Journal of Food Composition and Analysis 124 (2023) 105656

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Combination of homogeneous liquid–liquid extraction and vortex assisted dispersive liquid–liquid microextraction for the extraction and analysis of ochratoxin A in dried fruit samples: Central composite design optimization

Adil Elik[a], Özlem Ablak[a], Hameed Ul Haq[b], Grzegorz Boczkaj[b], Nail Altunay[a,*]

[a] Faculty of Science, Department of Chemistry, Sivas Cumhuriyet University, Sivas, Türkiye
[b] Gdansk University of Technology, Faculty of Civil and Environmental Engineering, Department of Sanitary Engineering, 80 – 233 Gdansk, G. Narutowicza St. 11/12, Poland

ARTICLE INFO

Keywords:
Sample preparation
Mycotoxins
Food analysis
Hydrophobic DESs
Microanalysis

ABSTRACT

This paper presents a new analytical procedure based on combination of homogeneous liquid–liquid extraction (HLLE) and vortex-assisted dispersive liquid–liquid microextraction (VA-DLLME) for the accurate and reliable determination of ochratoxin A (OTA) in dried fruit samples. To enable selective extraction of the OTA, six hydrophobic deep eutectic solvents (hDESs) were prepared and tested as extraction solvents. Optimization of DES volume, pH, NaCl amount, and mixing time affecting the efficiency of VA-DES-DLLME step was achieved by central composite design (CCD). Using optimized conditions, the working range was obtained in the range 0.4–350 ng mL^{-1} with an enrichment factor of 138. The limit of detection was 0.12 ng mL^{-1}. To evaluate the accuracy of the method, the samples were analyzed with both the HLLE-VA-DES-DLLME procedure and reference method. The precision of the method was investigated by intraday/interday studies. The robustness of the method was also evaluated by making minor changes to the optimized conditions. The HLLE-VA-DES-DLLME procedure was successfully applied to dried fruit samples and quantitative recoveries were obtained (92.1–99.2%) confirming its usefulness for implementation in routine analysis of food samples.

1. Introduction

Ochratoxin A (OTA) is a naturally occurring mycotoxin produced by certain species of fungi, primarily *Aspergillus* and *Penicillium*. It can contaminate a variety of food and feed commodities, including cereals, coffee, cocoa, wine, dried fruits, nuts, and spices (Wang et al., 2022). Exposure to OTA can occur through ingestion of contaminated food and beverages. The toxin is stable and resistant to heat, so it can maintain its primary form in food after its processing and cooking. Its persistency during storage and accumulation in certain food products over time has been also reported (Scott, 2005, Lee et al., 2023). OTA is considered a potential human carcinogen. It has been associated with kidney toxicity, thus its presence is a suspected risk factor for kidney diseases, including Balkan endemic nephropathy. Studies in animals have also shown reproductive and developmental toxicity, immunotoxicity, and genotoxicity (Cicoňová et al., 2010, García-Pérez et al., 2021).

Permissible limits for OTA in food and beverages differ depending on the country and the specific food product. Different regulatory agencies have established their guidelines and regulations to ensure food safety and protect consumers from excessive exposure to OTA. In the European Union (EU), the maximum limit for ochratoxin A in roasted coffee beans is 5 μg/kg, in wine 2 μg/kg, in cereals and cereal products and dried vine fruits 10 μg/kg (Yazdanfar et al., 2022). In the United States, the Food and Drug Administration (FDA) has not established specific limits for OTA in food products. However, it set guidance levels for various commodities as 20 μg/kg for raw grains, 5 μg/kg for finished grain products, and 2 μg/kg for roasted coffee beans were reported (Cai et al., 2020). Monitoring and control measurements are performed to ensure that food products comply with these regulations and to minimize consumer exposure to OTA. To reduce the risk of OTA contamination, it is important to implement good agricultural practices, proper storage conditions, and effective control measures throughout the food supply chain. This includes monitoring and management of fungal growth in crops, implementing proper storage and drying techniques, and conducting regular testing for mycotoxins (Dhanshetty and Banerjee, 2019, Arrúa et al., 2019, Girma and Sualeh, 2022).

* Corresponding author.
E-mail address: naltunay@cumhuriyet.edu.tr (N. Altunay).

https://doi.org/10.1016/j.jfca.2023.105656
Received 13 July 2023; Received in revised form 26 August 2023; Accepted 28 August 2023
Available online 29 August 2023
0889-1575/© 2023 Elsevier Inc. All rights reserved.

Analytical methods are used to determine the concentration of OTA, ensuring compliance with regulatory limits and assessing the safety of the analyzed products. There are several methods available for the analysis of ochratoxin A in food samples, including high-performance liquid chromatography (HPLC) (Zareshahrabadi et al., 2020), UV-spectrophotometry (Serra et al., 2004), liquid chromatography-mass spectrometry (LC-MS) (Zhou et al., 2023), gas chromatography-mass spectrometry (GC-MS) (Zhang et al., 2019) and capillary electrophoresis (CE) (Ragab & El-Kimary, 2021). It is clear, that sample preparation is crucial in analytical protocols. It plays a vital role in ensuring accurate and reliable results. In the current study, sample preparation should ensure the isolation of the OTA from the sample matrix, removal of interfering substances, and enrichment of the target analyte for accurate and sensitive detection. Deep eutectic solvents (DESs) gained high attention as alternative solvents in various extraction techniques, including dispersive liquid-liquid microextraction (DLLME). DESs are an emerging type of solvent frequently used for microextraction in sample preparation. They are composed of an eutectic mixture of two or more components, typically a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA). DESs have gained attention in recent years as potential alternatives to conventional organic solvents in various extraction and separation processes, including sample preparation (Elahi et al., 2022, Haq et al., 2022, Haq et al., 2021). DESs offer several advantages such as versatility, environmental friendliness, enhanced solubility, and selectivity. They require mild extraction conditions, are easy to synthesize, are least toxic, and have tunable properties (Elik et al., 2023, Altunay et al., 2023b, Haq et al., 2023, Elahi et al., 2022). In sample preparation techniques, DESs have been explored for various applications, including extraction, preconcentration, and derivatization of analytes. They offer enhanced selectivity for specific analytes and can be tailored to suit the desired extraction properties. DES-based DLLME has been demonstrated to be effective in the extraction of various analytes from complex matrices. DESs are often composed of naturally occurring or readily available components. Their low toxicity and biodegradability contribute to a greener sample preparation process (Elik et al., 2023, Altunay et al., 2023b, Shahvalinia et al., 2022). The components of DES can be selected and combined in various ways to tailor the solvent properties (e.g., polarity, viscosity, density) to the specific analytes of interest. Several studies on DES-based DLLME revealed them to provide an efficient extraction of a wide range of analytes from complex matrices. DES can be used in smaller amounts compared to traditional organic solvents, which reduces the overall consumption of potentially harmful solvents and minimizes waste generation. The DLLME technique is relatively simple to perform and can be easily adapted for various analytes (Pinheiro et al., 2021, Altunay et al., 2023a, Moslemzadeh et al., 2020).

