnalyst 5.0 to form PLS-DA graphs and compared with the one presented using MS-DIAL. The final results showed that both XCMS and MS-DIAL achieved to separate the dataset for the groups known as in a different profile (Fig. 1). However, the data processing strategies applied for XCMS were more selective than the ones for MS-DIAL.

Table 1. Peak numbers were obtained as a result of data analysis.

XCMS		MS-DIAL	
APPLIED TEST	NUMBER OF PEAKS	APPLIED TEST	NUMBER OF PEAKS
Number of peaks found with optimized xcms values	5470	The number of peaks found with the MSDial program	64040
Number of peaks with a value of r>0.90	3055	Number of peaks found in at least 50% of the samples	3515
Number of peaks remaining in the 95% confidence interval in the t-test result	2636	Number of peaks remaining in the 95% confidence interval in the t-test result	2991
The number of peaks whose amount changes at least 1.5 times	2276	The number of peaks whose amount changes at least 1.5 times	2493
Number of peaks matching at least one metabolite	118	Number of peaks matching at least one metabolite	192

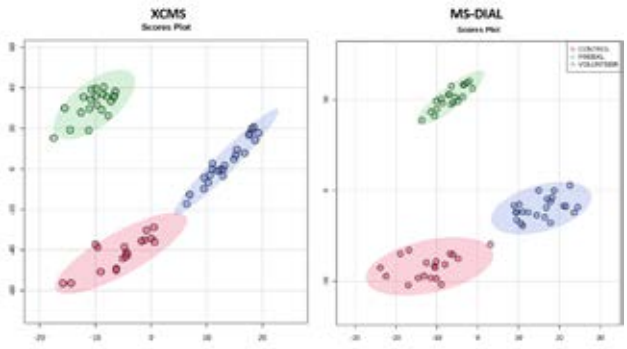

Fig. 1. The PLS-DA analysis of the results obtained with XCMS and MS-Dial

Keywords: Metabolomics, data analysis, XCMS, MS-Dial, metaboAnalyst

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PT-041

Anti-Cancer Effects of Exopolysaccharides Derived From Lactiplantibacillus plantarum EIR/IF-1, Isolated from Breastfed Infant Fecal Mıcrobıota

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Cancer, which affects many people around the world, draws attention as the first leading cause of death after cardiovascular diseases. Data obtained from studies carried out in recent years have made the microbiota a very important research area in cancer research. The aim of this study is to determine the anti-cancer effects of *Lactiplantibacillus plantarum* EIR/IF1- derived exopolysaccharides.

Lactiplantibacillus plantarum EIR/IF1- originated from infant fecal microbiota was cultured in MRS media for 24 h and the cell free supernatant was used for the extraction of release exopolysaccharides (EPS-r). The sugar amount in EPS-r was determined according to the Dubois method, while its sugar content was evaluated using FTIR and HPLC analysis. Anti-cancer effects of the EPS-r were assessed on various cancer cell lines (B16F10, HT-29 and Hep40) using MTT assay.

EPS-r showed cytotoxic effects on B16F10 (human skin melanoma cell line), HT-29 (human colorectal adenocarcinoma cell line) and Hep40 (human hepatocellular carcinoma cell line) cells lines at concentrations of 1 µg/mL, 25 µg/mL and 10 µg/mL, respectively. The HPLC chromatogram showed that glucose was the highest saccharide with a concentration of 5.674 g/100 g. Galactose and fructose were found in EPS-r at 4.476 g/100 g and 0.23 g/100 g, respectively. The presence of various monosaccharides indicates that the EPS-r is a heteropolysaccharide. According to FTIR spectroscopy, the peak at 1070 cm−1 characterized the molecule as a polysaccharide.

Our results showed that EPS-r extracted from *Lactiplantibacillus plantarum* displayed anti-cancer effects on different cancer cell lines. To be used as a natural, safe and effective treatment strategy, *in vivo* experiments has to be carried out.

Keywords: Cancer, anti-cancer, exopolysaccharide, microbiota

PT-042

Development and Easy Applicable Sample Pre-Treatment Procedure for Sensitive Analysis Sibutramine and Fluoxetine in Adulterated Herbal Products

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Obesity is one of the most important health problems that threaten the whole world. Irregular diet, unhealthy foods, and sedentary life are the most important causes of this problem. Many civilian organizations are making efforts to combat obesity (Loss and Yeo, 2022). The history of pharmacological research for slimming drugs dates back more than a century. Medications used to cause weight loss can reduce appetite, increase satiety, reduce the absorption of nutrients, or increase energy consumption (Hieu et al., 2021)

A new preconcentration and determination method including chromatographic (HPLC-DAD) analyzes after Fabric Phase Sorptive Extraction (FPSE) has been developed to monitor the trace amounts of Sibutramine and Fluoxetine molecules found at trace levels in herbal slimming products. In the proposed method, fluoxetine and sibutramine analytes were extracted into the solid sorbent phase with the help of newly synthesized fabric phase membranes in the presence of pH 10 buffer. Before the samples were transferred to vials, they were filtered with a 0.45 µm PTFE injector-tip filter, and then they were placed in the HPLC autosamplers. After optimization of the experimental variables such as pH, desorption solvent and amount, analytical parameters of the developed method such as linear range, enrichment factor and detection limit were studied and determined according to official guidelines.

In the developed method; Fluoxetine and Sibutramine molecules were analyzed after FPSE at a wavelength of 225 nm for Fluoxetine and 265 nm for Sibutramine, using a DAD detector with gradient elution of pH 5.0 acetate buffer (0.6%) and Methanol (70:30). The detection limits obtained for each type of drug molecules under optimized conditions were 4.25 ng mL^{-1} for fluoxetine and 8.43 ng mL^{-1}, respectively. In the repeated measurements made with model solutions containing 100 ng mL^{-1} drug molecules, the RSD % values were found below 6.0%.

Acknowledge: This study includes some data obtained from a part of doctorate thesis of Ms.Aslıhan Gürbüzer and it was suppurted by Erciyes University Scientific Research Project Unit with project id:12123.

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Keywords:, Fluoxetine, sibutramine, fabric phase sorptive extraction, herbal slimming products, HPLC

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PT-043

Metabolomics Profiling Using Dual Stationary Phase Columns

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To understand the physiology of a biological system is only possible to analyze all metabolites in living organisms. However, analysis of metabolites that show great physical and chemical differences cannot be carried out with a single method. The analysis of the highest number of metabolites should be performed in a short analysis period, taking into consideration of time and analysis costs. For more extensive metabolomics profiling, chiral metabolites analysis should also be performed.

In the literature, the chiral compounds were generally analyzed after a derivatization step. Although derivatization has advantages such as achieving better peak shapes and increasing sensitivity, there are disadvantages such as adding a synthetic step prior to separation, interferences from the derivatizing agent, and the possibility of not completing the derivatization. In addition, it is practically impossible to perform derivatization for only one group in a place where thousands of metabolites are analyzed at the same time in metabolomics analyses because of difficulties in derivatization and identification of metabolites of other metabolites having the same functional group. Considering all these problems, there is a need for methods where simultaneous analysis of all chiral metabolites is carried out without derivatization.

Within the scope of our study, chiral and non-chiral stationary phase compounds containing two-module column systems were developed and chiralomics analysis was performed. For this purpose, metabolomic analyzes were carried out in positive and negative ionization modes in a binary stationary phase system in which HILIC and cellulose-based chiral stationary phases were connected in series with LC-qTOF-MS. At the end of the analysis, the number of metabolites obtained from the two-module stationary-phase chromatographic system was more than the number of metabolites (4160/5764 in positive mode and 2972/2937 in negative mode) obtained from the single stationary-phase system. The higher number of metabolite annotations may not only be due to chirality but also that the simultaneous usage of a second separation mechanism may have separated metabolites with similar physicochemical characteristics better than single stationary phase.

Keywords: Metabolomics, chirality, LC-qTOF-MS, dual stationary phase

PT-044

Development of Chromatographic Method for Sensitive and Reliable Analysis of Fingolimod And Citalopram Molecules

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Mental disorders that often accompany Multiple skleroz (MS); depression, dysthymic disorder, bipolar disorder, anxiety disorders (generalized anxiety disorder, panic disorder, obsessive-compulsive disorder), psychosis, substance and alcohol abuse. Although depression is the most common and investigated psychiatric comorbid mental disorder in MS patients, it is known that bipolar disorder is more common in these patients than in the normal population (Alping et al., 2020).

In this study, it was aimed to develop a new sample pretreatment procedure for Fingolimod as an MS drug and Citalopram as an Antidepressant drug based on solid phase-based extraction and HPLC determination. A fabric phase sorptive extraction(FPSE) based chromatographic method for sensitive analyses both drug active molecules have been developed for the simultaneous analysis. Separation and preconcentration procedure were performed using the fabric phase extraction method, and then the analysis of target molecules was performed with the HPLC-DAD system. Firstly, the synthesis and characterization of fabric phase materials to be used as solid phase support material were carried out step by step. By using the synthesized sorbent material, favorable conditions for adsorption and desorption were determined for the pre-separation enrichment of fingolimod and citalopram. For this purpose, analytical parameters such as pH, adsorption time, eluent type and volume, adsorbent amount were optimized. The accuracy and validation of the method has been confirmed by recovery studies with model solutions and real samples. The detection limit, quantification limit, precision,

accuracy and enrichment factor of the analytical method developed in a certain concentration range were calculated by using model solutions. Then, recovery studies were performed on synthetic urine and urine samples taken from healthy volunteers, and simultaneous determination conditions were monitored and evaluated.

In the developed method; determination of fingolimod and citalopram molecules in HPLC was carried out by isocratic elution of 50% Acetonitrile, 40% pH 3 buffer and 10% methanol mixture. The wavelength was 238 nm for fingolimod and 213 nm for citalopram in HPLC-DAD analysis. The limit of detection (LOD) obtained under optimized conditions is 7.46 ng mL^{-1} for the fingolimod and 5.97 ng mL^{-1} for citalopram molecules. Three repetitive measurements were made with model solutions containing 500 ng mL^{-1} drug molecule and RSD % values were found as 4.9 for fingolimod and 4.4 for citalopram. Recovery values for synthetic urine solutions were in the range of 98.5-103.3 for both molecules.

Acknowledge: This study includes some data obtained from a part of doctorate thesis of Ms.Esra Durgun and it was suppurted by Erciyes University Scientific Research Project Unit with project id:12425.

Keywords:, Fingolimod, citalopram, fabric phase sorptive extraction, urine samples, HPLC

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PT-045

An Experimental Search to Better Understand What Pooled Samples Provide to Untargeted Metabolomics

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Metabolomic studies, along with the scientific and technological developments that have emerged in recent years, have found a place in various research areas, especially the early diagnosis and treatment of diseases. Metabolites are the general name given to low molecular weight chemical compounds that participate in or emerge because of biochemical reactions occurring in the human body. All the metabolites found in the body are expressed by the term metabolome. Metabolomics, on the other hand, can be defined as the measurement of as many metabolites as possible by advanced analytical and statistical methods and comparing them with the control group on a determined variable, such as disease status or treatment type, to determine possible variables at the metabolome level, to detect metabolites whose amount changes, to find metabolic pathways affected by the disease state (López-López 2018, Jacob 2019).

In this study a dataset obtained from our previous clinical studies was used to compare the results for individual samples and pooled samples. Individual samples are the ones obtained from 24 group Control (C) members and 24 group Test (T) members. They were analyzed using Q-TOF LC/MS in a positive ion mode while the chromatographic column was a Zorbax C18 (1.8 µm, 100 x 2.1 mm) and a gradient elution program using acetonitrile and water both including 0.1% formic acid. The pooled samples are the ones which can be defined as the homogenous mixture of individuals samples for each group and they were analyzed in an identical conditions with the individual ones. The raw data for both pooled and individual groups were processed using XCMS and the Excel table containing the list of the normalized peak areas were used. A correlation occurred with the dilution factor of the pooled samples and normalized peak areas (Kaplan 2020). The peaks having areas not in a correlation with dilution factor ($R<0.9$) were excluded for pooled samples. The results for C and T group of the pooled samples and individual samples were compared with the 't-statistics test' to determine the peaks statistically different ($p<0.05$) between groups. When the peaks having a fold change (FC) value bigger than 1.5 evaluated, it was seen that the numbers for the comparison of pooled and individual samples were almost identical (Tab. 1). Some final examination must be performed on further studies to better understand how pooling the samples effect the results. Pooling the samples for each group to support individual data might be an alternative way to obtain initial results on Q-TOF LC/MS based metabolomics studies.

Tab. 1. Comparison of the pooled and individual samples results processed in an identical way.

	Using Consecutive Dilution		Without Consecutive Dilution	
	Individual	**Pool**	**Individual**	**Pool**
Total peak	5470	5470	5470	5470
Corelated peaks with dilution factor	3055	3055	-	-
t-Test ($p<0.05$)	2636	2701	4539	4594
t-Test, Fold Change ($p<0.05$, FC>1.5)	2276	2248	3531	3504

Keywords: Data analysis, XCMS, metabolomics, Q-TOF LC/MS

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PT-046

Could Metabolomics Shed Light on The Potential Connection Between a mRNA Vaccine and Leukemic Hematopoiesis?

