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ARTICLE

Application of magnetic solid-phase extraction for sensitive determination of anticancer drugs in urine by means of diamino benzidine tetrachlorohydrate modified magnetic nanoparticles

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Abstract

Background The analysis of drug active molecules and residues in the treatment of cancer is important for the sustainability of human life and therapeutic effects. For this purpose, a new magnetic sorbent was developed to use in solid phase extraction prior to conventional high-performance liquid chromatography (HPLC) analysis of Paclitaxel (PAC) and Gemcitabine (GEM) molecules.

Methods In this study, a separation and pre-concentration approach based on magnetic solid phase extraction (MSPE) was proposed for PAC and GEM by means of using a newly synthesized magnetic sorbent. After the MSPE procedure, an HPLC system with a diode array detector (DAD) was used to analyze trace amounts of PAC and GEM anticarcinogenic drugs in urine samples. Surface modification of magnetic Fe_3O_4 nanoparticles was carried out by diaminobenzidinetetrachloro hydrate (DABTC) for the first time and a useful sorbent was obtained for MSPE experiments.

Results In the proposed method, PAC and GEM molecules were retained on the c in the presence of a pH 5.0 medium and desorbed to 300 µL of acetonitrile: methyl alcohol (1:1) eluent phase before HPLC–DAD analysis. Under the optimized conditions, the limit of detection (LOD) values for PAC and GEM were 1.38 and 1.44 ng mL^{-1} while the enhancement factor for PAC and GEM were 139.5 and 145.3, respectively. The relative standard deviations (RSD %) for PAC and GEM were below 3.50% in inter-day repeated experiments by means of model solutions containing 100 ng mL^{-1} drug active ingredients.

Conclusions Synthesis and characterization of DABTC-Fe_3O_4 nanoparticles were performed using suitable methodologies. Optimization of MSPE was done step by step. And finally, the developed method was successfully applied to urine samples with quantitative recoveries in the range of 99.0% and 105.0%.

Keywords Paclitaxel · Gemcitabine · Magnetic solid phase extraction · HPLC · Urine samples

Abbreviations

3-APTES	3-Aminopropyl triethoxysilane
ACN:MeOH	Acenotrile: Methyl Alcohol
BR buffer	Britton–Robinson buffer
DABTC	Diaminobenzidinetetrachloro hydrate
DABTC-Fe_3O_4 NPs	Diaminobenzidinetetrachlorohydrate modified iron oxide nanoparticles
DAD	Diode array detector
EDX	Energy dispersive spectroscopy-energy dispersive X-ray
EF	Enhancement factor
FE-SEM	Field emission-scanning electron microscope
FTIR	Fourier Transformed Infrared Spectroscopy
GEM	Gemcitabine
LOD	Limit of detection
LOQ	Limit of quantification
MSPE/HPLC	Magnetic solid phase extraction/high-performance liquid chromatography
NPs	Nanoparticles

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PAC	Paclitaxel
PF	Pre-concentration factor (PF)
PTFE	Polytetrafluoroethylene
RSD	Relative standard deviation

Introduction

Cancer, known as malignant tumors, is a disease characterized by uncontrolled proliferation of cells, invasion of surrounding tissues, and metastasis in near and distant organs due to a defect in the control system that governs the proliferation and differentiation of cells [1, 2]. It has become an increasing problem in today's society and has become a serious threat to our lives. Although many methods are applied for the treatment of this disease, chemotherapy is the most commonly used and most important treatment method [3]. In the chemotherapy method, drugs containing anticarcinogenic drug active ingredients such as paclitaxel (PAC) and gemcitabine (GEM) are frequently used [4, 5]. Drugs with anticarcinogenic effects and many other drug-active substances are excreted in the urine after use and are rapidly mixed into the environment [6, 7]. Therefore, post-use follow-up of these active pharmaceutical ingredients is important both in terms of monitoring the degradation products of anti-carcinogens and their potential to cause environmental pollution [8].