Central composite design (CCD) is a statistical experimental design method used for optimizing analytical methods. It is widely employed in method development and optimization to determine the optimal operating conditions or factors that will yield the desired response or results (Wang et al., 2013).

In this work, a novel method has been developed for the analysis of OTA in dried fruit samples. The method is based on hydrophobic deep eutectic solvents (hDES) enabling efficient extraction of the OTA. After vortexing and centrifugation, the hDES phase, containing the extracted OTA, was separated and subjected to UV spectrophotometry for quantification. Hexafluoroisopropanol and carnitine-based hDES provided the best performance in the extraction of OTA. In this method, CCD was used as a tool for the optimization of essential extraction parameters. ANOVA statistical model was used for the calculation of analytical parameters. The developed method was validated for selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy. A new sample preparation microextraction-based protocol for OTA determination is presented. The method demonstrated good linearity, sensitivity, and accuracy for the analysis of OTA in dried fruit samples. The described method is sensitive, highly selective, environmentally friendly, cost-effective, faster and requires mild extraction conditions.

2. Materials and methods

2.1. Chemicals

All chemicals used throughout the studies were of at least analytical purity and no further purification steps were applied. Stock solution (50 mg L^{-1}) of ochratoxin A (OTA) was prepared by dissolving the appropriate amount of its reagent (Sigma Aldrich, St. Louis, MO, USA) in methanol (Merck, Darmstadt, Germany) and kept in the refrigerator at − 4 °C. Working solutions were freshly prepared by daily sequential dilution of the stock solution. Ethanol (EtOH), methanol (MeOH), tetrahydrofuran (THF) and hydrochloric acid (37%, w/w) were purchased from Sigma Aldrich, while acetonitrile (ACN), sodium chloride (NaCl), sodium hydroxide (NaOH), and potassium hydroxide (KOH) were purchased from Merck. For the preparation of hydrophobic DESs, tetraoctylammonium bromide (N_{8888}-Br), menthol, decanoic acid, tetrabutylammonium chloride (N_{4444}-Cl), betaine, undecylenic acid and lactic acid were purchased from Sigma; while carnitine, levulinic acid, hexanoic acid, hexafluoroisopropanol (HFI) and thymol was purchased from Merck. A citrate-phosphate buffer solution (40 mM, pH 5.6) was prepared by dissolving an appropriate amount of sodium phosphate dibasic (anhydrous) and citric acid in 100 mL water.

2.2. Instrumentation

The instruments used in sample preparation and extraction steps are given below. An SK5210LHC model ultrasonic bath (Kudos, Shanghai, China) producing 52 kHz frequency was used in the preparation of the samples. In the extraction step, a centrifuge (Universal-320 model, England) was used to separate the phase containing OTA from the sample solution. A digital pH meter (Sartorius PB20, Gttingen, Germany) was used to adjust the pH of the aqueous solutions. Spectrophotometric analysis was carried out on a UV-1800 dual-beam spectrophotometer (Shimadzu, Tokyo, Japan) equipped with microcapacity (400 μL) quartz cell with a 1.0-cm optical path. Ultra-pure water was obtained from a Milli-Q water system (Millipore, Madrid, Spain). The STATISTICA package Design-Expert® version 12.0.1 was used for the experimental design, statistical analysis, and evaluation of the optimization step.

2.3. Dried fruit samples

In the present study, all dried fruit samples (apple, mandarin, green tea, fig, apricot, kiwi, plum, pear, peach, and mulberry) were collected from local markets in Sivas/Turkey. First, the dried fruit samples were powdered using a laboratory blender. The samples were then stored at 4 °C in a refrigerator until the HLLE-VA-DES-DLLME procedure was applied.

2.4. Preparation of hydrophobic DESs

The hDESs were prepared by the addition of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) type chemical compounds at a certain molar ratio to the glass vials and mixing them on the magnetic stirrer until a homogeneous solution was obtained. For some DESs, they were additionally heated (40–80 °C). In this study, hexanoic acid, levulinic acid, HFI, menthol, undecylenic acid, and decanoic acid were used as HBD, while N8888-Br, N4444-Cl, carnitine, lactic acid, thymol, and betaine were used as HBA. The preparation conditions and molar ratio for each hydrophobic DES are presented in Table 1.

Table 1
Composition, preparation conditions and abbreviation of hydrophobic DESs.

Abbreviation	HBD	HBA	Molar ratio	Condition of preparation
DES-1	Hexanoic acid	N_{8888}-Br	1:2	heating at 80 °C for 30 min
DES-2	Levulinic acid	N_{4444}-Cl	1:2	mixing with mechanical stirring at 350 rpm at 60° C
DES-3	HFI	Carnitine	1:3	heating at 80 °C in screw-cap pressure tube
DES-4	Menthol	Lactic acid	1:2	heating at 50 °C for 15 min
DES-5	Undecylenic acid	Thymol	1:3	heat and stirred magnetically at 60 °C
DES-6	Decanoic acid	Betaine	1:3	heating at 40 °C

N8888-Br: Tetraoctylammonium bromide, N4444-Cl: Tetrabutylammonium chloride, HFI: hexafluoroisopropanol

2.5. Developed HLLE-VA-DES-DLLME procedure

The experimental steps of the HLLE- VA-DES-DLLME procedure are as follows. First, the powdered dried fruit samples (10 g) were transferred to conical tubes including 7 mL of 1.5 mol L^{-1} HCl solution and 2.0 mL of THF. Then, the tubes were sonicated for 2 min at room temperature. Following the centrifugation step (3500 rpm 3 min), the solution phase containing the OTA was transferred to another conical tube. After the pH of the mixture was adjusted to pH 5.6 using citrate-phosphate buffer solution, DES-3 (485 μL) and NaCl solution (4.3 w/v %) were added to the tube to ensure separation of the OTA from the aqueous solution. In the final step, the tube was vortexed for 3.5 min. At this step, the DES-3 phase containing the OTA was collected on top of the aqueous solution. Afterwards, the DES-3 phase was transferred to micro cuvettes, and spectrophotometric measurements were made at 355 nm (see Fig. S1). All studies were carried out in parallel with the sample blank. The experimental steps of the developed method are presented in Scheme 1.