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Abstract

Leukemia, characterized by clonal expansion of hematopoietic stem cells (HSCs) and impaired differentiation, poses a significant health concern. The SARS-CoV-2 virus, responsible for COVID-19, enters host cells through the spike protein and angiotensin-converting enzyme 2 (ACE2) interaction. The Pfizer-BioNTech COVID-19 vaccine (BNT162b2) employs an RNA-lipid nanoparticle to deliver modified mRNA encoding a P2 mutant spike protein (PS 2). While mRNA vaccines are generally safe, their potential association with hematological disorders like leukemia remains uncertain. Metabolomic studies, focusing on identifying and quantifying metabolites, provide insights into disease-specific metabolic alterations and treatment monitoring. This study investigates the possible link between the BNT162b2 mRNA COVID-19 vaccine and leukemia development, postulating that the vaccine may induce leukemogenesis by influencing hematopoiesis. Metabolomic profiling was performed on bone marrow aspiration samples from three groups: leukemia patients with a history of the BNT162b2 vaccine, leukemia patients without the vaccine, and a control group without COVID-19 or the vaccine. Liquid-phase metabolites were collected, extracted by methanol and analyzed with C18 column using liquid chromatography/time-of-flight mass spectrometry. Metabolite identification involved peak picking, statistical transformations, and pathway analysis utilizing XCMS, MetaboAnalyst 5.0, and IMPaLA software. Significant alterations in metabolic pathways, including lipid metabolism, amino acid metabolism, the tricarboxylic acid (TCA) cycle, purine metabolism, arachidonic acid metabolism, steroid hormone levels, and retinoic acid derivatives, were observed. These findings suggest that exposure to the mRNA vaccine may impact leukemia development and treatment response. This study highlights the potential relationship between the BNT162b2 mRNA COVID-19 vaccine and leukemia development, emphasizing the significance of investigating metabolomic changes as potential markers for disease progression and therapeutic outcomes.

Keywords: SARS-CoV-2, COVID-19, The Pfizer-BioNTech COVID-19 vaccine, leukemia, hematopoiesis, metabolomics

I. Introduction

The transformation of white blood cells into a cancerous state during hematopoiesis results in leukemia, which is known for its ineffective immune cells and disruption of normal bone marrow function (Pinho & Frenette, 2019). SARS-CoV-2 is indeed a single-stranded RNA virus that is surrounded by a lipid membrane and nucleocapsid proteins (Tenchov & Zhou, 2022). When the spike protein binds to ACE2, it allows the virus to enter and infect the host cell, leading to the development of COVID-19 (Turk et al., 2020). Metabolomic studies in clinical settings involve the application of metabolomics to understand and analyze metabolic profiles in relation to human health and disease. It focuses on the identification and quantification of small molecules (metabolites) in biological samples such as blood, urine, or tissues, with the goal of detecting metabolic alterations that could be associated with specific diseases, monitoring treatment (Dudzik et al., 2018). There is limited knowledge about the possible oncogenic mechanisms of SARS-CoV-2, but some studies suggest that the virus may disrupt tumor suppressor proteins like pRb and p53, which could contribute to cellular proliferation and cancer risk. The Pfizer-BioNTech COVID-19 vaccine (BNT162b2) uses an RNA-lipid nanoparticle to deliver a modified mRNA encoding a P2 mutant spike protein (PS 2) into host cells after intramuscular injection. Overall, mRNA vaccines are considered safe and well tolerated, with only a low frequency of severe adverse events reported. However, the vaccines encode the spike protein of SARS-CoV-2, which can potentially be shed into the circulation and cause binding to ACE2 or other targets, leading to adverse events. The purpose of this study is to evaluate whether there is a difference in metabolomic profiles between leukemia patients who developed after mRNA COVID-19 vaccination and leukemia patients who have not received any BNT62b MRNA Covid 19 vaccination.

II. Materials and Methods

Collected fresh bone marrow samples were centrifuged at 3,000 rpm for 20 minutes after that the liquid phase (metabolite phase) was collected from the samples. Then, the metabolite phase was removed from its solvent in a vacuum centrifuge. The metabolite phase was then redissolved with 200 µL of 50:50 (v/v) water (Milli-Q): Acetonitrile (ACN). The dissolved samples were diluted 1/1 with mobile phase and taken into vials and then analyzed on Agilent 6530 Q-TOF LC-MS (Agilent Technologies, 184 Santa Clara, CA) liquid chromatography/time-of-flight mass spectrometry instrument. Water and acetonitrile, both containing 0.1% formic acid, will be used as the mobile phase. Analyzes will be performed using a reverse phase chromatography column (1.7 µm, 2.1 x 100 mm) using gradient elution at 0.2 mL/min flow. For the MS device, the scanning range will be set to 100-1700 m/z, the column temperature will be 30 °C, the drying gas temperature will be 350 °C and the capillary voltage will be 4000 V. The MS device will be operated in positive ion mode and all samples will be given to the system in random order in duplicate. For the identification process. Peak picking, statistical transformations and pathway analysis steps performed with using XCMS, MetaboAnalyst 5.0 and IMPaLA softwares.

III. Results and Discussion

In this study, the metabolic profiles of bone marrow samples belonging to 7 Control (C) (neither COVID-19 mRNA vaccine nor leukemia history) and 7 (ASL) (after exposure to mrna vaccine and leukemia onset) and 2 leukemia (LO) (leukemia without COVID-19 and mRNA vaccine history) groups were compared. A total of 2296 peaks were found for the injected samples. 394 of these peaks were calculated as $p<0.05$ and $FC>1.5$. When 394 peaks were loaded onto the MetaboAnalyst 5.0 platform, the number of metabolites released was recorded as 198. The number of 198 metabolites matched in The Human Metabolome Database (HMDB) through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was 37 and those associated with the disease. PLS-DA scores were created from data loaded into MetaboAnalyst 5.0 (Fig 1).

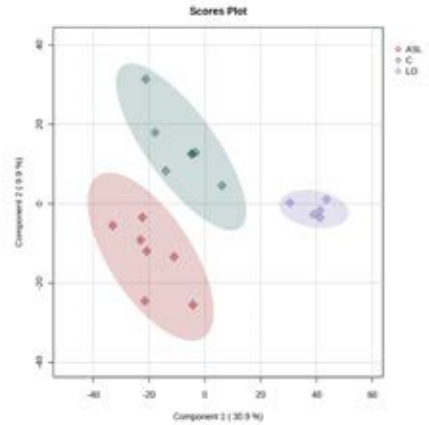

Fig. 1. PLS-DA scores between ASL, LO and C

Several metabolites were found to be significantly decreased in both the ASL/CO and LO/CO groups compared to the control group. These included phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), and several amino acids such as leucine, isoleucine and valine. The decrease in these metabolites could be indicative of altered lipid metabolism and protein degradation, potentially as a result of the leukemia or the mRNA vaccine exposure. In addition to lipid and amino acid metabolism, also identified alterations in several other metabolic pathways, including the tricarboxylic acid (TCA) cycle and purine metabolism. Specifically, metabolites involved in the TCA cycle, such as citrate and succinate, were decreased in both the ASL/CO and LO/CO groups compared to the control group. Regarding purine metabolism, study found decreased levels of inosine, which is involved in the salvage pathway of purine metabolism. in both the ASL/CO and LO/CO groups compared to the control group. In addition to the mentioned findings, our study found that several metabolites, including several PCs and LPCs, were significantly decreased to a greater extent in the ASL/CO group compared to the LO/CO group. mRNA COVID-19 vaccine exposure might lead to alterations in arachidonic acid metabolism in the bone marrow microenvironment, which could have implications for leukemia progression and response to therapy.

IV. Conclusion

The differences in metabolomics between post-vaccine and not vaccine leukemias are complex and multifactorial. and likely involve a combination of factors. The vaccination process itself triggers a complex and dynamic cascade of immune responses. including the activation of various immune cells and the release of cytokines and chemokines. which can have profound effects on cellular metabolism. These effects may include changes in the expression and activity of metabolic enzymes. leading to alterations in metabolic pathways.

The unique properties of mRNA vaccines, such as those used for COVID-19, may also contribute to the observed differences in metabolomics. It is also possible that other factors, such as genetic variations, lifestyle and environmental exposures, may play a role in the observed differences in metabolomics between post-vaccine and not vaccine leukemias. Genetic variations can influence the expression and activity of metabolic enzymes and lifestyle and environmental factors can influence metabolism through diet, physical activity and exposure to toxins and pollutants. Therefore, the interplay between genetic, lifestyle and environmental factors, in conjunction with the immune response and mRNA vaccine properties, likely contribute to the observed differences in metabolomics. Overall, the metabolomics pathway analysis revealed several differences in the levels of metabolites involved in various metabolic pathways between the non-vaccine leukemia group and the post-mRNA vaccine groups.

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PT-047

Optimization of the Novel Antiviral Drug Molnupiravir by LC-ESI-MS/MS

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Coronavirus disease (COVID-19) is an infectious disease caused by severe acute syndrome coronavirus 2 (SARS-CoV-2). The first case was detected in December 2019 in Wuhan city of China's Hubei province. As of May 11th 2023, 688.115.265 confirmed cases and 6.873.356 deaths have been reported by the World Health Organisation (http-1, 2023).

Molnupiravir is a board spectrum, directly acting oral antiviral agent that acts on the RNA-dependent RNA polymerase (RdRp) enzyme (Singh, Singh, Singh, & Misra, 2021). Molnupiravir, which received emergency use approval from the FDA on December 23th, 2021 for the use in adults with mild or moderate COVID-19 disease in several countries, including the USA, Japan and those in the EU (Syed, 2022).

The recommended dose of Molnupiravir administrated after the diagnosis of COVID-19 and within 5 days of the outbreak of symptoms is 800 mg every 12 hours for 5 days (http-2, 2023).

Molnupiravir is a synthetic nucleoside derivative and works by inhibiting certain RNA viruses through copying errors during RNA replication (Ashour et al., 2022).

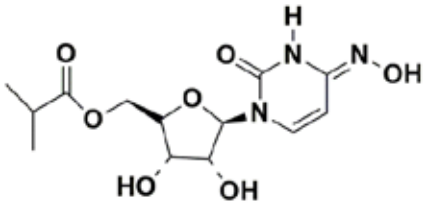

*Figure 1.*The molecular structure of Molnupiravir

Since it is a newly developed antiviral, there are not enough studies in the literature. Therefore, the aim of this study was to develop a new and rapid method for the analysis of Molnupiravir by LC-ESI-MS/MS. Supelco Ascentis® Express C2.7) ,18 µm, 10 cm × 2.1 mm) HPLC Column was used as stationary phase. Water and acetonitrile both of containing 0.1% formic acid were used as mobile phase. MS parameters were optimised such as fragmentor voltage and collision cell voltage.

Keywords: Molnupiravir, COVID-19, LC-ESI-MS/MS, method optimisation.

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PT-048

Simultaneous Determination of Ruscogenin, Neuruscogenin, Trimebutine and Parabens Using HPLC

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Abstract

Ruscogenin (RUS), neuruscogenin (NRUS), trimebutine (TB) are the most commonly used compounds in hemorrhoidal disorders. On the other hand, the protective role of parabens in pharmaceutical dosage forms, their levels must be monitored during stability studies. The determination of these compounds is interesting in many areas such as the production of pharmaceutical preparations, quality control stages, and activity studies. An HPLC method was developed for the simultaneous determination of paraben mixture and its active ingredients in in pharmaceutical semisolid dosage forms. The chromatographic separation was achieved on a C18 column (ACE-121-2546) using gradient elution. The optimized mobile phase consisted of sodium dihydrogen phosphate buffer (pH=3.9) in purified water (A) and ACN (B). The flow rate was kept constant at 1.0 mL/min for the first 8 minutes, then 2 ml/min at the 14th minute and the column was kept at ambient temperature. The injection volume was 10 µL, and UV detection was set for 200 nm. The developed method has been validated and the results obtained satisfactorily precise, simple, linear, specific, sensitive, and accurate. Finally the developed method was applied to determine the ruscogenin, neuruscogenin, trimebutine, methyl paraben and propyl paraben in pharmaceutical dosage forms.

Keywords: Ruscogenin, Neoruscogenin, Trimebutine, Propylparaben, Methylparaben, Determination, Ointments, HPLC, Validation

I. Introduction

Hemorrhoids are a normal part of our body and are cushions of blood vessels surrounding the anal canal. Also, the term "hemorrhoids or hemorrhoidal disease" is often used to refer to pads that enlarge and cause discomfort. Hemorrhoidal diseases occur with variable frequency (4-86% of people) in various studies, with no difference between men and women.(Bermejo San José & Álvarez Sánchez, 2006) A treatment that can completely eradicate hemorrhoids has not been defined in the literature. Semi-solid dosage forms containing RUS and TB active substances are frequently used in the treatment of hemorrhoids (Fig.1). Semi-solid dosage forms containing RUS and TB active substances are frequently used in the treatment of hemorrhoids. In addition, manufacturers often prefer to use methyl paraben (MP) and propyl paraben (PP) preservatives to increase the shelf life of these semi-solid dosage forms (Fig.1). In this study, a reverse phase high performance liquid chromatography method was developed to determine the RUS, NRUS, TB, MP and PP substances contained in pharmaceutical preparations with semi-solid dosage form.