Urine samples are very important for monitoring anti-carcinogens and degradation products [9]. The complexity of urine samples and low concentrations of drug molecules are a challenge for analyzers [10]. Although it is sometimes possible to perform analyzes with techniques such as liquid chromatography-mass spectrometry (LC–MS) [11], LC–MS/MS, gas chromatography–mass spectroscopy (GC–MS) [12], capillary electrophoresis-UV–Vis spectroscopy (CE-UV) [13], capillary electrophoresis-laser-induced fluorescence spectroscopy (CE-LIF) [14] and Raman spectroscopy [15], which most of them are expensive hybrid systems, both the installation and operating costs of these devices are quite high. In addition, expert user knowledge is needed both in the analysis and in the interpretation of the results. Although the HPLC analysis system found in almost every laboratory is cheaper and easier to use than the analysis systems mentioned above, its direct use in the determination of low-concentration analytes in the complex matrix environment is often not possible [16, 17]. Therefore, the samples that are planned to be analyzed should be subjected to a sample preparation method that includes separation and preconcentration steps before their analysis [18, 19].

Liquid–liquid micro(extraction), solid phase micro(extraction), co-precipitation, cloud point extraction, and magnetic solid phase extraction are the most used sample preparation methods [20–23]. Among them, the magnetic solid phase extraction (MSPE) method based on the use of nano absorbents with magnetic properties has been the focus of attention of analytical chemists in recent years as an important sample preparation method due to its many advantages, including high adsorption capacity, reusability, simple and quick experimental operation, high efficiency and no necessary expensive laboratory equipment's for extraction stages [24, 25].

In this study, which was designed with the awareness of the necessity and difficulty of monitoring anticancer drug active substances in urine samples, a magnetic solid phase extraction method using 3-Aminopropyl triethoxysilane (3-APTES) and diaminobenzidinetetrachloro hydrate (DABTC) modified Fe_3O_4 magnetic nanoparticles were combined with the HPLC–DAD analysis system. This method, which offered a high enrichment factor, low detection limit, and high accuracy, was successfully applied to the analysis of PAC and GEM drug active ingredients in urine samples.

Materials and methods

Standards and materials

All reagents used during the experiments were of analytical grade and were purchased from Sigma or Merck companies. All solutions used were prepared with ultrapure water with 18.2 MΩ.cm resistance obtained from MP Minipure Dest Up device. Britton Robinson (BR) electrolyte solutions ranging from pH 2.0 to 12.0 were used in the experimental studies. For this, stock BR solution was prepared, containing H_3BO_3, H_3PO_4, and CH_3COOH acids with 0.05 M concentration. To prepare the desired buffer solution in appropriate pH ranges according to the acidity constants, 0.1 M NaOH was added dropwise under control with the help of a pH meter and adjusted to the desired pH. The chemicals used in magnetic particle synthesis were bought from Sigma Aldrich: $FeCl_3{\cdot}6H_2O$ (CAS Number: 10025-77-1), $FeSO_4{\cdot}7H_2O$ (CAS Number: 7782-63-0), 3-aminopropyl triethoxy silane (3-APTES), (CAS Number: 919-30-2), DABTC were obtained Merck, Supelco with CAS Number: 868272-85-9.

50 mg of analytically pure grade PAC (Sigma Aldrich, CAS Number: 33069-62-4) and GEM (Sigma Aldrich, CAS Number: 122111-03-9) were weighed and taken into a flask, dissolved with 50 mL of methanol (Sigma Aldrich, CAS Number: 67-56-1) and made up to 100 mL, transferred to a dark glass bottle and stored at + 4 ℃. The calibration standards and the other model solutions were prepared by diluting these main stock solutions with methanol.

Equipment

Shimadzu (Prominence) HPLC (Kyoto, Japan) device was used for all chromatographic measurements. It is equipped with LC 20 AD quaternary pump, SPD-M20 A PDA detector, DGU-20A vacuum degasser, and CTO-10 AS VP column furnace. All separations and determinations were performed on a reverse phase C18 column (Inertsil ODS-3, 250 mm × 4.6 mm × 5 µm). Evaluation of chromatograms was done using LC Solution 2.0 software.

Synthesis of DABTC-Fe_3O_4 NPs

In the production of Fe_3O_4 nanoparticles, the co-precipitation method, which we have applied in previous studies and is also well-known in the literature, was used [26, 27] with some minor modifications. Briefly, 0.745 g of $FeCl_3 \cdot 6H_2O$ and 0.383 g of $FeSO_4 \cdot 4H_2O$ salts were first dissolved in 50 mL of 0.1 M HCl and then 100 mL of 50% (v/v) ethanol solution was added while mixing at 85 ℃ at 600 rpm. The synthesis reaction was carried out in an inert medium with nitrogen gas. By adding 20 mL of ammonia solution dropwise to the vigorously mixing solution, Fe_3O_4 NPs formed, and Fe_3O_4 NPs were collected from the resulting black solution with the help of the applied external magnetic field, washed with a certain proportion of water/alcohol mixture and dried in an oven at 60 °C for 6 h [28].