2.6. Experimental design

The central composite design (CCD) was applied to optimize the important parameters in the VA-DES-DLLME step, which was performed after the HLLE step. A five-level CCD was applied for the optimization of DES-3 vol (A: 200–600 μL), pH (B: 3–6), NaCl amount (C: 1.5–4.5 w/v %) and mixing time (D: 1–5 min). In addition, experiments were carried out at the star points (±α), apart from the lower and upper values of the working ranges of all variables. Detailed data from the CCD are presented in Table S1. According to the CCD, the effect of the variables on the recovery of OTA was adapted according to the following quadratic equation - 1.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

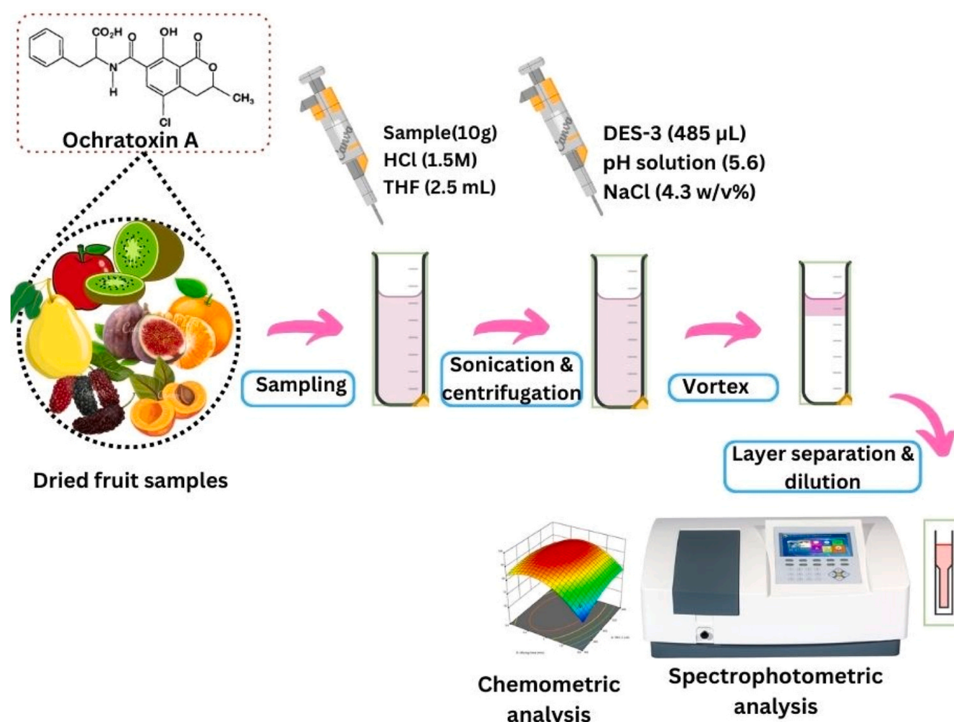
Where Y was the analytical result, X_1, X_2, and X_3 were variables, β_{11}, β_{22}, and β_{33} were quadratic coefficients, β_1, β_2, and β_3 were linear coefficients, β_{12}, β_{13}, and β_{23} were coefficients of interaction and β_0 was a constant.

3. Results and discussion

3.1. Optimisation of parameters in HLLE step

3.1.1. Effect of the sample medium

Organic and inorganic species in dried fruit samples do not allow direct OTA analysis, as they affect the results due to their matrix effects. They also reduce the extraction of the OTA into the DES phase and consequently reduce the efficiency of the method. Therefore, the HLLE



Scheme 1. Experimental steps of the developed method.

step was applied to reduce the matrix effect and allow easy and selective extraction of the OTA into the DES phase. First, the effect of the sample medium on the recovery of the OTA was investigated using alkaline (KOH and NaOH) and acidic (HCl) solutions. In this study, these solutions were analyzed in equal volumes and concentrations. The results in Fig. S1 revealed that the recovery of the OTA was more efficient in an acidic medium. Therefore, further studies were carried out in an acidic medium. In an acidic medium, the carboxylic acid group of ochratoxin A are in the non-dissociated form (as CO_2H). The lack of charge increases its solubility in organic solvents like DES. In an acidic medium, the hydrogen bond acceptor may interact with the carboxylic group of ochratoxin A via hydrogen bonding. The concentration and volume of the HCl solution should be optimized as it can affect the extraction efficiency of the method in the HLLE step. Therefore, the effects of the concentration and volume of the HCl solution on the recovery of OTA were studied in the range of 0.25–3 mol L^{-1} (see Figs. S2) and 1–8 mL (Fig. S3), respectively. According to the results, the best recovery of the OTA was obtained when 7 mL of 1.5 mol L^{-1} HCl solution was used. In particular, the low recovery of the OTA at low HCl concentrations can be attributed to insufficient HCl to transfer the OTA from dried fruit samples to the aqueous phase.

3.1.2. Effect of extraction solvent type and volume

In the HLLE step, the type of extraction solvent has a significant impact on the efficiency, selectivity and phase separation of the method. The extraction solvent used in the HLLE step will be used as the dispersion solvent in the next VA-DES-DLLME step. Therefore, the extraction solvent used must be miscible with the extraction solvent used in the VA-DES-DLLME step. Based on these explanations, acetone, MeOH, EtOH, THF, and ACN were tested in equal volumes as extraction solvents in the HLLE step. The results in Fig. S4 show that the best recovery of the OTA is achieved when THF is used. In the current study, THF plays two key roles: (1) as a polarity modifier of extraction solvent in the HLLE step and (2) as a dispersing solvent in the VA-DES-DLLME step. Therefore, the THF volume has a significant impact on the performance of the method. Therefore, the effect of THF volume on the recovery of the OTA was studied in the range of 0.5–4 mL. The results in Fig. S5 showed that 2 mL of THF volume was sufficient for quantitative recoveries – it corresponds to the volumetric ratio (water: THF) in extractant as 1:2 v/v. THF functions as an aprotic solvent in these investigations, selected to facilitate the separation of phases. The findings of this study show that it promotes phase separation, and also amplifies the percentage of analyte recovery. Such aprotic solvents tend to engage with water to a greater extent than DES. When THF molecules interact with water, the affinity of water molecules for DES diminishes, leading to the clustering and isolation of water molecules (Haq et al., 2022).

The interaction of OTA and DES involves hydrogen bonding. Ochratoxin A contains functional groups such as hydroxyl, carbonyl and carboxyl groups that can act as hydrogen bond donors or acceptors. When OTA is in contact with the DES, hydrogen bonding interactions can occur between the functional groups of OTA and the HBD and HBA components of the DES. Ochratoxin A contains a carboxyl group that is in neutral form (COOH) in an acidic environment. It enhances the solubility of OTA in organic solvents like DES and provides hydrogen bonding interactions between OTA and DES components. DESs are designed to have intermediate polarity between traditional organic solvents and water. This intermediate polarity can match well with the polarity of OTA, allowing for efficient extraction. Overall assessed polarity of the DES sometimes does not define its selectivity. According to available literature, it is known that often, only one of the DES components ensures effective interaction with the analyte providing high selectivity of the separation system. This observation was clearly documented in the case of DES application as stationary phases for chromatographic separations (Momotko et al., 2021; Momotko et al., 2022).