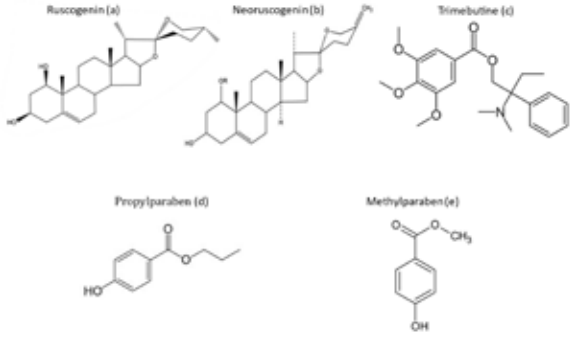

Fig. 1: Chemical structures of ruscogenin (a), neoruscogenin (b), trimebutynin (c), propylparaben (d) and methylparaben (e)

II. Material and method

HPLC analyses were performed on a Shimadzu HPLC system. Chromatographic separations were carried on a C18 column (ACE-121-2546). The mobile phase comprised sodium dihydrogen phosphate buffer (pH=3.9) (A) and ACN (B) in a gradient mode as follows: 0–8. min, 80% B; 8-17. min, 95–0% A; 7.75–8.5 min, 0% A; 8.5–8.51 min, 0–95% A; 8.51–10 min, 95% A. The flow-rate was 0.225 mL min−1. The column temperature was set at 25 °C. The injection volume was 10 µL and 200 nm was selected as the detection wavelength for the diode array detector. Peak identity was confirmed by retention time comparison. The 3 µg/mL standard stock solutions of RUS, NRUS, TB, MP and PP were separately prepared in methanol. The calibration standard solutions were daily prepared from the standard stock solutions by diluting with the mobile. For the analysis,10 g of the cream (Proctolog®) was accurately weighed and transferred into 25 mL flask. Then 10 mL of methanol was added and sonicated for 30 min in an ultrasonic water bath at 50 °C with occasional stirring. The sample was cooled to room temperature and filtered through a syringe strainer (Millex-LG, filter, 0.20 µm, Hydrophilic, PTFE, 25 mm). Then the volume was made up to 25 mL with methanol. Appropriate volumes were taken from the final solution, diluted with ACN:buffer (pH:3.9) mixture (80:20 v/v), and analyzed with the developed RP- HPLC method. The developed method was validated with selectivity, linearity, sensitivity, accuracy and precision, robustness studies.

III. Results and discussion

Different pH values (3, 3.5, 4, 4.5, 5, 5.5, 6 and 6.5) of 20 mM

sodium dihydrogen phosphate (NaH_2PO_4) buffer were tested. The obtained values were evaluated in terms of peak resolution, symmetry, retention factor and capacity factor. As a result, the optimum pH value was chosen as 4. The system suitability of the developed RP-HPLC method under optimum analysis conditions by injection of 50 µg/mL standards (n=6) was evaluated in terms of retention time (Rt), column efficiency (theoretical plate number, N), capacity factor (k'), resolution (R), peak purity index (PPI) and tailing factor parameters (Tf) (Tab 1) The linear ranges of compounds analyzed in the developed method are 1- 150 µg/mL for MP, RUS, 5-200 µg/mL for PP, NRUS, and 10- 200 µg/mL for TB. The detection limit of the method was 0.07, 0.28, 0.07, 0.45, 0.02 µg/mL for MP, TB, PP, NRUS and RUS, respectively.

Table 1: System suitability parameters for the developed RP-HPLC method.

	Rt	k'	N	Rs*	Tf	PPI
MP	6.7	1.239	18052	5.32	1.459	0.9999
TB	7.9	1.636	16396	2.86	1.265	0.9904
PP	8.5	1.844	32263	16.23	1.387	0.9998
NRUS	12.4	3.142	29375	4.88	1.457	0.9972
RUS	13.5	3.500	129600	-	1.33	0.9963

For selectivity studies, a solution of placebo and standards (50 µg/mL) was prepared and analysed (Fig 2). No distracting peaks were detected in the retention times of the analytes.

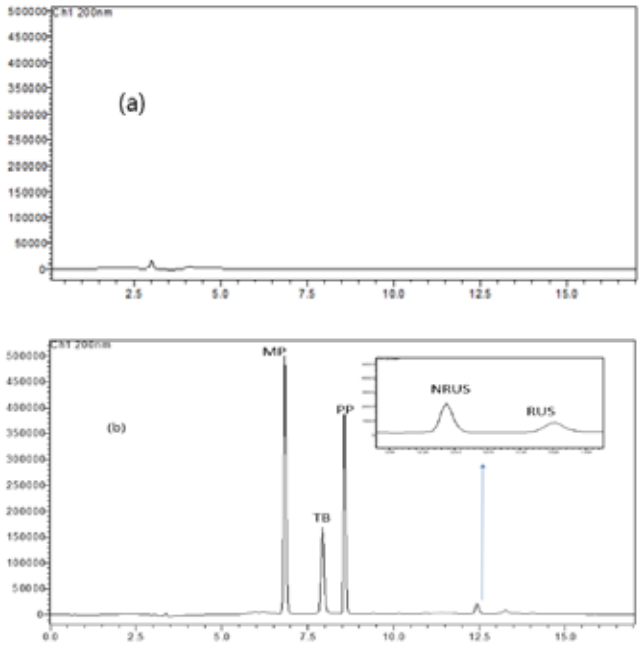

Fig 2: Chromatograms obtained under optimum chromatographic conditions; (a) Chromatogram of placebo of proctolog cream (b) MP, TB, PP, NRUS and RUS standard solution (50 µg/mL).

For intraday and interday precision and accuracy studies, three replicates of standard solutions of RUS, NRUS, TB, MP and PP (in four different concentrations covering the linear range) were prepared and analyzed using the proposed method. The low RSD (<2.09%) and RE (1.83%) values indicate that the method is precise and accurate.

The recovery study was carried out by performing analyses on three solutions of different concentrations. In this study, 80%, 100% and 120% of the standard MP, TB, PP, RUS and NRUS within linear ranges were added to the placebo. Recovery values for all analyzed compounds were between 98 and 102% and the relative standard deviation values (RSD) were within the limit or <2%.

For robustness tests of the developed method, a nine-stage fractional factor design including seven experiments was applied under optimized conditions (Table 6). The results of the analysis were statistically compared with the ANOVA test and p values of the regression coefficient and regression equation were calculated. The results were not statistically difference ($p \geq 0.05$). The lack of significant effects of small changes on peak area, retention time and peak symmetry indicates the robustness of the developed method.

IV. Conclusion

Through the developed method, the five analytes were eluted and determined in a total time lower than 17 min. The method has been validated and the results obtained satisfactorily precise, simple, linear, specific, sensitive, and accurate.

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PT-049

Determination of Methylation Levels in the Promoter Region of the Soluble Epoxide Hydrolase Gene in Patients with Diabetic Retinopathy

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Soluble epoxide hydrolase (sEH) is an enzyme that degrades epoxyeicosatrienoic acids (EETs), which are essential lipid signaling molecules involved in various physiological processes, including regulation of blood pressure, inflammation, and glucose metabolism. Recent studies have suggested that sEH and EET levels may be implicated in the pathogenesis of diabetes mellitus (DM) and diabetic retinopathy (DR). Our study aimed to investigate the methylation status of the sEH gene (EPHX2) promoter region in patients with DM and DR.

To achieve this goal, DNA samples from the blood samples of 200 DR patients, 200 DR patients, and 200 age- and sex-matched healthy controls. Methylation levels of the EPHX2 gene promoter in the DNA samples were determined by methylation-sensitive high-resolution melting analysis.

Our results showed no significant difference between the DR, DM, and control groups regarding methylation levels in the promoter region of EPHX2.

In conclusion, these findings suggest that methylation of this gene's promoter region may not play a significant role in developing DM or DR. However, further studies are needed to elucidate the precise molecular mechanisms underlying EET metabolism in DM and DR.

This work was supported by TÜBİTAK under project number 220S106.

Keywords: Diabetes mellitus, diabetic retinopathy, epoxyeicosatrienoic acids, soluble epoxide hydrolase.

PT-050

Development and Validation of Dispersive Liquid-Liquid Microextraction Followed by Micellar Electrokinetic Chromatography-Tandem Mass Spectrometry for Determination of Six Anticancer Drugs in Human Plasma

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Globally, breast cancer is the most common cancer. Despite recent therapeutic advances, clinical outcomes in breast cancer treatment can still be improved. Therapeutic drug monitoring of breast cancer drugs is an optimistic way for this purpose, but analytical methods are still scarce. Micellar electrokinetic chromatography coupled with tandem mass spectrometry (MEKC-MS/MS) is a promising technique, which is emerging as an alternative to LC-MS technique due to its ability to analyze a wide range of compounds in a highly efficient and environmentally friendly manner. There are few LC-MS/MS methods reported for the simultaneous determination of abemaciclib, ribociclib, palbociclib, anastrozole, letrozole and fulvestrant in human plasma [1,2]. However, here we report a first-ever MEKC-MS/MS method for these drugs. Six drugs were separated by employing ammonium perfluorooctanoate (APFO) as a volatile, MS-compatible surfactant. The method's sensitivity was greatly improved by the optimization of classical ESI source parameters, sheath liquid interface parameters and Agilent Jet Stream parameters. Furthermore, sweeping as an online sample concentration technique was performed by dissolving the sample in the background buffer without the APFO. Plasma samples were prepared for the analysis by protein precipitation with acetonitrile followed by the dispersive liquid-liquid microextraction with isopropanol and chloroform. Together with sweeping, sample concentration factors between 26 and 51 were achieved. The method was fully validated according to ICH and EMA guidelines with respect to selectivity, specificity, stability, linearity, range, accuracy and precision, carry-over and matrix effect. The method was shown to be linear in the therapeutic ranges of all drugs with LOQ values as low as 0.2 ng/mL. Due to the use of deuterated internal standards for three analytes, the method showed remarkable accuracy (93.3-105.1 %) and precision (RSD < 12.1 %) as well as a minor, but highly reproducible matrix effect

between different plasmas (RSD < 12.7 %). Analytes were stable in plasma in all conditions. No carry-over was observed in blank samples after the injection of the highest concentration level. In further studies, the validated MEKC-MS/MS method will be applied to real patients' plasma samples for the purpose of therapeutic drug monitoring.

This work has been fully supported by the Croatian Science Foundation through projects UIP-2019-04-8461 and DOK-2021-02-4595, and the European Regional Development Fund, project Farminova, KK.01.1.1.02.0021.

Keywords: Dispersive liquid-liquid microextraction, micellar elektrokinetic chromatography, mass spectrometry, anticancer drugs, human plasma

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PT-051

Light Exacerbates Local Effects Induced by pH Unfolding in Monoclonal Antibodies

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Monoclonal antibodies (mAbs) are an essential class of therapeutic proteins for the treatment of cancer, autoimmune diseases, asthma, viral infections, and other diseases, including central nervous system disorders, infectious and cardiovascular diseases. These protein drugs are exposed to several stresses during their production, storage, and administration. Such stresses can lead to subtle modifications of the proteins or cause denaturation with loss of biological activity. Modifications can be of chemical nature such as oxidation, deamidation, or formation of adducts. The specific position of sensitive amino acid residues in polypeptide chain sequence and therefore in the 3D structure of the antibody dictates the consequences and the extent of the injuries. For example, the exposure to acidic pH is a normal procedure routinely used in antibody manufacturing for the viral inactivation. The effects of acidic pH on the protein can range from partial or total structural denaturation to fragmentation of acid sensitive peptide bonds. Another critical stress factor is the light because mAbs contain a significant number of aromatic and cysteine residues, quite sensitive to the light effects. In certain circumstances, the accidental exposure to light could cause an injury to the protein. Here we have studied the consequences of the contemporary exposure to acidic pH and light on the conformation and sensitivity to chemical modifications of three marketed mAbs. Molecules after injuries were characterized by spectroscopic (intrinsic and extrinsic fluorescence, circular dichroism in the far and near UV) and biochemical (size exclusion chromatography, mass spectrometry) techniques, showing that variations of the pH are potentially dangerous for mAbs since irreversible unfolding occurs at different pH in relation to the protein structure and pH exposure time. Light exacerbates certain local effects probably increasing the susceptibility to chemical modifications of residues normally hidden in the core structure, but temporarily exposed. Moreover, extensive protein aggregation is observed leading to reduced availability of the protein drug.

Keywords: monoclonal antibody, protein drug, viral inactivation, photostability, artificial sunlight.

PT-052

Evaluation of Extractive Probes Congruent with Thermal and Solvent Desorptions for Targeted and Untargeted Analysis

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Solid phase microextraction (SPME) is a versatile sample preparation and sampling technique. One of the most important advantages of SPME is its applicability for in-vivo, in-vitro, and on-site applications. Although various commercial SPME samplers are present, a universal sampler that is capable of extracting both polar and nonpolar analytes, volatiles and non-volatiles and being compatible with solvent and thermal desorption is not present. In this study, hydrophilic lipophilic balanced (HLB) polymer embedded in amorphous fluoropolymer PTFE/AF 2400 was explored as potential universal SPME coating with volatile and non-volatile compounds having wide range of polarities. Creatinine, 1,2-distearoyl-sn-glycero-3-phosphocholine,

glutamine, arginine, tryptophan, glutamic acid, leucine, guanine, riboflavin, and cholesterol were selected as analytes for the evaluations with non-volatiles under direct immersion extraction conditions. The separation and quantitative analysis of the probed analytes was achieved in liquid chromatography-mass spectrometry (LC-MS) under HILIC conditions. Using HLB/PTFE coated SPME fibers, several parameters were optimized including extraction (60 min) and desorption (120 min) times and desorption solvent (ACN/MeOH/H_20 (40:40:20, v:v:v)). Using 2 mm coated fibers, SPME-LC-MS method was validated in 10% fetal bovine serum (FBS) in phosphate buffered saline (PBS) to show its potential applicability for cell line studies. The limit of quantification (LOQ) calculated using matrix matched internal standard calibration varied between 150 and 500 ng/mL. The evaluation of HLB/PTFE fibers for volatiles was performed with head space extraction followed by thermal desorption to GC-MS. For this part of the study, benzene, toluene, n-octane, benzaldehyde and 2-ethyl-1-hexanol were selected as probe analytes. The sampling and desorption time was optimized as 10 minutes and 5 minutes at 250°C, respectively. Because all the analytes were desorbed directly to the column, SPME-GC-MS method provided significantly lower LOQ levels compared to solvent desorption with LOQ of 0.5 ng/mL for all analytes. The obtained results of the study suggest that HLB/PTFE sampler is a promising tool towards preparation of a universal SPME fiber.