2.0 g of dried Fe_3O_4 NPs were dispersed in 50 mL of 50%(v/v) isopropyl alcohol solution and 1.0 mL of 6 M NH_3 ammonia was added to the reaction medium. 1.0 mL of 3-aminopropyl triethoxy silane (3-APTES) solution was added dropwise to the resulting mixture and the surfaces of Fe_3O_4 NPs were modified with 3-APTES. During the coating phase of the particle surfaces with DABTC, 100 mg of DABTC was dissolved in 60 mL of Ethanol: Methanol (1:1), which can increase the interest of magnetic particles to target molecules in the light of our experience and literature information. After stirring for 6 h at room temperature, the obtained material was washed several times with an ethanol/water mixture and then left to dry in an oven at 60 ℃.

Characterization studies

Bruker AXS D8 simple cubic lattice Kα radiation ($\lambda = 0.15406$ nm) X-ray powder diffractometer with scanning range (2θ) ranging from 5° to 90° was used to examine the crystallographic structure of the synthesized magnetic particles. Fourier transform infrared spectrophotometer (FTIR, Perkin-Elmer, USA) and Raman spectrophotometer (WITec Alpha 300) were used to identify the structure and chemical bonds of the adsorbent. The morphology of the patterns and EDX spectrums for the synthesized materials were obtained by field emission-scanning electron microscope (FE-SEM, Gemini 550).

Magnetic solid phase extraction method

40 mL of the sample or the standard was transferred into a 50 mL falcon tube including 50 mg of DABTC-Fe_3O_4 NPs. 2 mL of pH 5.0 BR buffer was added to adjust the pH and the falcon tube was completed to 50 mL with distilled water. Then, the tubes were placed on an orbital shaker at 100 rpm for 40 min. After the time expired, the tubes were placed in the neodymium magnet assembly and the aqueous phase was separated from the solid phase with the help of a pipette. After separating the aqueous phase, 300 µL of acetonitrile:methyl alcohol (1:1) was added to the solid phase for the desorption of target molecules from the surface of NPs and the tubes were vortexed for 40 s to accelerate this procedure. The solvent phase was separated again by using an external magnet and transferred to HPLC vials after filtration by a 0.45 µm injector tip filter.

Results and discussion

Characterization of solid phase material

FTIR

For the synthesized magnetic-based solid phase sample, FTIR spectrum varying in the wavelength range of 4000–450 cm^{-1} was given in Supplementary File, Figure S2. The peak, which is seen as a broad band at a wavelength of 3445.7 cm^{-1}, is a –NH (hydroxyl) stretching vibration band corresponding to N–H bonds of DABTC. Since it is known that the free hydroxyl groups give a sharp peak between 3645–3610 cm^{-1}, the spectrum seen at 3445.7 cm^{-1} ibroader, showing that there is an intermolecular H bond. The peaks observed at 1789.3 and 1645.3 cm^{-1} wavelengths show the presence of a peak representing C=O and C=C stretching vibrations, respectively. It is known that the peak observed at a wavelength of 562.6 cm^{-1} belongs to the specific Fe–O vibrational peak originating from Fe_3O_4. The peaks seen at 1347, 1150, 1110.8, and 998.2 cm^{-1} wavelengths belong to C–OH, C–N, and C–O vibrations.

During the production of the DABTC-Fe_3O_4 NPs, images were obtained with field emission scanning electron microscopy at different magnifications and EDX analyzes were performed before and after the functional group coating step.