3.1.3. Effect of mixing type and time

To provide effective extraction of the dried fruit samples proper mixing must be applied. In this context, mixing steps such as vortexing, sonication, orbital shaking, and hand mixing were applied for 2 min and the results are presented in Fig. S6. According to the results, the highest recovery (96.1%) of the OTA was obtained when sonication was applied. Following this step, the effect of sonication time on the recovery of the OTA was studied in the range of 1–6 min. The results in Fig. S7 show that 2 min of sonication time is sufficient to effectively disperse THF into the sample solution.

3.2. Optimisation of parameters in VA-DES-DLLME step

3.2.1. Selection of suitable DES

The extraction solvent plays a key role in ensuring the selective and reliable extraction of the target analyte from the sample solution. Therefore, different DESs must be prepared and investigated for the extraction of the OTA. In this study, six hydrophobic DESs were prepared and tested for the extraction of the OTA. The results in Fig. S8 show that the best recovery (95.4%) of the OTA was obtained using DES-3 prepared from a mixture of HFI and carnitine. In addition, the recovery of the OTA for DES-1, DES-2, DES-4, DES-5 and DES-6 was 88.1%, 61.2%, 79.7%, 86.2% and 68.8%, respectively. Based on the results obtained, DES-3 was chosen as the extraction solvent for the CCD step.

3.2.2. Effect of molar ratio of DES

The most important factor affecting the formation of DES is the molar ratio of the components. Since the main factor forming DES is the H-bond formed between the components, the most appropriate DES formation should be investigated by mixing the components in different molar ratios. Based on these explanations, the DES-3 was prepared by mixing HFI and carnitine in different molar ratios (see Fig. S9). Then, each prepared DES-3 was investigated for the recovery of the OTA. The results show that the best recovery of the OTA was obtained using a 1:3 molar ratio of DES-3 components. Further excess of HBD in DES (1:4) does not provide a transparent mixture. Therefore, a 1:3 molar ratio of DES-3 was chosen for the CCD step.

3.2.3. Optimization of important variables using CCD

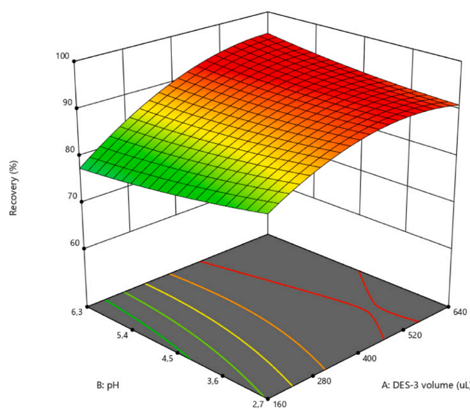
Optimization of important variables (DES-3 vol, pH, NaCl amount, and mixing time) affecting the VA-DES-DLLME step was performed using five-level CCD. The results of the experimental studies of the five-level CCD are presented in Table S2. Based on these results, the effects of the variables and the established CCD on the extraction of the OTA were evaluated by statistical analysis (see Table 2). Statistical evaluations were made for the 95% confidence level, i.e. p-value must be less than 0.05 for the established CCD and variables to be significant for the extraction of OTA. Based on the explanations, the model was significant when viewed in Table 1 because its p-value was < 0.0001. Moreover, linear, quadratic and binary interactions were all significant and contributed to the recovery of the OTA. Because their p-values were less than 0.05. The determination of the variable that contributes the most to the recovery of the OTA depends on the F-value. Accordingly, as the F-value increases, the contribution of the variable to the CCD also increases. According to the results in Table 2c, those contributing the most to the recovery of the OTA for linear, binary, and quadratic interactions are A (F-value: 634.88), CD (F-value: 2455.26), and D^2 (F-value: 1925.16), respectively. The effect of uncertain errors on the recovery of the OTA is evaluated by the Lack of Fit P-value. For uncertain errors to be meaningless to the CCD, the Lack of Fit P-value must be greater than 0.05. Also, the smaller the Lack of Fit F-value, the less the uncertain errors contribute to the CCD. In light of these explanations, the P-value and F-value of Lack of Fit were 0.46 and 1.17, respectively. These results confirmed that uncertain errors are meaningless for the established CCD.

The agreement between the experimental results and the predicted values of the established CCD is evaluated with the R^2 values. The R^2

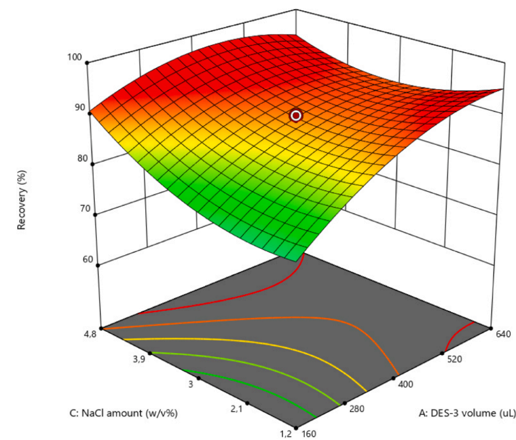
Table 2
ANOVA for quadratic model.

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	1586.97	113.35	631.10	< 0.0001	significant
A-DES-3 vol	102.54	102.54	570.90	< 0.0001	
B-pH	114.03	114.03	634.88	< 0.0001	
C-NaCl amount	0.8475	0.8475	4.72	0.0463	
D-Mixing time	43.93	43.93	244.59	< 0.0001	
AB	49.00	49.00	272.81	< 0.0001	
AC	90.25	90.25	502.46	< 0.0001	
AD	56.25	56.25	313.17	< 0.0001	
BC	90.25	90.25	502.46	< 0.0001	
BD	6.25	6.25	34.80	< 0.0001	
CD	441.00	441.00	2455.26	< 0.0001	
A^2	45.41	45.41	252.83	< 0.0001	
B^2	1.36	1.36	7.56	0.0149	
C^2	44.87	44.87	249.81	< 0.0001	
D^2	345.79	345.79	1925.16	< 0.0001	
Lack of Fit	1.89	0.1886	1.17	0.4591	not significant
Fit Statistics					
R^2	0.9983	Predicted R^2	0.9930	Adjusted R^2	0.9967

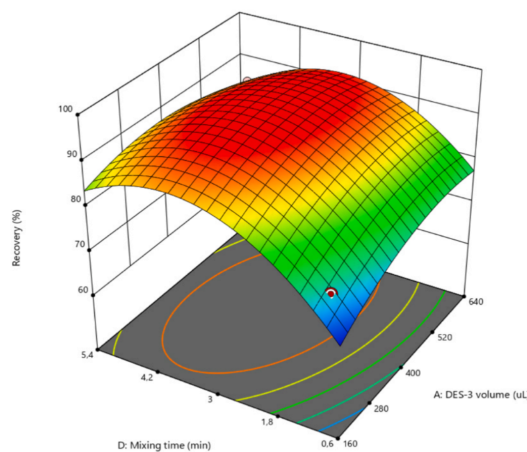
values are desired to be close to 1. Predicted R^2 refers to the model's capacity to predict the recovery of the OTA. According to the predicted R^2, the CCD shows that it predicts the results at a rate of 99.3%. For the established CCD to be valid, the difference between predicted R^2 and adjusted R^2 must be less than 0.2. In addition, the high correlation between the obtained results and the prediction of the established CCD is seen in Fig. S10. As a result of all these explanations, the established CCD analyzed the effect of the variables on the recovery of the OTA according to the following full quadratic Eq. 3.