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Keywords: Solid phase microextraction, metabolomics, liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry

PT-053

Toward a Better Understanding of the Effect of the Light Glucose-Induced Chemical Modifications on the Structure and Biological Activity of a Monoclonal Antibody

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Monoclonal antibodies (mAbs) have rapidly escalated as biopharmaceuticals into cancer treatments in these last years, mainly for their target specificity and stimulation of reliable anti-tumoral responses [1]. During their real-life, they are potentially unstable macromolecules under shaking, temperature fluctuations, humidity, and indoor and outdoor light exposure. All these stressors can occur throughout all stages of mAbs production, transport, storage, handling, and administration. It is important to highlight that the physical and chemical modification of mAbs can lead not only to the loss of their bioactivity, but also to the enhancement of their immunogenicity with increasing risks of severe hypersensitivity reactions [2]. Additionally, mAbs administered intravenously are diluted in 0.9% NaCl or in 5% glucose solutions and consequently the excipients are diluted too decreasing their specific role of protection, i.e., against light modifications. The photostability of Nivolumab, the active principle of Opdivo ®, a medicine used to treat adults with a type of lung cancer called squamous non-small cell lung cancer has been studied. The chemical modifications detected by LC-MS/MS after the light stressor are here reported, with particular attention on the diluting solutions used for its administration to patients. Moreover, the physico-chemical properties and the rate of formation of non-native aggregates have been characterized. Finally, the biological activity under light stress conditions were assessed by using a cell test. By combining different analytical and biochemical techniques, we provided a deeper comprehension of the stability of mAbs after the dilution of the pharmaceutical form in the presence of light stress.

Keywords: Monoclonal antibody, photostability, sterile glucose

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PT-054

Untargeted Q-TOF LC/MS Metabolomics For Autism Spectrum Disorder

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Abstract

Autism spectrum disorders (ASD) is a neurodevelopmental condition characterized by limited interests and/or repetitive motor behaviours as well as the presence of social communication and interaction difficulties. However, some children later identified with ASD initially show a period of apparently typical development followed by a considerable loss of previously established skills somewhere in the second year of life, a phenomenon termed "regression". Metabolomics is the identification and quantification of small molecule metabolites in tissues, cells and physiological fluids over a given time period. Metabolomics is a promising tool for identifying potential biomarkers and possible pathogenesis of ASD. This study aimed to identify the pattern of metabolic complexity of ASD using metabolomics. Plasma samples of 25 children having developmental regression of ASD, and 27 children without any developmental regression were compared with 21 healthy controls using Q-TOF LC/MS. After the plasma samples were collected, a protein precipitation was performed using methanol to prepare the metabolite fractions. Separations were carried out with a Zorbax C18 (1.8 µM, 100 x 2.1 mm) column. The column temperature was set at 35 °C and the flow rate was 0.2 mL min^{-1} while using a gradient elution program including acetonitrile and water (%0,1 formic Acid) in different ratios in a timeline. The raw data was processed by XCMS program running under the R programming language, and peaks that statistically differed between groups were picked and detected. As a result of Q-TOF LC/MS analysis, 60 metabolites were indicated to be statistically ($p<0.05$) and relatively ($FC>1.5$) different between regression positive and negative groups.When a pathway analysis was conducted, it was observed that these metabolites were involved in lipid metabolism, sphingolipid metabolism, phospholipid biosynthesis, signal transmission pathway, steroid and bile acid metabolism.

Keywords: Q-TOF LC/MS, metabolomics, autism spectrum disorder, XCMS

I. Introduction

Autistic Disorder syndrome is a behaviourally - defined characterized by qualitative impairments of social interaction and communication accompanied by restricted, repetitive and/or stereotyped patterns of behavior and interests. Although individuals with ASD are very different from one another, the disorder is characterised by core features in two areas—social communication and restricted, repetitive sensory–motor behaviours—irrespective of culture, race, ethnicity, or socioeconomic group. Developmental regression in autism is one of the most challenging features of this disorder. Regression is described by parents of children with ASD as apparently normal development for the first 1 to 2 years of life, followed by an abrupt or gradual loss of previously acquired skills. The developmental skills that are typically reported to regression ASD are language and/or social communication (Williams, Brignell, Prior, Bartak, & Roberts, 2015). The first two years, or 2 years, are critical for early detection and treatment of autism symptoms in infants because children's brain development is faster before 2 years of age and their behavior control and response to interaction is more efficient, but most autism diagnoses are made at age 3 years. In this sense, early diagnosis is of great importance. To date, the causes of regression in autism are unknown. Potential factors, such as epilepsy, epileptiform EEGs and early childhood immunizations have not supported causal, or even correlational linkages (Al Backer, 2015). Etiologic factors underlying the complex neurodevelopmental condition autism spectrum disorder (ASD) are poorly understood. Because of its noninvasive accessibility and since it has already shown potential for containing discriminative metabolites, we decided to explore the metabolome of children's plasma as deeply as possible Q-TOF LC/MS-based study. The data in the literature were combined with the ones that we obtained in the present study to find novel biomarkers.

II. Material and method

Plasma samples were collected in the Department of Child and Adolemental Mental Health and Diseases of Hacettepe University (Children between the ages of 2 and 6) and stored at -80°C until the day of analysis. Briefy, 200 µL of plasma were extracted with 600 µL of methanol. After 90 s of vortex, the samples were centrifuged for 10 min (9,000 rpm, 4 °C) and the supernatant was collected. Then, the supernatant was removed from its solvent in a vacuum centrifuge. Samples are dissolved with MeOH:water (50:50) and diluted with mobile phase (1:1) before taken into vials and injected into Agilent 6530 LC/MS Q-TOF. A Zorbax C18 (1.8 µM, 100 x 2.1 mm) column was used as the chromatography column. The column temperature was set at 35 °C and the flow rate was 0.2 mL min^{-1} while using a gradient elution program using Water and acetonitrile, both containing 0.1% formic acid mixture in different ratio The analysis started with 10% acetonitrile and increased linearly to 90% acetonitrile within 10 min. The acetonitrile ratio was then reduced linearly to 10% until the 17th minute and the ratio was kept constant till the 25th minute. The scanning range for the MS device is set to

100-1700 m/z. The column temperature was set to 35 °C, the drying gas temperature to 350 °C and the capillary voltage to 4000V. The MS device was operated in positive ion mode. The raw chromatograms were processed using XCMS. The identification was performed using MetaboAnalyst 5.0.

III. Results and discussion

In the literature, there are several omic studies including proteomics, lipidomics, and metabolomics were conducted on individuals with regressive and non-regressive autism. Total secreted amyloid precursor protein and secreted amyloid precursor protein-a levels were reported as significantly higher in regression positive groups (Li et al., 2022). Thus, sleep problems and neurodevelopment were reported to be associated with energy metabolism, while neurodevelopment was associated with purine metabolism and aminoacyl-tRNA biosynthesis (Brister et al., 2022). In a lipidomic study on individuals with autism, the effects of myelin and myelin lipids on autism were investigated. Ceramide, one of the main components of mylelin, was observed to be significantly decreased (Ju et al., 2021). As a result of our Q-TOF LC/MS analysis, lipid metabolism, sphingolipid metabolism, phospholipid biosynthesis, signal transmission pathway, steroid and bile acid metabolism were found to be affected as a result of the development of regression in ASD. These results were in a corelation with the previously published ones. The PLS-DA graph given in Figure 1 present the difference between groups.

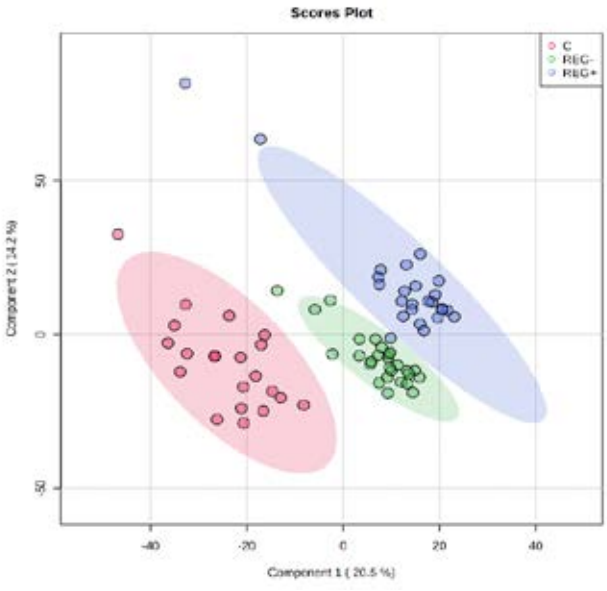

Fig. 1: PLS-DA graphs to show the differences of the groups in metabolome level (C: red, REG-: green, REG+: blue)

IV. Conclusion

In this study, autism disorder plasma samples were considered using Q-TOF LC/MS based metabolomics approach. PLS-DA graph shows that, there are significant difference between healthy group samples with autism samples. Regression positive and non-regressive samples were in a different metabolome profile when the results were presented using PLS-DA graphs. Metabolic pathway analyses confirm, the metabolic pathways associated with ASD and the results were in a corelation with the previously published ones.

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PT-056

Development and Evaluation of HLB/PTFE Thin Film Samplers for Extraction of Selected Pesticides From Complex Samples Prior to Their GC-MS Determination

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The use of pesticides cannot be prevented in modern life due to the limited natural sources and farming areas. However, their application should be monitored using maximum permissible levels set as safe levels by local and international authorities to protect public and environmental health. Among various techniques used for pesticides monitoring, solid phase microextraction (SPME) is a promising technique with features such as easy adaptation to different matrices (fruits/vegetables and their juices), application practicality and possibility of developing novel extractive phases with tuned properties towards selected groups of analytes. In the present study, hydrophilic lipophilic balanced polymer embedded in amorphous fluoropolymer (HLB/PTFE)-based thin film samplers which have large extractive surface area compared to

SPME fibers were prepared and used to extract selected pesticides (diazinon, chlorpyrifos-methyl, malathion, methyl parathion, trifluralin and carbaryl) from apple juice samples. Because HLB (extractive phase) and PTFE (glue) are both thermally and chemically inert under typical instrumental and sample preparation conditions, the extractive sampler is suitable for thermal and solvent desorption. As a first step, GC-MS method was developed and optimized for the separation and quantitation of the selected pesticides. Next, the optimum conditions for extraction were studied in terms of sample pH, ionic strength, matrix effect, extraction and desorption times. In the final method the following conditions were found as optimum: sample volume: 20.0 mL diluted with 20.0 mL of water, NaCl concentration: 10% (w/v), extraction time: 60 min, desorption time: 30 min, desorption solvent: methanol, desorption volume: 0.60 mL. Validation of the method with the optimized conditions showed that the limit of quantitation (LOQ) for the selected analytes is in the range of 1.0 to 5.0 ng/mL which is lower than MRLs set for these pesticides and indicates the success of the method. Finally, the preliminary investigations performed on agarose gel model (mimicking solid samples) indicated that TFME sampler can be used for the sampling from the surface of solid samples, such as a surface of the crops, directly on field without requiring of sample collection.

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Keywords: Pesticides determination, solid phase microextraction, GC-MS

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PT-057

Phenolic and Flavonoid Compounds and Biological Activities of Teucrium brevifolium: in silico and in vitro Evaluation

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The genus *Teucrium*, a member of the family Lamiaceae, has approximately 300 species all over the world, mostly in the Mediterranean region. *Teucrium* has 34 species (46 taxa) in Turkey, and 16 of these taxa are endemic. *Teucrium* species have been used in folk medicine due to their antidiabetic, anti-inflammatory, antiulcer, and antibacterial properties (Twaij et al, 1987). The main secondary metabolites isolated from *Teucrium* species to date are flavonoids, phenolics and diterpenoids (Topcu and Eris, 1997). The studies showed that *Teucrium* species demonstrate a number of biological effects including antimicrobial, antioxidant, and antifungal activities. It can be observed that secondary metabolites are responsible for those biological activities. In the present study, anticholinesterase (anti-Alzheimer) and antioxidant activities of the methanol extract of *Teucrium brevifolium* were investigated. In addition, flavonoid and phenolic compounds of the extract were detected and identified by LC-MS/MS techniques. Also, both biological activities and toxicological effects of the detected compounds were examined *in silico* by PASSonline, LAZAR Toxicity, ADMETlab, CLC-Pred prediction programs. Antioxidant activity of the extract was investigated through five different *in vitro* assays (Total Phenolic content, Total Flavonoid content, DPPH free radical and ABTS cation radical scavenging activity, CUPRAC). Anticholinesterase (anti-Alzheimer) activity of the extract was examined by Ellman's method. According to the results, while the extract showed good antioxidant activity, its anticholinesterase activity was not promising. 14 flavonoids/phenolics were identified by LC-MS/MS in the extract. The most abundant compounds detected in the extract were hesperidin (14.86 mg/g), verbascoside (4.07 mg/g) and chlorogenic acid (3.69 mg/g), respectively. According to the

in silico results, these three most abundant compounds showed very high antioxidant activity but low activity against Alzheimer, which proves a correlation between *in vitro* and *in silico* results. In addition, all the compounds possess very low toxic effects. This is the first study revealing the phenolic and flavonoid compounds and biological activities of *Teucrium brevifolium* in both *in silico* and *in vitro* aspects.

Keywords: Anticholinesterase, antioxidant, flavonoids, *Teucrium brevifolium*.