FE-SEM images were shown in Fig. 1A for Fe_3O_4 nanoparticles, images with symbols 1B for DABTC-modified Fe_3O_4 nanoparticles. When the images of Fe_3O_4

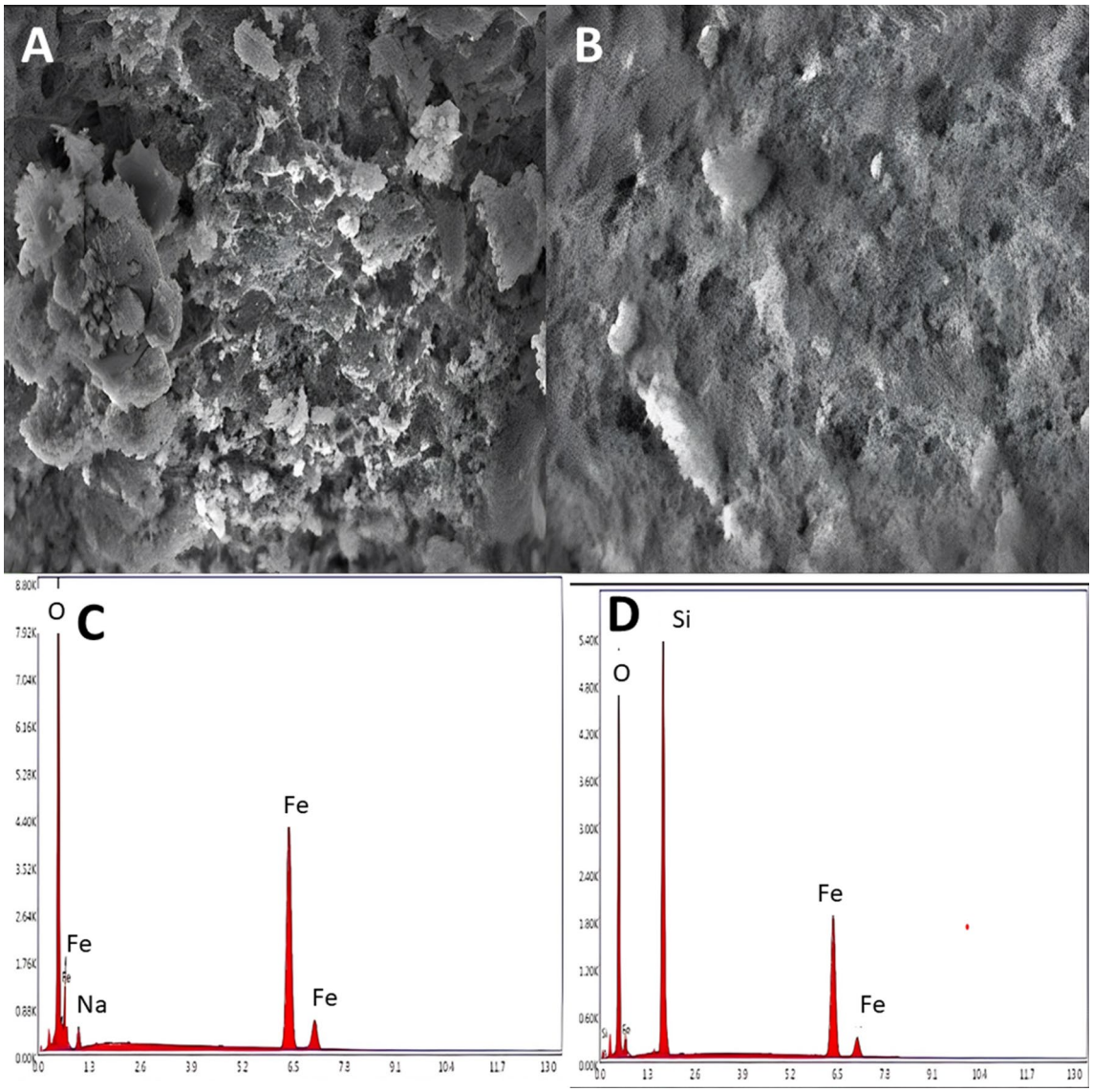

Fig. 1 FE-SEM images of Fe_3O_4 nanoparticles (**A**), diaminobenzidine tetrachlorohydrate modified Fe_3O_4 nanoparticles (**B**) and EDX spectrums of Fe_3O_4 nanoparticles (**C**) and diaminobenzidine tetrachlorohydrate modified Fe_3O_4 nanoparticles (**D**)

nanoparticles were examined, mixed-order nano magnetite structures with magnetite properties can be seen on the surface. In the images obtained for the nanomaterial obtained after the surface modification, it is seen that the surface morphology of the Fe_3O_4 nanoparticles has changed and the magnetite structure on the surface was surrounded by the binder species used in the synthesis phase. In addition, from the EDX spectra taken before (1-C) and after (1-D) the modification, it is seen that Fe_3O_4 nanoparticles and DABTC-modified Fe_3O_4 nanoparticles were successfully synthesized (Fig. 1C and D).