(a)



(b)



(c)

Fig. 1. (a-c). 3D surface response graphics for optimized variables, (b) pH and DES-3 vol; (c) NaCl amount and DES-3 vol; (d) Mixing time and DES-3 vol, (e) Mixing time and NaCl amount.

Table 3
Experimental data of analytical parameters.

Parameters	After the HLLE- VA-DES-DLLME method	Before the HLLE- VA-DES-DLLME method
Linear range, ng mL^{-1}	0.4–350	300–5000
Coefficient of determination (R^2)	0.9984	0.9991
Limit of detection, ng mL^{-1}	0.12	90.9
Limit of quantification, ng mL^{-1}	0.4	300
Enrichment factor	138	-
*Recovery%	96.7–98.3	-
*RSD%	1.2–2.9	-

* At concentrations of 1, 100 and 300 ng mL^{-1} of OTA (N = 3).

$$\text{Recovery (\%)} = +85{\cdot}90 + 2{\cdot}33\,A + 2{\cdot}46B - 0{\cdot}2119\,C - 1{\cdot}53D + 1{\cdot}75AB - 2{\cdot}38AC - 1{\cdot}87AD + 2{\cdot}38BC + 0{\cdot}6250BD + 5{\cdot}25CD - 2{\cdot}96\,A^2 + 0{\cdot}5119B^2 + 2{\cdot}94\,C^2 - 8{\cdot}17D^2 \quad (3)$$

The effect of binary interactions of variables on the recovery of the OTA was evaluated with surface response graphs (see Fig. 1(a-c)). The effect of pH and DES-3 vol on the recovery of OTA is presented in Fig. 1a. According to the results, high recoveries were obtained at almost all pH values when the volume of the DES-3 was in the range of 400–600 µL. In particular, the achievement of non-quantitative recoveries at low DES-3 volumes can be attributed to the insufficient volume of DES-3 required to extract OTA from the sample solution. The effect of NaCl amount and DES-3 vol on the recovery of OTA is presented in Fig. 1b. The results show that the % recovery of OTA is maximum when the amount of NaCl and the volume of DES-3 are in the range of 3.3–4.6 w/v% and 420–640 µL, respectively. In particular, a significant decrease in the recovery of OTA was observed as the amount of NaCl decreased. This showed that the salting-out effect is important for the effective extraction of the target analyte by the DES-3 from the sample solution. The effect of mixing time and DES-3 vol on the recovery of OTA is presented in Fig. 1c. According to the results, the recovery of OTA was quantified when mixing time and DES-3 vol were 2.5–4 min and 400–550 µL, respectively. The recovery of OTA was not quantitative as the DES-3 was not effectively dispersed into the sample solution, especially when the mixing time was less than 2 min

According to performed optimization based on CCD (see Fig. S11) the maximum recovery of OTA (91.8%) was obtained using DES-3 vol (485 µL), pH (5.6), NaCl amount (4.3 w/v%) and mixing time (3.5 min) for 3.75 mL of water/THF sample. As a result of three repetitive studies performed under these conditions, the recovery of OTA was calculated as 91.3%. This result showed that there was no significant difference between the recoveries predicted by the established CCD. Therefore, these conditions were used as the optimum for validation studies and analysis of dried fruit samples.

3.3. Quantitative aspects

The analytical figures of the HLLE-VA-DES-DLLME procedure were determined using optimized conditions (7 mL of 1.5 mol L^{-1} HCl, 2.5 mL of THF, 2 min of vortex time, pH 5.6, 485 µL of DES-3, 4.3 w/v% of NaCl solution and 3.5 min of vortex time). After the HLLE-VA-DES-DLLME procedure, the working range of OTA was 0.4–350 ng mL^{-1} with 0.9984 of R^2. The limit of detection (0.12 ng mL^{-1}) and limit of quantification (0.4 ng mL^{-1}) were calculated from 3Sb/m and 10Sb/m, respectively. Where, Sb is the standard deviation of ten replicate measurements of the sample blank, while m is the slope of the calibration curve obtained after the HLLE-VA-DES-DLLME procedure. Furthermore, the percent relative standard deviation (RSD%) and recovery of the HLLE-VA-DES-DLLME procedure for the 1, 10 and 300 ng mL^{-1} of OTA were in the range of 1.2–2.9% and 96.7–98.3%, respectively. The enrichment factor (EF) was calculated as 138 from the slope of the calibration graphs obtained before and after the LLE-VA-DES-DLLME procedure. Analytical data are presented in Table 3.

3.4. Selectivity

Optimization studies were carried out using model solutions. Therefore, the selectivity of the LLE-VA-DES-DLLME procedure for OTA should be investigated in the presence of different chemicals. In this context, the chemical species in Table S3 were added to the model solutions for the selectivity of the method, and then the analysis of OTA was carried out by applying the LLE-VA-DES-DLLME procedure. In this study, the tolerable limit was calculated for each chemical species added to the model solution. In addition, recovery and the RSD of OTA were also calculated. The tolerable limit was calculated from the ratio of the concentration of chemical species that provided a ± 5% change in the analytical signal to the amount of OTA spiked. The results in Table S3 show that the tolerable limit of the method has changed from 100 to 50000. In addition, for the studied chemical species, the LLE-VA-DES-DLLME procedure exhibited recovery in the range of 92.7–99.3% and RSD in the range of 1.7–3.6%. The high tolerable limit, quantitative recoveries and low RSD showed that the LLE-VA-DES-DLLME procedure exhibits high selectivity for OTA.

3.5. Robustness

The robustness of an analytical method is investigated with the possible impact of small changes in optimized conditions on the results. In this context, the robustness of the method was investigated by changing the optimized variables by ± 10%. The change applied for each variable and the resulting RSD and recovery are presented in Table 4. The results showed that the small changes made did not cause significant differences in the recovery and RSD of the OTA. These results demonstrated the robustness of the LLE-VA-DES-DLLME procedure.