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PT-058

Controlled Release of Ibrutinib from Collagen Nanobubbles

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Nanobubbles are basically nanosized bubbles designed to increase the structural stability and distribution of the transported drug to the targeted site.1 Due to their small size, they can penetrate the desired area from the bloodstream. The structure of the bubbles contains gas inside, surrounded by an outer shell.2 There are studies in the literature that include non-invasive, targeted drug delivery systems for nanobubbles3. In this particular study, perfluoropentane (PFP), which has low solubility in aqueous media and does not show toxic effects at low doses, is used as a gaseous core. When drug-loaded nanobubbles burst into a cavity with the effect of ultrasound, they cause temporary pores to form on the cell surfaces and increase the cellular uptake of the drug they carry. In this study, the biodegradability and excellent biocompatibility of a well known protein collagen were used to prepare nanobubbles for the release of Ibrutinib, which is used for the treatment of lymph cancer. In accordance with this purpose, characterization studies were accomplished such as Scanning electron microscopy (SEM), Fourier transform infrared (FTIR), Transmission electron microscopy (TEM). The release studies of collagen nanobubbles prepared at several drug doses were carried out in a Franz cell using a dialysis membrane at different pH (5.5.0-7.4) and temperature (4-40°C) range. In the release experiments with collagen nanobubbles, it was observed that approximately 70% of the drug was released within 6 days at pH 7.4, while the same result was reached within 24 h with collagen nanobubbles blasting by ultrasound. In the future studies, cell culture studies will be performed to demonstrate the effectivity of synthesized nanobubbles.

Keywords: Collagen, nanobubbles, ibrutinib

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PT-059

Comparative Evaluation of a Transdermal Patch form Drug in the Franz Cell Using Two Different Types of Synthetic Membranes

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Transdermal therapeutic systems (TTS) are devices that provide an alternative route for administering medication. It is defined as self-contained, discrete dosage forms that, when applied to the intact skin, deliver the drug through the skin at a controlled rate to the systemic circulation. Active substances can be delivered across the skin barrier. Pharmaceuticals can be delivered across the skin barrier using these devices. TTS work very simply in theory. A relatively high dose of a drug is applied to the inside of a patch, which is worn on the skin for an extended period of time (Pradeep Kumar Bolla, 2020).

The drug enters the bloodstream directly through the skin via a diffusion process. The drug has a high concentration on the patch but a low concentration in the blood, it diffuses into the blood for a long time, maintaining a constant concentration of drug in the blood flow. TTS are self-contained, discrete dose forms that, when applied to undamaged skin, transport the drug to the systemic circulation at a controlled rate through the skin. (Yang

Chen, 2014).

In this study, in vitro (IV) permeability experiments were performed by Franz diffusion system with 2 different synthetic polymeric membranes Strat-M (ST) membrane and Cellulose Acetate (CA) and HPLC separation technique to evaluate the TTS permeation characteristic to mimic the human body.

Based on the obtained results, 2 different membranes were compared. The permeability of the active substance is obtained higher percentage active substance permeate through the CA type membrane than ST membrane. After 24hours, results of 85.7% and 57.4% were obtained respectively. Results from the present investigation noted considerable differences in permeation of active substance across the ST and CA membranes, this study could serve as a ready reference to analytical scientists for selection of the membrane type to achieve desirable permeation profile. It should be noted that during the permeation profile studies, different membranes should be tried to select the best membrane parameter which effect directly the permeation profile.

Keywords: Permeation, cellulose acetate membrane, franz diffusion, Transdermal Therapeutic Systems (TTS)

PT-060

Multi-mycotoxin Analysis in Food Supplements by LC-MS/MS

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The term "Mycotoxins", represents a wide variety of low molecular weight compounds, that are the secondary toxic metabolites of various species such as *Aspergillus sp.* (Aflatoxins & Ochratoxins), *Fusarium sp.* (Fumonisins& Trichothecenes) and *Penicillium sp.* (Ochratoxins). The corresponding species generally colonizes on the agricultural products such as crops (eg. wheat, barley, rice), spices (eg. red&black pepper, turmeric). Consequently, the contamination of foods and feeds with mycotoxins can occur during the production, harvesting, storage and processing. Mycotoxins are identified as major causes of "Foodborne diseases" by World Health Organization, because they led many acute and chronic illnesses corresponding to consumption of mycotoxin-contaminated foods, and thus the maximum exposure limits are implemented by regulatory bodies, globally.

Exposure to common mycotoxins is occured through the direct consumption of raw food contaminated with mycotoxins, or the consumption of processed food products, manufactured from raw materials contaminated with mycotoxins.

Conventional HPLC techniques with fluorescence detection is generally employed in mycotoxin analysis. Because of the considerable interference of matrix originated substances, immunoaffinity chromatography (IAC), because of its high selectivity, is the most preferred sample preparation technique in the analysis of mycotoxins. In these techniques, antibodies that are specific for the target mycotoxin, are immobilized in an SPE cartridge, briefly. Beyond all advantages, IAC methods needed to be changed according to target analytes (eg. Aflatoxins, Ochratoxins).

LC-MS/MS, thanks to its high selectivity, and specificity provided by Multiple Reaction Monitoring (MRM) mode, makes it possible to apply a simple crude extraction step just before the analysis.

In this study, we developed a quantitative screening analysis method for the nine mycotoxins; Aflaloxins B1,B2, G1, G2, Fumonisins B1&B2, Ochratoxin A, Deoxynivalenol and Zearalenone. The method have an IDL (Instrument Detection Limit) of 0.25µg/L for Aflatoxins, 0.5µg/L for Ochratoxin A. Briefly, after a simple crude extraction step, three commercially available food supplements (turmeric extracts) were investigated for the mycotoxin contamination.

Keywords: Mycotoxins, dietary supplements, food contaminants, LC-MS/MS, crude extraction

PT-061

Evaluation of the Matrix Effect of Four Different Sample Matrices for 9 Neurotransmitters by Post-Column Infusion

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Abstract

Ion-suppressing or enhancing agents caused by the sample matrix, solvent, or LC-MS system components should be identified, analyzed, and action should be taken to correct or mitigate the problem. Considering ion suppression should form part of the optimization and validation of any quantitative LC-

MS method. Today, a sample analysis of catecholamines is done according to their relative abundance. Most catecholamines are identified based on positive electrospray ionization and multiple reaction monitoring measurements, and their relative abundances are obtained by utilizing the relative peak areas of these compounds. However, relative abudance, the ionization of different compounds in the ESI source with very different efficiency, and the severe ionization suppression effects of the matrix components can be misleading. The ionization efficiency of the analytes in the biological matrix (solvent, urine, plasma, brain homogenate) has been demonstrated. For the analysis of 9 catecholamine compounds (dopamine, acetylcholine, tyrosine, serotonin, tyramine, glutamic acid, γ-amino butyric acid, 3-methoxy tyramine hydrochloride, epinephrine) in positive electrospray ionization mode, the predicted abundance mismatch was compared with the actual abundance.

Keywords: LC-MS/MS, matrix effect, neurotransmitter, Post column infusion.

I.Introduction

Neurotransmitters changes that occur in the direction of decrease or increase in their levels in the regions where they are found are seen as the harbinger of many mood disorders, diseases, or irreversible events such as tissue deformation.

When looked closely at the chemical events that neurotransmitters direct in the brain tissue, almost all of them are electrochemical-based reactions. In addition, although the structures of these compounds are simple, the fact that they contain non-rigid electroactive functional groups supports these phenomena. Epinephrine (EP) and dopamine (DOPA) are the main catecholamines, and metanephrine (ME), vanilla mandelic acid (VA), and homothallic acid (HVA) are the resulting metabolites. Norepinephrine (NE) metabolites are of two types, HVA and VA. DOPA; it is also known that HVA is their final pathway when converting 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT). Serotonin (SER) synthesized from the amino acid tryptophan is converted to 5-hydroxyindole-3-acetic acid (5-HIAA). Mass spectrometry (MS) coupled to electrospray ionization (ESI) is extensively used to analyze various compounds in biological matrices. The ability to directly analyze samples by direct infusion (García-Sevillano, García-Barrera, Navarro, Montero-Lobato, & Gómez-Ariza, 2015) or flow injection experiments (Fuhrer & Zamboni, 2015) coupled with high-resolution MS resulted in a large increase in sample throughput. This technique has proven useful in the analysis of a wide range of samples, from human plasma (Schuhmann et al., 2012) to historic wines (Jeandet et al., 2015) to ecological samples (Hertkorn, Harir, Cawley, Schmitt-Kopplin, & Jaffé, 2016). However, standard substances are required for quantitative analysis due to the large differences in ionization efficiency observed in MS/ESI.

II. Material and method

All samples, materials and standards was purchased from Sigma Aldrich (USA). The prepared stability solution consists of a 0.08 M acetic acid solution. A 10 mL urine sample was taken and centrifuged at 4000 RPM for 10 minutes at 15 °C. 2 mL of supernatant was taken from this solution and filtered with a 0.22 µm polyvinylidene filter. 1 mL of this filtrate was taken and diluted with water to 10 mL volume. The synthetic urine solution is a model solution containing human urine's basic micro and macro components below their average content.

The study performed chromatographic separation using a 100 mm×4.6 mm Kinetex 2.7 µm HILIC Ascentis® Express (Sigma Aldrich, USA) column. The injection volume into the mobile phase pumped at a 0.5 mL/min flow rate is 1 µL. The optimum column temperature was chosen as 40.0±0.1 °C, and the autosampler thermostat temperature was chosen as 15±0.1 °C. The sample injection volume is optimized to be 1 µL.

Various matrices were injected into the analytical LC system. At the same time, a continuous post-column infusion of 50 ng/mL neurotransmitter standard solution at 50 µL/min via a T-linker with a Model 22 infusion pump was delivered to the analytical LC system. The mobile phase solution from the HPLC column and the flow from the infusion pump is combined with a zero dead volume 'T' junction and fed to the source of the mass spectrometer.

III.Results and discussion

The standard solution of each analyte was injected into the system with a mixture of water and acetonitrile containing 0.1% formic acid without a column, and the MS/MS parameters were optimized. After the MS optimization studies, the MS method developed by attaching the column was optimized. The maximum ionization peak obtained in the positive mode was observed in the Q3 scan, and therefore the ESI + MRM mode was chosen. The dwell time for the quantitative determination detected in ESI + MRM mode was 100 ms for all product ions. Tab.1 contains the optimized MRM parameters.

Tab. 1. Optimized MRM parameters

Compound	Main ion m/z	Daughter ion m/z	Dwell time (msec)	Q1 Pre Bias (V)	Collision energy (eV)	Q3 Pre Bias (V)
SER	177.10	160.05	100.0	-20.0	-12.0	-28.0
3-MT	168.05	150.95	100.0	-19.0	-12.0	-20.0
GLU	148.00	83.95	100.0	-10.0	-17.0	-15.0
ASET	146.05	86.85	100.0	-16.0	-16.0	-20.0
DOPA	154.05	136.95	100.0	-17.0	-14.0	-23.0
GABA	104.10	86.95	100.0	-11.0	-13.0	-16.0
EP	184.05	133.95	100.0	-13.0	-21.0	-22.0
TYRA	138.05	121.05	100.0	-20.0	-15.0	-20.0
TYRO	182.00	136.00	100.0	-13.0	-15.0	-24.0

In brain homogeneity analyses, it was observed that there

were interferences that increased the signals of GABA and GLU analytes too much. It is seen that there is an enhancing matrix effect in the ASET signal as well. On the other hand, no enhancing effect was observed in the TYRO signal. However, there is a reducing effect in the signals of other analytes. So the matrix effect plays a decisive role in analyzing neurotransmitter compounds in the mass detector. For this reason, it is essential to present effective sample preparation methods in body fluids and brain homogenate analyses of these compounds and to carry out recovery studies in which the matrix effect is demonstrated.

IV.Conclusion

The compounds have very efficient ionization characteristics in mass detectors. Although many methods are presented for analysis, a comprehensive study on the matrix effect that affects the precision and accuracy of the method has yet to be presented in the literature to date. For this reason, it has been demonstrated in the study mentioned above whether the matrix effect of the neurotransmitter compound to be analyzed is in the direction of decreasing or increasing the signal in the mass detector. It is seen that the effects of each analyte according to the environment are quite different. After that, considering this effect in neurotransmitter analysis, more efficient and highly reproducible analysis methods can be developed.

Acknowledgements: This research was funded by Anadolu University Scientific Research Projects Commission (Grant number: 2207S092).

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PT-062

Alternative Analytical Method and Validation to Pharmacopeia Methods for NDMA and NDEA Related Compounds of Losartan, Valsartan and Irbesartan Drug Products

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Abstract

Nitrosamines are human carninogenic chemical compunds which must be controlled in especially drug products that have sartan groups. European Pharmacopeia has different analysis method to detect these compounds by using LC-MS, GC-MS, GC-MS/MS. This study provides a new LC-MS method for the detection of two potential genetoxic impurities – NDMA, NDEA to analyze different drug products containing Losartan, Valsartan and Irbesartan compatible with their maximum daily doses by using QdA mass dedector. XSelect CSH C18 (150 x 4.6 mm, 5 µm) column, 0.1% (v/v) Formic acid in water, 0.1 % (v/v) Formic acid in acetonitrile are used. Column and autosampler temperature are 40 °C and 10 °C, respectively. Flow rate is 0.85 ml/min and samples are monitored by a QdA[4] detector in positive mode. Total analysis time is 17 minutes. NDMA and NDEA retention time are approximately 3 and 7 minutes, respectively.