Raman spectroscopy

Main Raman peaks were obtained for Fe_3O_4 and DABTC-modified Fe_3O_4 at wavelengths of 476, 894, 1590, 1713, 1954, 2505, 2871, 2891, 2914, 2936, and 3062 cm^{-1} (Supplementary File, Figure S3). Raman spectrum seen at a wavelength of 476 cm^{-1} belongs to Si–O or Fe–O species. In the spectrum seen at a wavelength of 894 cm^{-1}, a Raman shift is observed in the product after synthesis, and it is seen that the peak shifts to the wavelength of 895 cm^{-1}. Such shifts are also seen in the 1955 → 1954 cm^{-1}, 2505 → 2504 cm^{-1}, and 2871 → 2869 cm^{-1} Raman shifts.

Peak at the wavelength of 875 cm^{-1} represents the presence of aromatic chains. Again, the peaks seen at 1017 and 1069 cm^{-1} wavelengths represent the presence of C–C and aromatic chain-based Raman shifts. Peak at the wavelength of 1590 cm^{-1} is the Raman-active G band resulting from the in-plane vibrational mode. The peak seen at 1713 cm^{-1} peak belongs to the carboxyl peak originating from C=O. A very sharp peak visible at 2504 cm $^{-1}$, causes Raman scattering after being excited with a 532 nm laser source, and Metal-Oxide (MO) band energies and densities increase and create pressure on other peaks. Raman shifts at wavelengths 2914 and 2936 cm^{-1} belong to -CH_2 vibrational bands. A peak is seen at around 3300 cm^{-1} corresponds to the N–H band. The sharp Raman spectrum seen at the 3062 cm^{-1} peak also characterizes the -CH vibrational bands in the aromatic rings (Figure S3).

XRD analysis

XRD analysis was also taken for the characterization of Fe_3O_4 and modified Fe_3O_4 nanoparticles. Results were given in Supplementary File. The diffraction peaks obtained at $2\theta = 8.46°$, 21.52°, 29.48°, 34.86°, 35.47°, 57.06° and 63.23° are characteristic for Fe_3O_4 nanoparticles as seen in Supplementary File, Figure S4. The results obtained by using FTIR, SEM, Raman, and XRD techniques proved that Fe_3O_4 nanoparticles and DABTC-modified Fe_3O_4 nanoparticles were produced successfully.

Optimization of the developed MSPE method

Effect of pH of sample solution on adsorption efficiency of analytes

The pH of the sample solution, where the adsorption process takes place, is an important factor as it affects the adhesion of the analyte to the solid phase and the reactions between the species. To determine the optimum pH, 2 mL of BR buffer in the pH range of 2–10 was added to the model solution medium containing target molecules. After the adsorption process was completed, the magnetic particles were isolated from the solution with an external neodymium magnet.

By considering, the pKa values for GEM [32, 33] and PAC [34] are 3.8 and 10.4, respectively, it can be predicted that the molecules will be at unprotonated form beyond these values. Both the molecular structure of drug molecules and the surface properties of magnetic particles are affected by changes in pH. So, the most favorable conditions are desired by considering the pH of the medium to obtain the maximum transfer of drug molecules to the particle surface. The result of this optimization was shown in Fig. 2. It is seen that the highest extraction efficiency was achieved for both

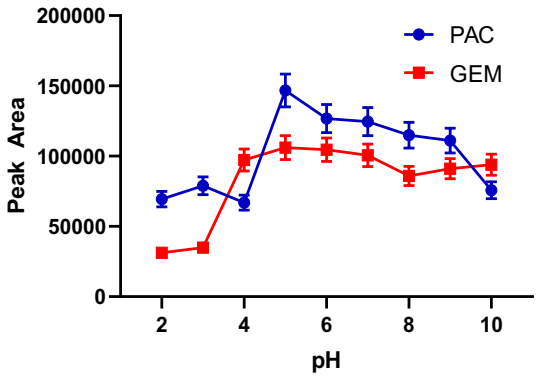

Fig. 2 Effect of sample solution pH on the adsorption efficiencies of PAC and GEM ($N = 3$)