3.6. Precision

Obtaining reliable results in the analysis of non-homogeneous samples is highly dependent on the sample preparation step. It is very important to use quality control samples (QC) when working with such non-homogeneous samples. In this context, a dried fig fruit sample was selected as the QC and the precision of the LLE-VA-DES-DLLME procedure was investigated by intraday and interday studies on this sample. In this study, three-level concentrations (5, 150 and 300 ng mL^{-1}) of

Table 4
Robustness of the HLLE- VA-DES-DLLME method for the analysis of OTA.

Variables	Optimal condition	Change condition	RSD (%)	Recovery (%)
DES-3 vol± 10%	485 µL	533 µL	1.5	94.4
		436 µL	1.3	95.8
pH ± 10%	5.8	6.4	1.6	93.1
		5.2	1.9	92.9
NaCl amount ± 10%	4.3%	4.7%	1.2	96.8
		3.9%	1.6	97.3
Mixing time ± 10%	3.5 min	3.9 min	1.8	94.4
		3.1 min	2.1	96.2

Table 5
Application results of the HLLE- VA-DES-DLLME method to dried fruit products (N = 3).

Sample	HLLE- VA-DES-DLLME method			Reference method	
	Added (ng mL^{-1})	Calculated (ng mL^{-1})	Recovery (%)	Calculated (ng mL^{-1})	*t-exp
Apple	-	<LOD	-	<LOD	-
	50	48.2 ± 1.8 * *	96.4	46.4	1.27
	150	147 ± 6	98.1	145	1.15
Mandarin	-	<LOD	-	<LOD	-
	50	46.6 ± 2.2	93.3	43.1	0.68
	150	143 ± 8	95.1	140	0.79
Green tea	-	2.8 ± 0.1	-	2.1	0.95
	50	51.2 ± 3.7	96.8	50.8	0.74
	150	151 ± 9	98.7	152	0.82
Fig	-	19.7 ± 0.3	-	20.6	1.22
	50	65.8 ± 2.7	92.1	66.3	1.14
	150	163 ± 8	95.3	165	0.92
Apricot	-	<LOD	-	<LOD	-
	50	48.9 ± 2.4	97.7	47.3	0.74
	150	149 ± 8	99.2	146	0.96
Kiwi	-	<LOD	-	<LOD	-
	50	47.9 ± 3.3	95.8	48.1	1.04
	150	146 ± 7	97.3	147	0.88
Plum	-	<LOD	-	<LOD	-
	50	48.3 ± 2.7	96.6	48.9	0.66
	150	147 ± 8	97.8	148	0.73
Pear	-	1.4 ± 0.4	-	1.9	1.44
	50	49.4 ± 3.3	95.9	50.6	1.26
	150	149 ± 6	98.3	151	0.95
Peach	-	8.3 ± 0.4	-	9.7	0.76
	50	55.5 ± 3.0	94.4	56.9	0.83
	150	152 ± 7	96.0	155	1.02
Mulberry	-	<LOD	-	<LOD	-
	50	48.4 ± 2.8	96.7	49.1	0.94
	150	148 ± 5	98.5	149	0.72

* *The criterion t-value established by two paired ANOVA analysis for 4-degree of freedom at 95% confidence limit where texp= (m1–m2)/ Spooled× [(n1 + n2)/n1 × n2]1/2 and Spooled= [(n1-1) Sm.12 + (n2-1) Sm.22 /(n1 + n2-2)]1/2
* * Mean ± standard deviation.

OTA were added to the QC sample. Subsequently, in the intraday study, the samples were analyzed in triplicate on the same day, while in the interday study, the samples were analyzed in triplicate on three consecutive days. As a result of the study, the RSD of the interday study was in the range of 2.9–3.8%, while the RSD of the intraday study was in the range of 3.4–4.1%. These results showed that there was no significant difference in precision between the studies. Table S4 shows the precision and accuracy of quality control samples.

3.7. Accuracy

The accuracy of the LLE-VA-DES-DLLME procedure was evaluated on the basis of recovery data of OTA spiked (50 and 150 ng mL^{-1}) dried fruit samples. The recovery was calculated according to the following equation-3

$$\text{Recovery}(\%) = \frac{\text{Change in the amount of the OTA measured}}{\text{Amount of the OTA spiked to the dried fruit solution}} x100 \quad (3)$$

From the analysis of dried fruit samples, the recovery was calculated between 92.1% and 99.2%. The results (see Table 5) confirm that it can be considered an effective and reliable method for the extraction and determination of OTA in different dried fruit samples where the sample matrix does not have a significant effect on the LLE-VA-DES-DLLME procedure.

3.8. Analysis of dried fruit samples

After detailed validation studies, the LLE-VA-DES-DLLME procedure was applied to 10 g of dried fruit samples prepared as in Section 2.3. In addition, the reliability of the results obtained from the LLE-VA-DES-DLLME procedure was evaluated by applying the reference method (Ruan et al., 2016) to the same samples. The results obtained are presented in Table 5. In samples of apple, mandarin, apricot, plum, kiwi, and mulberry, OTA could not be detected in both the LLE-VA-DES-DLLME procedure and the reference method. Also, the highest amount of OTA was found in fig (19.7 ± 0.3 ng mL^{-1}). Moreover, the t-exp used to evaluate the reliability of the results obtained from both methods was smaller than the t-critical (2.78) for four degrees of freedom at the 95% confidence level. This means that there is no significant difference between the results obtained from both methods. This suggests that the LLE-VA-DES-DLLME procedure can be safely applied to the analysis of OTA in complex matrices.

3.9. Comparison of the developed method with other approaches

There are several methods available for the analysis of OTA in food and feed samples. These methods can be broadly categorized into two main groups: instrumental methods and immunochemical methods. Several methods have been developed for OTA analysis in food. Enzyme-linked-immunosorbent-assay (ELISA) which is based on the enzyme, has the advantages of low cost, simple signal readout, and strong specificity. However, it is time-consuming (generally takes 5–8 h to perform the whole protocol), with the bias of false positive and negative results (Wei et al., 2023). Instrumental methods include liquid chromatography-tandem mass spectrometry, High-performance liquid chromatography (HPLC)-tandem mass spectrometry, HPLC with fluorescence detection, and spectrophotometric methods. In Table 6, a few recently developed methods have been summarized. Important parameters (extraction system, LOQ, RSD%, linearity, extraction time) were compared. Among all reported methods, mass spectrometry-based methods are the most sensitive based on the lowest LOD. However, this method relies on complex sample preparation, adequate laboratory settings, large and expensive instruments, and experienced laboratory personnel, thus being unable to meet the requirements for on-site rapid detection (Yan et al., 2020). On the other hand, UV-spectrophotometric methods are easier, frequently available, and do not require more experienced laboratory personnel. However, these methods are associated with higher LOD and low selectivity. In this present method, hDES based extraction method was coupled with spectrophotometric determination, which made this method highly selective, and more sensitive. Furthermore this method is faster and applicable in wide range of concentration. Use of green and environment friendly DES in this method eliminate the use of toxic organic solvents. Additionally, this method gave highest recovery (99.2%) in a single cycle.