Keywords: NDMA, NDEA, losartan, valsartan, irbesartan, QDA, validation, pharmacopeia

I. Introduction

Nitrosamines are chemical compounds classified as probable human carcinogens on the basis of animal studies. EU regulators first became aware of nitrosamines in medicines in mid-2018 when nitrosamine impurities, including N-nitrosodimethylamine (NDMA), were detected in blood pressure medicines known as ‚sartans'[1]. The EMA and the FDA reported a major issue regarding the detection of a genotoxic impurity, NDMA (N-nitrosodimethylamine), and subsequently NDEA (N-nitrosodiethylamine), in current lots of valsartan, an API used to manufacture generic angiotensin receptor blockers (ARBs). Most ARBs have a chemical structure that includes a tetrazole group.[2]

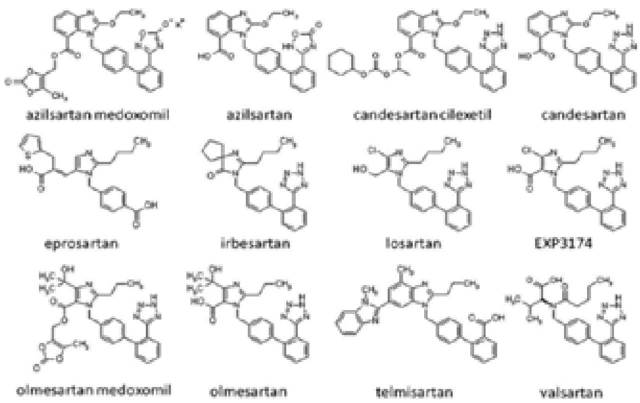

Fig.1: Structures of different Sartan groups

European Pharmacopoeia 2.5.42 N-Nitrosamine[3] in Actice Substances has three methods to analyze N-Nitrosamine. First method is LC-MS (liquid chromatography coupled with mass spectrometry), second method is GC-MS (gas chromatography coupled with mass spectrometry) and third method is GC-MS/MS (gas chromatography coupled with mass spectrometry). In this study, a new LC-MS method has been developed and validated for the simultaneous analysis of sartan group drug products and its NDMA, NDEA impurities, then its applicability in pharmaceutical preparations that contain Losartan, Valsartan and Irbesartan active ingredients has been proven. In this method special QdA mass dedector is used.

II. Material and method

In the developed method, XSelect CSH C18 (150 x 4.6 mm, 5 µm) column and 0.1% (v/v) Formic acid in water and 0.1 % (v/v) Formic acid in acetonitrile were used as stationary and mobile phases, respectively. Chromatographic separations were carried out at 40 °C column temperature, 10 °C autosampler temperature and samples were monitored by a QdA[4] detector with positive mode at 0.85 ml/min flow rate. Total analysis time was finalized as 17 minutes, NDMA retention time was approximately 3 minutes and NDEA retention time was approximately 7 minutes.

III. Results and discussion

According to validation results, the linearity range was obtained as 0.0000060 – 0.000192 mg/ml for NDMA, 0.0000054 – 0.000054 mg/ml for NDEA the limit of quantitation (LOQ) and the limit of dedection (LOD) were 0.0000060 mg/ml for NDMA, 0.0000054 mg/ml for NDEA and 0.0000024 mg/ml for NDMA, 0.00000168 mg/ml for NDEA, respectively. Percentage relative standard deviation values (% RSD) obtained in intra-day and inter-day repeatability studies were determined as 1.20% for NDMA, 3.96% for NDEA and 0.28%, 6.97% for NDMA, 3.68% for NDEA and 0.28% respectively, and the average recovery value was found 96.36% for NDMA and 96.08% for NDEA.

Detailed analytical method validation study to control the presence of NDMA and NDEA impurities of drug products which have sartan group was conducted in the Ali Raif Pharmaceuticals according to the ICH guidelines[5] and pharmacopoeias. The validated method was used for different pharmaceutical forms containing Losartan, Valsartan and Irbesartan for the control of NDMA and NDEA genetoxic impurities in compatible with maximum dailiy doses of substances.

IV. Conclusion

Specificity, LOD, LOQ, linearity range, accuracy, precision, robustness and solution stability parameters were validated. All data obtained are within the acceptance criteria. This shows that the developed method is extremely suitable for the routine NDMA and NDEA analysis of drug products which have different sartan groups in terms of especially speed and convenience. This method is also alternative to methods in European Pharmacopoeia thanks to its dedector type and this dedector is preferrable in terms of its cost and accessibility for the routine analyses.

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PT-063

UV/Vis Spectrophotometer Determination of Cadmium in Tap Water with Matrix Matching Calibration Strategy after Preconcentration with Vortex Assisted Liquid Phase Microextraction

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Accumulation of cadmium (Cd) in the body causes deterioration of the bone structure, kidney dysfunction and some cancer types [1]cadmium, chromium, copper, and nickel were determined in 25 tea samples from China, including green, yellow, white, oolong, black, Pu'er, and jasmine tea products, using high-resolution continuum source graphite furnace atomic absorption spectrometry. The methods used for sample preparation, digestion, and quantificational analysis were established, generating satisfactory analytical precisions (represented by relative standard deviations ranging from 0.6% to 2.5%. Cadmium is considered among chemicals with the potential to cause different cancer types [2]cadmium, mercury and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO. Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years. Cadmium compounds are currently mainly used in re-chargeable nickel-cadmium batteries. Cadmium emissions have increased dramatically during the 20th century, one reason being that cadmium-containing products are rarely re-cycled, but often dumped together with household waste. Cigarette smoking is a major source of cadmium exposure. In non-smokers, food is the most important source of cadmium exposure. Recent data indicate that adverse health effects of cadmium exposure may occur at lower exposure levels than previously anticipated, primarily in the form of kidney damage but possibly also bone effects and fractures. Many individuals in Europe already exceed these exposure levels and the margin is very narrow for large groups. Therefore, measures should be taken to reduce cadmium exposure in the general population in order to minimize the risk of adverse health effects. The general population is primarily exposed to mercury via food, fish being a major source of methyl mercury exposure, and dental amalgam. The general population does not face a significant health risk from methyl mercury, although certain groups with high fish consumption may attain blood levels associated with a low risk of neurological damage to adults. Since there is a risk to the fetus in particular, pregnant women should avoid a high intake of certain fish, such as shark, swordfish and tuna; fish (such as pike, walleye and bass. This element enters the metabolism through different ways such as air, food and water [3,4]. Hence, determination of this element at trace amount is very crucial. In this study, a sensitive analytical strategy was developed for Cd determination by UV-Vis spectrophotometer after complexation with ((Z)-4-bromo-2[(naphthalene-2-ylimino)-methyl]phenol ligand and preconcentration using liquid-liquid microextraction. Before the system analytical performance study, the pH value of the solution, the amount of buffer solution, the ligand volume and the vortex period were optimized to increase the extraction efficiency in order to get low detection limit. Under the optimum experimental conditions, system analytical performance studies were done to determine limit of detection/quantification (LOD and LOQ) and linear range. LOQ/LOQ values and linearity were recorded as 17/56 µg/L and 0.050 – 4.0 mg/L (with 0.9984 coefficient of determination). In order to determine the applicability and accuracy of developed method, recovery results were carried out. Recovery results were recorded in the range of 92.8 – 117.0% via matrix matching calibration strategy while recovery results were calculated between 92.9 – 105.9%. These recovery results proved the accuracy/applicability of developed method.

Keywords: Cadmium, microextraction, tap water, UV–Vis spectrophotometer, matrix matching

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PT-064

Capillary Electrophoresis and GC-FID Analysis of Omega-3 Fatty Acids in Food Supplements

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The study focuses on the analysis of dietary supplements containing omega-3 fatty acids. Such products are used by consumers for their antioxidant properties or to improve cognitive functions [1]. The objective of this study is to develop a method to characterize the fatty acid content of these dietary supplements by capillary electrophoresis (CE) with UV detection. The work focused on the analysis of the three main fatty acids, alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The dietary supplements analyzed are fish oils or vegetable oils, marketed in the form of capsules or soft gels. The omega-3s are present in these oils in the form of triglycerides and/or ethyl esters and not as free fatty acids. A saponification step was therefore optimized prior to CE analysis, using a 1M sodium hydroxide solution in ethanol (70%) followed by a cyclohexane extraction. Unlike chromatographic methods such as GC-MS or GC-FID which require esterification of fatty acids before analysis, CE is a fast, sufficiently sensitive, inexpensive method which requires very few sample preparation steps. It is therefore well suited to perform rapid fatty acid profiling in various commercial products. In this preliminary study, we compare GC/FID and GC/MS results with those of CE to characterize the levels of the three target fatty acids in the dietary supplements studied.

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Keywords: Capillary electrophoresis, GC-FID, Omega-3, fatty acid

PT-065

Metabolomic Analysis of Clinical *E. Coli* Strains in Urinary Tract Infection and Urosepsis Patients

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The most common cause of sepsis is bacterial infections, especially those related to the urinary tract. *Escherichia coli* accounts for 52% of cases and is most often responsible for urosepsis. It is estimated that UTIs account for approximately 10–20% of all community-acquired infections and about 40–50% of nosocomial infections. The presence of bacteria or their products in the urinary tract stimulates a rapid immune response, cytokines and the influx of neutrophils are observed, and pro-inflammatory interleukins (IL-6, IL-8, TNF-α) are produced, as well as nitric oxide. In addition, bladder epithelial cells show a high expression of TLR 4. In contrast, in renal epithelial cells, the expression of TLR 4 is low, which means that the response to uropathogenic bacteria is much weaker, and the kidney-blood placenta barrier may be overcome.

As part of the research, with the use of the Sciex qTOF 6600+ apparatus, extensive analyzes of metabolites characteristic of clinical *E. coli* strains responsible for UTI or urosepsis were carried out. The developed methodology made it possible to distinguish between metabolites inside and outside the cells and perform multidimensional statistical analyses, which in turn allowed for the selection of biomarkers capable of discriminating *E. coli* urinary tract infections. In our study, we examined research material of 248 *E. coli* isolates from blood and urine isolates from patients with suspected sepsis, of which patients with urosepsis and patients with *E. coli*-induced UTI were identified. Bacterial cultures of the isolated strains were prepared in an artificial urea medium and incubated at 37 °C overnight until OD600 was reached between 0.4 and 0.6. After OD measurement, the cultures were centrifuged (3600 rpm, 21 °C, 10 min), the supernatant consisting of external metabolites was separated, and 1 ml of a 50% methanol solution (-40 °C) was added to suppress the metabolic activity of the collected bacterial pellets. Samples

were then freeze-dried and resuspended in 50% cold methanol solution with internal standard and 1% FA to extract metabolites. After centrifugation, the supernatants were collected, dried and resuspended in 0.1% FA containing 1% ACN. The LC-MS analysed the solutions thus prepared.

The obtained analytical data were analyzed using MasterView, and MultiQuant software (Sciex, Canada) for identification with the spectral database NIST and the All-in-one Sciex database. On this basis, 450 metabolites were selected based on selection criteria - < 20 ppm mass error, intensity > 100 cps, isotope distribution and MS /MS spectra similarity. The obtained data were used to determine the differences in the concentration of metabolites and the activity of metabolic pathways between UTI and the uroseptic strains. Statistical analysis was performed using Mathematica and MetaboAnalyst software with support from KEGG databases to determine metabolic pathways. Using PCA and VIP-score analysis, we proposed the model allowing for the discrimination of UTI and uroseptic *E. coli* strains. In addition, our study suggests that the increased concentrations of metabolites observed in the urine of urosepsis patients may be derived from bacteria and not from human metabolic activity.

Keywords: Metabolomics, urosepsis, LC-MS

PT-066

Nanostructured Lipid Carrier (NLC) Formulations of Paliperidone for Treatment of Schizophrenia

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Paliperidone (PAL), which is the major active metabolite of risperidone, is a member of the benzisoxazole derivative class of atypical agents. It binds to both dopamine D_2 and serotonin 5-HT_{2A} receptors, and antagonism at these receptors is responsible for its therapeutic activity in schizophrenia. In 2007, it was approved by the U.S. Food and Drug Administration for the acute and maintenance treatment of schizophrenia (Marder et al., 2007; Carvajal et al., 2009). The aim of the present research is to produce the nanostructured lipid carrier (NLC) formulations of PAL and to developed a validated HPLC method for the determination of PAL in NLCs. NLCs consist of mixture of solid and liquid lipids, resulting in a partially crystallized lipid system that provides higher drug loading efficiency compared to solid lipid nanoparticles. Due to the biocompatibility, low toxicity, ease of preparation as well as high stability, NLCs have found extensive use in numerous pharmaceutical applications (Gomaa et al., 2021). In this study, first of all, a stability-indicating high pressure liquid chromatography (HPLC) method with a DAD detection system was developed for the quantitative analysis of PAL. Efficient chromatographic separation was achieved on SunFire® C18 column (250 mm × 4.6 mm, 5 µm) by isocratic elution with mobile phase comprising of 0.05 M Monobasic Phosphate Buffer (pH 3.8): Acetonitrile (70:30) (Jones et al., 2009). The flow rate of the mobile phase was 1.0 mL/min with a column temperature of 25 °C. DAD detector was used for the analysis and detection was carried out at 280 nm. The developed method was validated according to ICH guidelines with respect to accuracy, precision (repeatability and intermediate precision), detection limit (LOD), quantification limit (LOQ) and linearity. The HPLC method showed linearity over the range of 0.5 to 10 µg/ml with r^2 = 0.9995. The accuracy of the method in terms of recovery study was 100.25-101.40%. The repeatability and intermediate precision for the determined concentrations exhibited RSD% values below 2%. In the second step, NLC formulations of PAL were prepared using the high-pressure homogenization method. Briefly, NLC formulations were prepared using 150 mg of Tripalmitin (TP) as solid lipid and 150 mg of Oleic acid (OA) as liquid lipid with different surfactants such as Tween 80, sodium cholate, sodium deoxycholate, Gelucire 50/13 and Poloxamer 188 at a 1% w/v concentration. The nanoparticles prepared have a particle size ranging from 111.6 ± 0,500 to 142.4 ± 1,756 nm with a low PDI values. The encapsulation efficiency (EE%) of the NLC formulations was assessed indirectly, by measuring the amount of non-encapsulated drug in the supernatant (Cunha et al., 2022). For this purpose, PAL-loaded NLCs were placed in an Amicon® Ultracel-50 K (Millipore, Ireland) centrifugal filtration unit and centrifuged at 10,000 rpm for 20 minutes. Subsequently, the separated supernatant was diluted with methanol and analyzed by HPLC to determine the amount of non-encapsulated PAL. The EE% values of NLC formulations varied depending on the type of the surfactant. The highest EE% was obtained for the NLC formulation prepared with sodium deoxycholate (96.16%).