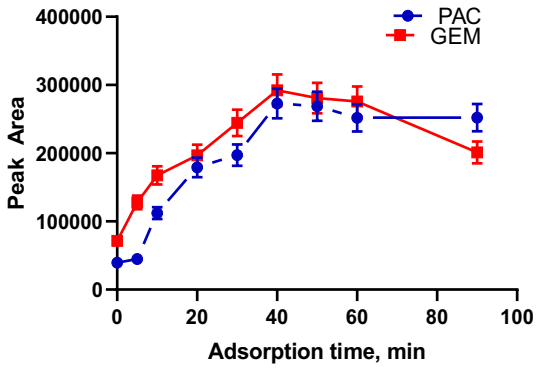

Fig. 3 Effect of sample solution shaking time on the adsorption efficiencies of PAC and GEM ($N = 3$)

molecules at pH 5. It provides that the most favorable form of drug molecules for maximum adsorption carries out at pH 5. The chemical and electrostatical interaction of GEM needs its unprotonated form while PAC is in protonated form. So, in the next optimization steps, the pH of the sample solution was adjusted to 5.

Effect of shaking time on adsorption efficiency of analytes

The effect of the shaking time on the extraction efficiency of analytes from the sample solution phase to the solid phase material is another important parameter due to the effect of the contact time of the adsorbent with all molecules in the sample solution. Transferring target molecules onto the surface of magnetic particles is depending on various parameters. Orbital shaker, magnetic stirrer, and some other simple laboratory equipment are used to increase interactions between drug molecules and particles. All of these tools accelerate the adsorption process. An orbital shaker was used at a certain rate (50 rpm) for this purpose and shaking time was scanned in the range of 0–90 min. It was

seen that the best extraction efficiency was obtained for the shaking time of 40 min as can be seen in Fig. 3. The shaking time of 40 min was chosen as the optimum value for the next experiments.

Effect of eluent solution on desorption efficiency of analytes

A solvent should be used to determine the analyte components without damaging the HPLC device by desorbing the analytes adsorbed to the adsorbent. In this study, some solvents were tried in the selection of solvents used for this purpose in terms of being suitable for the running phase of the HPLC system and being strong enough to quantitatively desorb the components adsorbed to the adsorbent. After the adsorption step was completed under optimum conditions, 1 mL of various solvents including methanol, acetonitrile, ethanol, 2-propanol, 50% methanol, acetone, water, *n*-hexane, acetonitrile:methyl alcohol (ACN:MeOH) (1:1) and pH:5.0 BR buffer solution was added into different tubes for the desorption of analytes. The resulting mixtures were vortexed for 60 s. The analyte components adsorbed to the solid phase were allowed to desorb into these solvents, and liquid phase samples of this solvent were taken with an injector, filtered through a 0.45 µm PTFE membrane filter, put into vials, and submitted to the HPLC device. The results of the elution process were shown in Fig. 4. As can be seen, the best extraction efficiency was obtained with ACN/MeOH solvent for both drug molecules. ACN/MeOH was used as the eluent in the next enrichment steps.

The amount of solvent for the desorption of target molecules from the surface of the magnetic directly affects the enrichment factor, so the volume of the solvent used is very important in MSPE experiments. To achieve a high enrichment coefficient, the solvent volume must be at the lowest value. Otherwise, as the solvent volume increases, the enrichment coefficient decreases due to dilution. However, the dissolution of low amounts of solvent and analyte species attached to the solid phase cannot be completed, and filtration cannot be performed before putting them into vials. For this reason, volume optimization was performed by adding solvent in the range of 200–1250 µL of ACN/MeOH solution. At the end of these processes, the analyte components adsorbed to the solid phase were transferred to the solvent ACN/MeOH, and these solvent liquid phase samples were taken with an injector, filtered through a 0.45 µm PTHE membrane filter, placed in insert vials and placed in the HPLC device. When the results obtained in Fig. 5 were examined, the optimum eluent volume was chosen as 300 µL.