Table 6
Comparison of the method with other approaches.

Extraction method	Analytical method	Linearity (ng mL^{-1})	LOD (ng mL^{-1})	EF	RSD (%)	Extraction time (min)	References
[a]UA-MIP-SPE	[b]LC-TMS	2.5–100 μg kg^{-1}	1.15 μg Kg^{-1}	33.3	15	45	(Jayasinghe et al., 2020)
[c]MOF-DSPE	[d]HPLC-TMS	83–1 mg L^{-1}	24.8 mg L^{-1}	—	5	> 72 h	(Mohebbi et al., 2022)
[e]SPE	[f]HPLC-FD	0.1–75	0.1	—	2	10	(Kholová et al., 2020)
[g]FPSE	[f]HPLC-FD	1.5–25 ng/g	0.09 ng/g	—	≤ 11.0	> 60	(Olia et al., 2023)
[h]MCOF-SPE	HPLC	0.1–800	0.03	99.6	0.2–5.5	20	(Yang et al., 2023)
[i]MMOF	HPLC-MS	1–100	0.28	—	5	10	(Wei et al., 2023)
HLLE-VA-DES-DLLME	UV-Spectrophotometric	0.4–350	0.12	138	≤ 2.4	5.5	*Current study*

[a] UA-MIP-SPE: Ultrasound assisted-molecularly imprinted polymer-solid phase extraction. [b]LC-TMS: Liquid chromatography-tandem mass spectrometry. [c]MOF-DSPE: Metal-organic framework-dispersive solid phase extraction. [d]HPLC_TMS: High-performance liquid chromatography-tandem mass spectrometry. [e]SPE: Solid phase extraction. [f]HPLC-FD: High-performance liquid chromatography with fluorescence detection. [g]FPSE: Fabric phase sorptive extraction. [h]MCOF-SPE: Magnetic covalent organic framework-solid phase extraction. [i]MMOF: magnetic metal-organic framework.

4. Conclusions

The research findings confirm the successful utilization of hDES for extracting OTA from dried fruit samples. The hDES-based method demonstrated high efficiency and selectivity in extracting the desired analyte. Combining hDES-based VA-DLLME with UV spectrophotometry proved to be a suitable approach for OTA analysis. UV spectrophotometry, a widely available and cost-effective method, provided a straightforward and reliable means of quantification. The developed method underwent comprehensive validation, including assessments of linearity (0.4–350 ng mL^{-1}), LOD (0.12 ng mL^{-1}), LOQ, precision (3.4–4.1%), and accuracy (92.1–99.2%). The results indicated that the method met the required criteria for reliable OTA analysis in dried fruit samples. The implications of the study are significant in terms of food safety, specifically in the analysis of OTA in dried fruit samples. The developed method, which combines hDES-based VA-DLLME and UV spectrophotometry, offers an efficient and accessible approach to the detection and quantification of OTA. It ensures the quality and safety of dried fruit products. Moreover, the utilization of hDESs, derived from renewable and environmentally friendly components, reduces the ecological impact of the extraction process, aligning with the principles of green chemistry. The cost-effectiveness of the method, facilitated by the use of readily available and inexpensive starting materials for DES synthesis, makes it suitable for routine analysis in laboratory settings.

CRediT authorship contribution statement

Adil Elik: Supervision, Experimental design**. Özlem Ablak:** Investigation**. Hameed Ul Haq**: Conceptualization, Writing – review & editing**. Grzegorz Boczkaj**: Writing – review & editing**. Nail Altunay:** Investigation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105656.

References

Altunay, N., Haq, H.U., Castro-Muñoz, R., 2023a. Optimization of vortex-assisted hydrophobic magnetic deep eutectic solvent-based dispersive liquid phase microextraction for quantification of niclosamide in real samples. Food Chem., 136646

Altunay, N., Hazer, B., Lanjwani, M.F., Tuzen, M., Haq, H.U., Boczkaj, G., 2023b. Ultrasound assisted dispersive solid phase microextraction using polystyrene-polyoleic acid graft copolymer for determination of Sb (III) in various bottled beverages by HGAAS. Food Chem. 425, 136523.

Arrúa, A.A., Mendes, J.M., Arrúa, P., Ferreira, F.P., Caballero, G., Cazal, C., Fernández Ríos, D., 2019. Occurrence of deoxynivalenol and ochratoxin A in beers and wines commercialized in Paraguay. Toxins 11 (6), 308.

Cai, Y., McLaughlin, M., Zhang, K., 2020. Advancing the FDA/office of regulatory affairs mycotoxin program: new analytical method approaches to addressing needs and challenges. J. AOAC Int. 103 (3), 705–709.

Ciconová, P., Laciakova, A., Mate, D., 2010. Prevention of ochratoxin A contamination of food and ochratoxin A detoxification by microorganisms-a review. Czech J. Food Sci. 28 (6), 465–474.

Dhanshetty, M., Banerjee, K., 2019. Simultaneous direct analysis of aflatoxins and ochratoxin A in cereals and their processed products by ultra-high performance liquid chromatography with fluorescence detection. J. AOAC Int. 102 (6), 1666–1672.

Elahi, F., Arain, M.B., Khan, W.A., Haq, H.U., Khan, A., Jan, F., Boczkaj, G., 2022. Ultrasound-assisted deep eutectic solvent-based liquid–liquid microextraction for simultaneous determination of Ni (II) and Zn (II) in food samples. Food Chem. 393, 133384.

Elik, A., Fesliyan, S., Gürsoy, N., Haq, H.U., Castro-Muñoz, R., Altunay, N., 2023. An air-assisted dispersive liquid phase microextraction method based on a hydrophobic magnetic deep eutectic solvent for the extraction and preconcentration of melamine from milk and milk-based products. Food Chem. 425, 136573.

García-Pérez, E., Ryu, D., Lee, C., Lee, H.J., 2021. Ochratoxin A induces oxidative stress in HepG2 Cells by impairing the gene expression of antioxidant enzymes. Toxins 13 (4), 271.

Girma, B., Sualeh, A., 2022. Prevalence and importance of the mycotoxin and ochratoxin A (OTA) in coffee: a review. J. Genet. Environ. Resour. Conserv. 10 (2), 88–100.

Haq, H.U., Balal, M., Castro-Muñoz, R., Hussain, Z., Safi, F., Ullah, S., Boczkaj, G., 2021. Deep eutectic solvents based assay for extraction and determination of zinc in fish and eel samples using FAAS. J. Mol. Liq. 333, 115930.