Keywords: HPLC, nanostructured lipid carrier, analytical validation.

Table 1. Particle size, PDI, zeta potential, and %EE of the NLC formulations.

Formulation Code	Surfactant Type	Particle size ± SD	PDI ± SD	Zeta Pot ± SD	EE%
M1	Tween 80®	118.9 ± **1.007**	0.233 ± **0.007**	-41.1 ± 0.208	58.34
M2	Na cholate	111.6 ± **0.500**	0.159 ± **0.006**	-64.2 ± 3.04	89.64
M3	Na deoxycholate	142.4 ± **1.756**	0.320 ± **0.014**	-83.4 ± 2.93	96.16

M4	Gelucire 50/13*	125.6 ± **1.397**	0.232 ± **0.004**	-34.7 ± 2.72	53.27
M5	Poloxamer 188*	137.7 ± **1.365**	0.181 ± **0.013**	-38.4 ± 0.608	58.88

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PT-067

Three Cyclodextrins Mixture in CEKC: A Long Travel for Resolution of 8 Diastereoisomers Derivative, a Short Trip to Resolution of 4 Diastereoisomers and 2 Enantiomers Derivatives

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The patented compounds under investigation in this work were all drug candidates for diabetes treatment. Worldwide, more than 415 million people have diabetes. The Takeda G-protein-coupled receptor 5 (TGR5) has recently been identified as an attractive therapeutic target for the prevention and/or treatment of type 2 diabetes. Following an initial screening with cells over-expressing hTGR5, a series of dihydropyridone derivatives was identified as candidates for the optimization phase.

Here the long road to obtain the full baseline separation of a three chiral center dihydropyridone analogue is presented through a capillary electrokinetic chromatography method (CEKC).

A background electrolyte comprising three cyclodextrins was found to successfully separate the eight isomers. First an anionic cyclodextrin, the SBE-β-CD, was selected to allow partial resolutions of the eight isomers. Then, the effects of different parameters such as the nature and concentration of the other cyclodextrins added as dual systems were examined. Finally, a triple CD-system consisted of 15mM SBE-β-CD plus 15mM γ-CD and 40mM HP-γ-CD in a 50 mM borate background electrolyte at pH 10, was found to successfully separate the eight isomers.

Subsequently, this triple CD-system was tested towards two-chiral centers dihydropyridone derivatives and chiral dihydropyridone derivatives as presented below. This system was found to sucessfully resolved the four diastereoisomers and the two enantiomers of these drug candidates for diabetes treatment.

Keywords: Diastereomer, chiral, CEKC, cyclodextrin

PT-068

Simultaneous Analysis of Nitrosamines in Pharmaceutical Products by Liquid Chromatography APCI-Triple Quadrupole Mass Spectrometry

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Reotek Cihazlar Ltd. Şti.

With a bad reputation, N-Nitrosamines, regarded as potential carcinogenic compounds by The International Agency for Research on Cancer (IARC). Individuals could be exposed this compounds in every part of the daily life; from water, diet (eg. processed meat products), personal care products, toys, baby products such as teats and the medications as well.

However the first detection of Nitrosamines dates back over a century, these compounds regarded as "potential carcinogenics" at the mid-1950's. By the start of 1970's (1972) "N-Nitroso compounds" included in IARC monographs as potential carcinogens

In general, the presence of nitrite and nitrosable compounds

such as secondary amines in an acidic environment, can be defined as favorable conditions for nitrosamine formation.

In pharmaceucital products, apart from the external factors (eg. contamination), the formation of Nitrosamines could be associated with the synthesis procedure of API's. Suitable contiditons for the formation of nitrous acids and precence a nitrosable compound, is the one of these scenerios.

Nitrosamines are first encountered in some valsartan, an angiotensin II receptor blocker, batches. In July of 2018, US-FDA and European Medicines Agency (EMA) announces Nitrosodimethylamine (NMDA) and Nitrosodiethylamine (NDEA) as new carcinogenic impurities, caused a great number of recalls globally.

The analysis of N-Nitrosamines are generally conducted with hyphenated mass spectrometric techniques. Both of the liquid direct injection and head space injection/GC-MS&GC-MS/MS successfuly utilized for the analysis of Nitrosamines in some API's (eg. losartan), but some of the APIs like Ranitidine, an H2 blocker, could be degraded to some Nitrosamines at high temperatures, consequently the amount of some certain Nitrosamines overestimated in quantitative manner (eg. NDMA). An LC-MS/MS system equipped with an Atmospheric Pressure Chemical Ionization source is a versatile tool for the Nitrosamine analysis. APCI covers a wider range of Nitrosamines by the meaning of ionisation than ESI (Electrospray ionization)).

In this study, an APCI-LC-MS/MS method is developed for quantitative analysis of NDMA and NDEA in commercially available medications, with an IDL (instrument detection limit) of 5µg/L, and this method is also adapted for targeted screening of NMBA (N-nitroso-N-methyl-4aminobutanoic acid) & NDBA (N-nitrosodibutylamine).

Keywords: N-Nitrosamines, nitrosable compounds, LC-MS/MS, carcinogenic impurity

PT-069

Separation of Enantiomers on Novel Chiral Cyclic Sulfoxides in High-Performance Liquid Chromatography Using Cellulose-Based Chiral Stationary Phases with Acetonitrile and Aqueous-Acetonitrile as Mobile Phases

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The goal of our study was separation of enantiomers of novel chiral cyclic sulfoxides in high-performance liquid chromatography. Cellulose-based chiral columns were used in combination with acetonitrile and aqueous-acetonitrile as mobile phases. In order to study separation of enantiomers and detect possible reversal of enantiomer elution order based on mobile phase or structure of a chiral selector the study was planned in the following way:

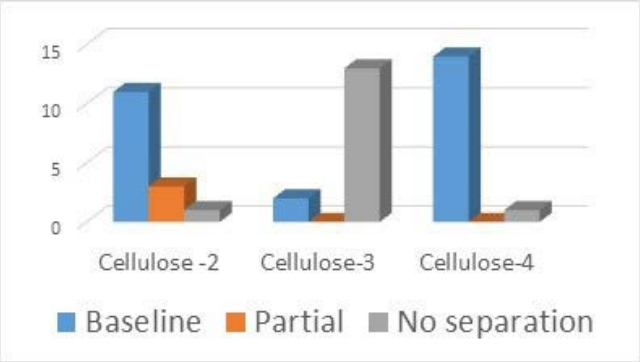
1) Separation of enantiomers of nonracemic (spiked) samples on different chiral columns and with different mobile phases.

2) Optimization of mobile phases by addition of various amount of water to acetonitrile.

Based on the obtained results we could determine which chiral selector is most suitable for separation of enantiomers of novel chiral sulfoxides under present study (Fig. 1). Specifically, Lux Cellulose-4 with cellulose tris(4-chloro-3-methylphenylcarbamate) as a chiral selector appeared to be the most successful chiral selector. Lux Cellulose-3 with cellulose tris(4-methylbenzoate) as a chiral selector exhibited very limited chiral recognition ability. This indicates the importance of having hydrogen bonding-donor functionality in the structure of a chiral selector as a key structural element for chiral recognition ability towards this specific group of chiral analytes. Several examples of enantiomer elution order reversal were also observed.

Keywords: Enantiomers, enantioseparation, chiral stationary phase

Fig. 1 Separation statistics of enantiomers of novel chiral sulfoxides on cellulose-based chiral columns with acetonitrile as a mobile phase.



Separation statistics of enantiomers of novel chiral sulfoxides on cellulose-based chiral columns with acetonitrile as a mobile phase.

PT-070

Innovating a Novel HPLC Method for Validated Assay of Quercetin in Nano Drug Delivery Systems

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Quercetin (QUE), is one of the most important flavonoids which has diverse biological actions including antioxidant properties, anti-inflammatory, antimicrobial, and anticancer activities. It is also reported to have anti-proliferative effect on human ovarian, stomach, and breast cancer cells. Despite its valuable biological properties, the main drawback of QUE is its insolublility in water, which impedes its use as an effective treatment against several diseases [1]. QUE has highly hydrophobic nature and its clinical use is restricted by its poor absorption. However, alternative strategies such as polymeric nanoformulations can improve its solubility, bioavailability, and targeted delivery to the tumor site, which in turn enhances the therapeutic capability [2]. This study aims to develop and validate a simple and selective high-performance liquid chromatography (HPLC) method for the determination of QUE. The chromatographic system was Agilent 1260 Infinity-II LC. The analyses were performed on a C18 column (EVOC18) (150x4.6 mm, 5 µm particle size) at 45°C with UV detection at 370 nm. The mobile phase was composed of methanol:water (50:50, v/v, H 3 PO 4 0.1%, pH2) mixture, and flow rate was set to 1 mL/min. Optimized chromatographic conditions were validated according to the International Conference on Harmonization (ICH) guideline [3]. Regarding to the ICH guideline, evaluated parameters were system suitability, specificity, linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and robustness. The stock solution of QUE was prepared in methanol. The linearity of the method was determined by selected concentration points. The calibration equation was evaluated by determination coefficient, slope and intercept by using Graph- Pad, InStat 3.0 (GraphPad Software, Inc.). The accuracy of the method was assessed by performing the recovery study at three different concentrations of QUE. The inter-day precision studies and intra- day precision studies were also carried out with the same 3 concentrations. All results evaluated by mean, standard deviation (SD) and relative standard deviation (RSD%) values. The analytical method was further used for the determination of QUE in newly developed polymer nanoparticles made of poly(l-lactide-co-caprolactone-co-glycolide) (PLCG), which is a copolymer of polylactic acid, polycaprolactone, and polyglycolic acid. Solvent evaporation method was used to prepare QUE loaded PLCG nanoparticles. Beforehand, PLCG and QUE were dissolved in acetone. The oil phase was added to the aqueous phase containing Pluronic F-127 (P-127) or Polyvinyl alchohol (PVA). Subsequently, the solution was ultrasonicated on ice bath. The mixture was stirred using magnetic stirrer for the evaporation of organic solvent. The formed nanoparticles were then washed with MilliQ water twice. Further, the nanoparticles were centrifuged. Entrapment Efficiency (EE%) was determined by indirect method. The average particle size and size distribution (PDI value) and also zeta potential of the PLCG nanoparticles were measured by zeta sizer (Nano ZS, Malvern Inst., Malvern, UK). The validated method was successfully applied for the assay of QUE entrapped in newly developed polymeric PLCG nanoparticles.

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Keywords: Quercetine, HPLC, PLCG

PT-071

Some Technical Aspects of Enantioselective High-Performance Liquid Chromatography Tandem Mass Spectrometry

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Since the number of chiral illicit drugs grows steadily the role of enantioselective analysis in forensic toxicology becomes increasingly important. Enantioselective high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) meets current requirements in this field more or less adequately and has the potential to assist in determination of cause of death and aid in the correct interpretation of substance abuse. As our experience shows there are some technical issues the scientists have to consider in order to correctly perform enantioselective HPLC-MS/MS experiment and to interpret experimental results adequately. This presentation summarizes our observations. The enantiomers of over 100 chiral substances were separated on various polysaccharide-based chiral columns in HPLC and detected with serially connected UV-VIS and MS/MS detector. The effect of a chemistry of a chiral selector, mobile phase composition, sample solvent and sample concentration on the peak shape, separation efficiency and ratio of response to both enantiomers was evaluated. It was found that mobile phase gradient (composition and especially the flow-rate), as well as sample concentration may drastically affect peak shape and ratio of response of MS detector to two enantiomers of a given chiral compound. Optimization of collision energy can provide a solution of this issue in a certain degree. Exactly the same response to both enantiomers of a given chiral compound is not warranted with MS-detector. Experimental conditions, especially mobile phase composition and flow rate has to be carefully optimized in order to reduce the effect of these factors on peak shape and ratio of MS-detector response of two enantiomers. For quantitative analysis independent calibration has to be performed for both enantiomers. In addition, the same response cannot be assumed for non-deuterated and deuterated compounds.

Keywords: Enantioselective analysis, HPLC-MS/MS, methorphan, methylone.

PT-072

Optimization of High-Performance Liquid Chromatographic Method for Impurity Analysis in Antituberculotic Drug Isoniazid

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Isoniazid (isonicotinic acid hydrazide), is an antibiotic used for the treatment of tuberculosis. Isoniazid was first synthesized in 1952 and currently it is on the World Health Organization's List of Essential Medicines as critically important for human medicine. For active tuberculosis, it is often used together with rifampicin, pyrazinamide, streptomycin or ethambutol. For latent tuberculosis, it is often used alone. This dug has serious side effects including liver inflammation and acute liver failure. It is assumed that some impurities and degradation products are responsible for the abovementioned side effects of the drug. Therefore, a reliable analytical method for purity testing of isoniazid is required. Currently available official high-performance liquid chromatographic (HPLC) methods for the analysis of isoniazid drug substance and formulations do not allow the separation of all relevant impurities. In addition, these methods are based on mobile phase gradients and require long analysis times (over one hour). Together with long analysis times, a high amount of harmful organic reagents are required. In addition, isoniazid is instable in some organic solvents used as mobile-phase components and this may impair the analytical results [1]. Based on these shortcomings our goal was to develop a new method for separation of isoniazid and its major impurities by using state-of-the-art HPLC technology enabling adequate separation of the drug and its major impurities with a short analysis time and low consumption of organic solvents. This goal was achieved by using HPLC column packed with small particles made of superficially porous silica and ultra-high-performance liquid chromatographic setup.