Effect of vortex time on desorption efficiency of analytes

In the desorption of the analytes adsorbed to the adsorbent, a vortex process was applied. To optimize the vortexing time, which has an effect on the desorption of the analyte in the solvent, vortexing times ranging from 0 to 90 s were applied to the samples prepared under the same conditions. The results obtained were shown in Supplementary File, Figure S5. Since the highest extraction efficiency was obtained with the 60-second vortex mixing process, this time was chosen as the optimum value.

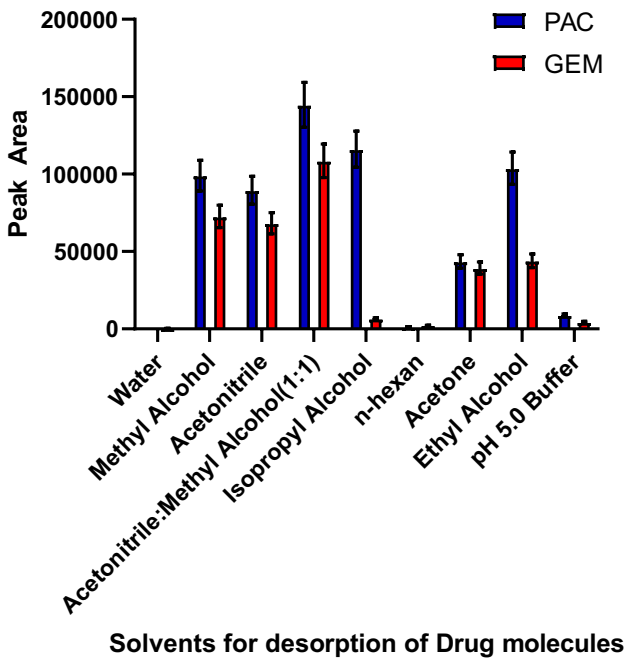

Fig. 4 Effect of eluent solution on the desorption efficiencies of PAC and GEM ($N=3$)

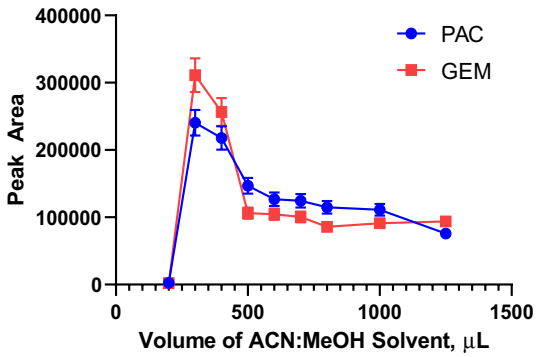

Fig. 5 Effect of eluent solution volume on the desorption efficiencies of PAC and GEM ($N=3$)

Analytical performance of the proposed method

Analytical validation of the MSPE-HPLC–DAD method was carried out after the developed magnetic solid phase extraction procedure was optimized systematically. Analytical figures of merit such as enhancement factor (EF), pre-concentration factor (PF), relative standard deviation (RSD), the limit of detection (LOD), the limit of quantification (LOQ), linear range, and correlation coefficient were calculated by considering correctness and sensitivity of the method.

The developed MSPE-based methodology was applied to model solutions containing increasing concentrations of PAC and GEM drug molecules to determine the linear working range. The linear calibration curves for both molecules were found in the range of 5.0–750.0 ng mL^{-1}. The linearity of the method describes the direct proportionality between the concentration of target molecules in model solutions and peak areas. All calibration standards in the linear range were tested for 3 replicate analyses. The linear equation is Peak Area = 1425.14 × [Pac] + 32.18 with 0.9954 correlation coefficient for PAC while Peak Area = 1671.21 × [Gem] + 12.42 with 0.9873 correlation coefficient for GEM.

The validation of the method was carried out according to International Conference on Harmonization guidelines [35–37]. LOD and LOQ values were calculated using data obtained from calibration plots.

Pre-concentration factors (PF) were calculated by using the ratio of the initial solution volume (50 mL) to the last elution solvent volume (0.3 mL). The enhancement factors (EF) were found from the ratio of the slope of the calibration curve of the analytes after the MSPE application to that of the prior MSPE application. The relative standard deviations (RSD%) were found by applying the MSPE method for seven repetition analyses, which includes 100 ng mL^{-1} of drug molecules. RSD % values for both molecules are lower than 5% as an indicator of the precision of the proposed method. All the analytical figures of merit were reported in Table 1.