Haq, H.U., Bibi, R., Arain, M.B., Safi, F., Ullah, S., Castro-Muñoz, R., Boczkaj, G., 2022. Deep eutectic solvent (DES) with silver nanoparticles (Ag-NPs) based assay for analysis of lead (II) in edible oils. Food Chem. 379, 132085.

Haq, H.U., Wali, A., Safi, F., Arain, M.B., Kong, L., Boczkaj, G., 2023. Natural deep eutectic solvent based ultrasound assisted liquid-liquid micro-extraction method for methyl violet dye determination in contaminated river water. Water Resour. Ind. 29, 100210.

Jayasinghe, G.T.M., Domínguez-González, R., Bermejo-Barrera, P., Moreda-Piñeiro, A., 2020. Ultrasound assisted combined molecularly imprinted polymer for the selective micro-solid phase extraction and determination of aflatoxins in fish feed using liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1609, 460431.

Kholová, A., Lhotská, I., Uhrová, A., Špánik, I., Machyňáková, A., Solich, P., Šatínský, D., 2020. Determination of ochratoxin A and ochratoxin B in archived Tokaj wines (vintage 1959–2017) using on-line solid phase extraction coupled to liquid chromatography. Toxins 12 (12), 739.

Lee, H.J., Kim, H.D., Ryu, D., 2023. Protective effect of alpha-tocopherol against ochratoxin a in kidney cell line HK-2. J. Food Prot. 86 (5), 100082.

Mohebbi, A., Nemati, M., Farajzadeh, M.A., Mogaddam, M.R.A., Lotfipour, F., 2022. High performance liquid chromatography-tandem mass spectrometry determination of patulin and ochratoxin a in commercial fruit juices after their extraction with a green synthesized metal organic framework-based dispersive micro solid phase extraction procedure. Microchem. J. 179, 107558.

Momotko, M., Łuczak, J., Przyjazny, A., Boczkaj, G., 2021. First deep eutectic solvent-based (DES) stationary phase for gas chromatography and future perspectives for DES application in separation techniques. J. Chromatogr. A 1635, 461701.

Momotko, M., Łuczak, J., Przyjazny, A., Boczkaj, G., 2022. A natural deep eutectic solvent-protonated L-proline-xylitol-based stationary phase for gas chromatography. J. Chromatogr. A 1676, 463238.

Moslemzadeh, M., Larki, A., Ghanemi, K., 2020. A combination of dispersive liquid–liquid microextraction and smartphone-based colorimetric system for the phenol measurement. Microchem. J. 159, 105583.

Olia, A.E.A., Mohadesi, A., Feizy, J., 2023. A fabric phase sorptive extraction protocol combined with liquid chromatography-fluorescence detection for the determination of ochratoxin in food samples. Food Anal. Methods 16 (5), 974–984.

Pinheiro, F.C., Aguirre, M.Á., Nóbrega, J.A., González-Gallardo, N., Ramón, D.J., Canals, A., 2021. Dispersive liquid-liquid microextraction based on deep eutectic solvent for elemental impurities determination in oral and parenteral drugs by inductively coupled plasma optical emission spectrometry. Anal. Chim. Acta 1185, 339052.

Ragab, M.A., El-Kimary, E.I., 2021. Recent advances and applications of microfluidic capillary electrophoresis: a comprehensive review (2017–mid 2019). Crit. Rev. Anal. Chem. 51 (8), 709–741.

Ruan, C., Diao, X., Li, N., Zhang, H., Pang, Y., Liu, C., 2016. Determination of ochratoxin A and citrinin in fruits using ultrasound-assisted solvent extraction followed by dispersive liquid–liquid microextraction with HPLC with fluorescence detection. Anal. Methods 8 (7), 1586–1594.

Scott, P.M., 2005. Biomarkers of human exposure to ochratoxin A. Food Addit. Contam. 22 (s1), 99–107.

Serra, R., Mendonça, C., Abrunhosa, L., Pietri, A., Venâncio, A., 2004. Determination of ochratoxin A in wine grapes: comparison of extraction procedures and method validation. Anal. Chim. Acta 513 (1), 41–47.

Shahvalinia, M., Larki, A., Ghanemi, K., 2022. Smartphone-based colorimetric determination of triclosan in aqueous samples after ultrasound assisted-dispersive liquid–liquid microextraction under optimized response surface method conditions. Spectrochim. Acta Part A: Mol. Biomol. Spectrosc. 278, 121323.

Wang, L., Hua, X., Shi, J., Jing, N., Ji, T., Lv, B., Chen, Y., 2022. Ochratoxin A: Occurrence and recent advances in detoxification. Toxicon 210, 11–18.

Wang, X., Yang, G., Li, F., Feng, Y., Ren, G., Han, X., 2013. Evaluation of two statistical methods for optimizing the feeding composition in anaerobic co-digestion: Mixture design and central composite design. Bioresour. Technol. 131, 172–178.

Wei, Q., Huang, C., Lu, P., Zhang, X., Chen, Y., 2023. Combining magnetic MOFs as a highly adsorbent with homogeneous chemiluminescent immunosensor for rapid and ultrasensitive determination of ochratoxin A. J. Hazard. Mater. 441, 129960.

Yan, X.L., Xue, X.X., Luo, J., Jian, Y.T., Tong, L., Zheng, X.J., 2020. Construction of chemiluminescence aptasensor platform using magnetic microsphere for ochratoxin A detection based on G bases derivative reaction and Au NPs catalyzing luminol system. Sens. Actuators B: Chem. 320, 128375.

Yang, X.S., Zhao, J., Ma, T.T., Li, Z.Y., Wang, L.L., Ji, S.L., Gong, H.S., 2023. Magnetic covalent organic framework for effective solid-phase extraction and HPLC determination of ochratoxin A in food. LWT 179, 114639.

Yazdanfar, N., Mahmudiono, T., Fakhri, Y., Mahvi, A.H., Sadighara, P., Mohammadi, A. A., Yousefi, M., 2022. Concentration of ochratoxin A in coffee products and probabilistic health risk assessment. Arab. J. Chem. 15 (12), 104376.

Zareshahrabadi, Z., Bahmyari, R., Nouraei, H., Khodadadi, H., Mehryar, P., Asadian, F., Zomorodian, K., 2020. Detection of aflatoxin and ochratoxin A in spices by high-performance liquid chromatography. J. Food Qual. 2020, 1–8.

Zhang, X., Li, M., Cheng, Z., Ma, L., Zhao, L., Li, J., 2019. A comparison of electronic nose and gas chromatography–mass spectrometry on discrimination and prediction of ochratoxin A content in *Aspergillus carbonarius* cultured grape-based medium. Food Chem. 297, 124850.

Zhou, W., Wieczorek, M.N., Pawliszyn, J., 2023. High throughput and automated solid-phase microextraction and determination by liquid chromatography-mass spectrometry for the analysis of mycotoxins in beer. Food Chem. 425, 136557.