Keywords: JPLC, ısoniazid, ımpurity analysis, pharmaceutical

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PT-073

Deep Eutectic Solvent Based Microextraction Method for the Preconcentration/Extraction of Palladium from Wastewater Samples Prior to Slotted Quartz Tube – Flame Atomic Absorption Spectrometry Measurement

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Palladium is classified as a noble metal and used in various industries such as jewelry, dental and medical equipment manufacturing, automotive and electronics due to its physical/chemical properties [1]. However, its widespread usage in these industries has led to environmental contamination, even though the levels of palladium in the environment are typically low [2]. Although it is not commonly considered a toxic heavy metal, anthropogenic sources of palladium can have significant effects on the environment and human health [3]. Consequently, it is essential to develop sensitive analytical methods that utilize preconcentration/extraction procedures to detect trace amounts of palladium in the environment to prevent its toxicity. In this study, a new method was created to measure trace amounts of palladium using the lab-made slotted quartz tube (SQT) supported flame atomic absorption spectrometry (FAAS). This method involves using a deep eutectic solvent based (DESb) liquid phase microextraction method (LPME) to preconcentrate palladium for better sensitivity. The DESb-LPME-SQT-FAAS system was optimized to improve the detection limit of the system, resulting in quantification and detection limits of 24.7 and 7.4 μg L^{-1}, respectively, which are compatible to other studies. By comparing the limits of detection with the other methods, the FAAS detection power was enhanced by almost 50-fold. The method was tested with a spiked wastewater sample and the recovery of palladium in this medium was found to be between 85 and 91%, demonstrated that the method is applicable and accurate to detect palladium in complex matrices. Overall, this new method is efficient, sensitive, and easy to use for accurately detecting trace amounts of palladium [4].

Keywords: Palladium, SQT, FAAS, Deep Eutectic Solvent, Wastewater

PT-074

Development an Analytical Method for the Trace Determination of Rhodium in Soil Sample Matrices by Flame Atomic Absorption Spectrometry after Manganese Ferrite Nanoparticle based Dispersive Solid Phase Extraction

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Rhodium (Rh) which is known as one of the rare and valuable elements belongs to the platinum group metals and is generally found in association with palladium, platinum, and other precious metals [1]. Rhodium has many industrial applications due to its physical and chemical properties [2]. One of these areas is the automotive industry, and monitoring the release of rhodium from catalytic converters is crucial for human health and the environment [3,4]. Hence, it is necessary to develop an analytical strategy to determine rhodium at low levels in soil samples which are frequently penetrated by rhodium. In present study, a dispersive solid phase extraction based on manganese ferrite ($MnFe_2O_4$) nanoparticles was developed for the preconcentration of Rh ions in soil samples prior to flame atomic absorption spectrophotometry measurement. After the optimization of all parameters that affect extraction efficiency, system analytical performance values such as limit of detection, limit of quantitation, linear working range, coefficient of determination and percent relative standard deviation were determined. Recovery studies were performed with spiking experiments at different concentrations

on soil samples to investigate the applicability of the method. The recovery results were calculated using the matrix matching calibration strategy which is the best way to minimize the effects of possible interferences from matrix components [5].

Keywords: Rhodium, soil, preconcentration, manganese ferrite nanoparticles, flame atomic absorption spectrophotometry

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PT-075

An Innovative and Sensitive QTF-Based Panel Biosensor System for the Analysis of Leptin: A Potential Biomarker for Early Detection of Obesity

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Leptin is a peptide hormone with a molecular weight of 16 kDa and is released from adipocytes. The most important physiological effect is suppressing food intake according to the balance between free and protein-bound forms and accelerating basal metabolism (Zhang et al., 2021). Leptin level secreted in obese individuals is higher than normal individuals. Therefore, the leptin hormone is a potential biomarker for early diagnosis of obesity (Özcan ve Sezgintürk, 2020). Quartz Tuning Fork (QTF) method is a popular technique used in developing biosensors for various applications. QTF-based biosensors have a great attention in recent years due to their high sensitivity, low cost and ease of preparation. The QTF method involves the use of a quartz tuning fork as a transducer for biosensing applications. Quartz is a great material for QTFs due to its piezoelectric qualities, which allow it to transduce mechanical vibrations into electrical impulses (Dedeoğlu et al., 2019). In this study, QTFs with gold surface were treated with a thiol-containing modification agent using the gold thiol chemistry. A cross-linking agent was used for the successful immobilization of anti-leptin antibody on the thiol-modified QTF surface. Afterwards, the anti-leptin solution was covalently immobilized on the QTF surface. To prevent the non-specific interactions bovine serum albumin (BSA) solution was utilized. Finally, QTF surfaces were treated with leptin antigen and resonance frequency shifts were detected in the system. Shift values at this resonance frequency were plotted against leptin concentration. Optimization studies were carried out to determine ideal analysis conditions for leptin on the QTF-based measurement system. After the optimization studies, increasing concentrations of leptin protein were applied to the QTF panel system in order to determine the linear detection range of the QTF system. Characterization studies were carried out in order to determine the characteristics of the sensor system such as repeatability and reproducibility capacity. The QTF panel system was tested for real human serum and the capacity of the leptin detection was analyzed.

Keywords: Obesity, leptin, quartz tuning fork, biosensor

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PT-076

A Novel QTF-based Panel System for the Detection of Aflatoxin B1

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Aspergillus flavus produces aflatoxins, secondary fungal metabolites also known as mycotoxins. A. flavus produces four aflatoxin compounds: AFB1, AFB2, AFG1, and AFG2 (AFG2) (Xu et al., 2023). These substances constitute a considerable danger of food contamination because they pollute the dietary supplies of these animals. AFB1 is the most cancer-causing of the aflatoxins. AFB1 has been discovered to be an immunosuppressive agent (Gul et al., 2023). The Quartz Tuning Fork (QTF) system is widely used to produce various application biosensors. QTF-based biosensors have attracted considerable interest recently due to their high sensitivity, low cost, and simple construction. Because of its piezoelectric properties, quartz is an excellent material for QTFs since it can transform mechanical vibrations into electrical impulses and vice versa. This makes quartz an ideal choice for QTFs.In this study, a "Quartz tuning fork" (QTF) based biodetection system was developed for the detection of aflatoxin-B1 (AFB-1) (Di Gioia et al., 2023). This study was carried out with QTFs modified a gold surface with a mercapto compound and treated with the gold thiol chemistry. The anti-aflatoxin-B1 antibody was successfully immobilized on the thiol-modified QTF surface by using a cross-linking agent. Then the anti-aflatoxin-B1 solution was covalently immobilized on the surface of the QTF. The solution containing bovine serum albumin (BSA) was used in the next step to prevent non-specific interactions. Subsequently, resonance frequency changes were observed after AFB-1 antigen was applied to QTF surfaces. The resonant frequency shifts were displayed as a function of AFB-1 concentration. Optimization studies were conducted to determine ideal analysis conditions for the AFB-1 biosensor system on the QTF-based measurement system. Afterwards, the dynamic range was determined at increasing concentration values of AFB-1 in the QTF panel system. Sensor system properties, including repeatability and reproducibility, were studied in the characterization process. Finally, the proposed biosensor system was applied to real food samples.

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Keywords: Aflatoxin B1 (AFB-1), Quartz tuning fork (QTF), Biosensor

PT-077

A Novel and High-sensitive Quartz Tuning Fork Based Sensor System for C1-INH Determination

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Angioedema is a temporary localized swelling of the mucosal tissues that can occur anywhere in the body and lasts a few days. Hae (angioedema) poses a vital risk in the person's daily life due to recurrent attacks. It has been reported that some mechanisms related to cholinesterase 1 (C1-INH) inhibitors may play a role in late stage of the angioedema (Drouet et al., 2022). A quartz tuning fork (QTF) is a small, two-pronged device made of a thin piece of quartz crystal that vibrates at a specific frequency when an electrical voltage is applied to it (Vlassov et al.,2012). Due to their high sensitivity, low cost, and ease of preparation, biosensors based on QTF have attracted much interest in recent years. The piezoelectric properties of quartz make it an excellent material for QTFs, as it can convert mechanical vibrations into electrical signals and vice versa. QTFs are widely used in various fields, including scientific research, industrial process control, and medical diagnostics (Özgüzar et al.,2019). QTFs are sensors that have become popular recently and can be used as temperature, humidity, pressure and biosensors. In medical diagnostics, QTFs are used in biosensors to detect the presence of biomolecules such as DNA or proteins. In this study, QTF material with a gold surface was treated with an agent containing a thiol group. A crosslinker agent was also used for successful interactions between the anti-C1-INH antibody and the thiol-containing solution.

After the immobilization of the anti-C1-INH antibody, bovine serum albumin (BSA) protein was used as a blocking agent for hindering the nonspecific binding. After the incubation of the C1-INH antigen, resonance frequency values obtained on the QTF sensor system were measured. The resonance frequency shifts were determined and plotted against the antigen concentration. In order to design an ideal and stable QTF sensor system for C1-INH detection, optimization studies were carried out. The linear range, repeatability and reproducibility studies were investigated for the analytical characterization of the constructed sensor system.

Keywords: Biosensor, C1-INH, quartz tuning fork, angioedema

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PT-078

Comparison of Immunopurification Methods for Erythropoietins Analysis Anti-doping Control

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Erythropoietin receptor agonists (ERAs), a substance on the World Anti-Doping Agency (WADA) Prohibited List S2, are commonly used by athletes to enhance endurance performance. The current WADA technical document (TD2022EPO) specifies that the only method capable of screening and confirming all kinds of erythropoietin is sarcosyl polyacrylamide gel electrophoresis (SAR-PAGE), followed by Western blot to distinguish erythropoietin by molecular weight. Immunopurification (IP) of urine and serum/plasma samples is mandatory for all analysis by initial testing procedure (ITP) and confirmation procedure (CP) performed by SAR-PAGE.

To guarantee optimal selectivity and sensitivity in detecting erythropoietin, it is crucial to achieve high efficiency in IP and protein transfer. This is because it is important to prevent any possible cross-reaction of the secondary antibody with residual proteins that might be present in cleaned samples. In this study, our objective was to compare the results of different IP methods performed on the SAR-PAGE test. We optimized the IP conditions and compared the sensitivity and selectivity of blood and urine analysis using various IP methods. To achieve this, we used blank blood samples spiked with different levels of ERAs at MRPL levels, which were then immunopurified using the MAIIA anti-EPO gel column (1430) or anti-EPO mAb coated magnetic beads. Similarly, urine samples spiked with different levels of ERAs at MRPL levels were immunopurified using a StemCell ELISA plate after ultrafiltration and MAIIA anti-EPO gel column (1410). The purified samples were then analyzed with SAR-PAGE and subjected to an immunoblotting procedure, followed by chemiluminescent detection. The IP methods used showed no significant differences in terms of selectivity, reproducibility, and sensitivity. Both methods for each matrix are suitable for initial testing and confirmation procedures, reducing the likelihood of false positives. When selecting an appropriate method, convenience, time, and cost should be taken into account. In urine analysis, the time required for both methods is comparable, but the StemCell method is more cost-effective than the MAIIA kit (1410). As a result, the StemCell method followed by a single blot can be used regularly for initial testing procedures. For blood analysis, MAIIA (1430) is less time-consuming than magnetic bead methods, which are less expensive. Furthermore, the beads are durable and can be reused up to 20 times for at least 6 months without any reduction in efficiency or carryover (based on unpublished in-house data) through proper washing steps.

Keywords: Erythropoietin, SARPAGE, immunopurification, WADA, doping

PT-079

Integration of Thin Film Extractive Samplers with Surface Enhanced Raman Spectroscopy: Preliminary Investigation for Rapid Pesticides Determination

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Pesticides are chemicals that are used to protect the plants against pests and diseases, in agricultural production all over the world. However, the excessive use of pesticides has detrimental effects on the environment and human health. That is, for such risks to be reduced, rapid and on-site control of the amount and type of pesticide is essential. Hence, methods that can be used both for sampling and sample preparation directly on site which later can be coupled either with fast analysis approaches such as Raman Spectroscopy or more selective techniques such as chromatography coupled with mass spectrometry are in high demand. In this scope, PDMS which can be used as an extractive substrate for preconcentrating the analytes and at the same time as a substrate for Surface Enhanced Raman Spectroscopy (SERS) is appealing as an ideal material for developing such methods. With this motivation, a new thin film microextraction (TFME) based approach, which is capable of combining sampling and sample preparation with fast determination of pesticides was developed. For this purpose, poly(dimethylsiloxane) (PDMS) and silver nanoparticles containing PDMS (PDMS/Ag) were prepared using spin coating approach. Both thin films were used for the extraction of trifluralin and methyl parathion from aqueous solutions (pH 7.5 PBS) and analyzed with SERS and GC-MS for rapid screening and confirmatory analysis, respectively. The SERS results revealed the presence of trifluralin and methyl parathion characteristic peaks which were consistent with previous SERS reports suggesting the reliability of the method. Moreover, the results of the study suggested that pesticides cannot be detected from PDMS films using SERS approach if Ag nanoparticles are not present. Also, it has been found that addition of Ag nanoparticle solution on the surface of the film further enhances the SERS signal and this enhancement is more significant for the PDMS/Ag films. The results obtained with PDMS/Ag films (extraction conditions: 4.0 mL sample and 60 min of extraction time) suggested that both pesticides can be detected reproducibly at the lowest tested concentration (12.5 ng/mL) using SERS approach and can be further optimized to obtain detection limits at sub-ng/mL levels.

Keywords: Thin film microextraction, sample preparation, Surface Enhanced Raman Spectroscopy (SERS), gas chromatography-mass spectrometry, pesticides

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