Application to real samples

To check the feasibility and accuracy of the proposed DABTC-Fe_3O_4 NPs-based MSPE/HPLC method for real samples, standard addition-recovery studies were performed on synthetic urine and normal urine samples.

The synthetic urine sample was prepared using a literature protocol [28–30]. 6.25 g urea, 0.27 g $CaCl_2 0.2H_2O$, 0.25 g NH_3Cl, 0.4 g KCl, 0.35 g Na_2SO_4, 0.35 g KH_2PO_4, 0.73 g NaCl were weighed and dissolved in distilled water and the volume was completed to 250 mL in a volumetric balloon. Then, the pH of this solution was adjusted to 6 by using 0.1 M HCl solution. It was transferred to an amber-colored bottle and stored at + 4 ℃.

Normal urine samples were taken from a healthy volunteer were directly subjected to the developed method. The human urine samples were collected in a capped test tube from a healthy volunteer free from any kind of medication who had been informed about the perimental procedure and the nature of the study [30].

In the application step, PAC and GEM at the concentration levels of 100 ng mL^{-1} and 250 ng mL^{-1} were added in these samples and analyzed under optimized conditions. The spike recoveries of urine samples were analyzed in triplicate and independently. As shown in Table 2, the recoveries of PAC and GEM in urine samples ranged from 95.2 to 107.9%, with the RSD lower than 6.3%, which indicated the acceptable accuracy and repeatability of the determination results.

Conclusions

In this study, a novel magnetic solid phase extraction method based on DABTC-Fe_3O_4 NPs for separation and preconcentration of PAC and GEM prior to a conventional HPLC analysis was developed, validated and evaluated. For the first time in the literature, DABTC-Fe_3O_4 NPs was synthesized, characterized, and applied to the preconcentration and extraction PAC and GEM in urine samples. The most

Table 1 Analytical merits of the developed method

Parametre	Before MSPE		After MSPE	
	Paclitaxel	Gemcitabine	Paclitaxel	Gemcitabine
Linearity	1.0–20.0 μg mL^{-1}	1.0–20.0 μg mL^{-1}	5.0–750.0 ng mL^{-1}	5.0–750.0 ng mL^{-1}
LOD	0.32 μg mL^{-1}	0.33 μg mL^{-1}	1.38 ng mL^{-1}	1.44 ng mL^{-1}
LOQ	0.88 μg mL^{-1}	0.80 μg mL^{-1}	4.71 ng mL^{-1}	4.78 ng mL^{-1}
RSD (%)	4.1	4.5	3.2	3.5
Calibration sensitivity	10.218	11.501	1425.14	1671.21
Correlation coefficient (R^2)	0.9956	0.9965	0.9954	0.9873
Pre-concentration factor	–	–	166.7	166.7
Enhancement factor	–	–	139.5	145.3

Table 2 Application of the developed MSPE/HPLC procedure to synthetic urine and urine samples ($N=3$)

Sample	Added ng·mL^{-1}	Found[a] ng·mL^{-1}		RSD,%		Recovery, %	
		Paclitaxel	Gemcitabine	Paclitaxel	Gemcitabine	Paclitaxel	Gemcitabine
Synthetically urine	0	<LOD	<LOD	–	–	–	–
	25	23.8 ± 1.5	26.9 ± 1.9	6.3	7.1	95.2	107.6
	250	255.1 ± 12.5	240.5 ± 11.5	4.9	4.8	102.0	96.2
	500	539.5 ± 24.5	495.2 ± 20.4	4.5	4.1	107.9	99.0
Human urine	0	<LOD	<LOD	–	–	–	–
	25	24.2 ± 1.5	24.5 ± 1.1	6.2	4.5	96.8	98.0
	250	259.5 ± 12.7	260.1 ± 12.5	4.9	4.8	103.8	104.0
	500	512.4 ± 25.3	489.5 ± 18.6	4.9	3.8	102.5	97.9

[a]Mean ± standard deviation

important advantage of the developed MSPE/HPLC procedure is that it can be applied with low detection limit, high sensitivity, accuracy and repeatability as well as high efficiency and simplicity.

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Author contributions EA: investigation, formal analysis, HIU: data curation, and writing—original draft; UP: validation and data curation; EY and MS: writing—review and editing, conceptualization.

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Data availability The data presented in this study are available in the article or supplementary materials.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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