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Molecularly imprinted polymer as sorbent in micro-solid phase extraction of ochratoxin A in coffee, grape juice and urine

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ABSTRACT

A simple, environmental friendly and selective sample preparation technique employing porous membrane protected micro-solid phase extraction (μ-SPE) loaded with molecularly imprinted polymer (MIP) for the determination of ochratoxin A (OTA) is described. After the extraction, the analyte was desorbed using ultrasonication and was analyzed using high performance liquid chromatography. Under the optimized conditions, the detection limits of OTA for coffee, grape juice and urine were 0.06 ng g^{-1}, 0.02 and 0.02 ng mL^{-1}, respectively while the quantification limits were 0.19 ng g^{-1}, 0.06 and 0.08 ng mL^{-1}, respectively. The recoveries of OTA from coffee spiked at 1, 25 and 50 ng g^{-1}, grape juice and urine samples at 1, 25 and 50 ng mL^{-1} ranged from 90.6 to 101.5%. The proposed method was applied to thirty-eight samples of coffee, grape juice and urine and the presence of OTA was found in eighteen samples. The levels found, however, were all below the legal limits.

1. Introduction

Ochratoxin A (OTA) (Fig. 1) is a mycotoxin that is produced by fungi of the genera *Aspergillus* and *Penicillium* that exhibits considerable adverse health effects to human and animals. OTA commonly occurs in sub-tropical and temperate climates and can be found in a number of food products, including cereals, beer, coffee beans, cacao, spices, dried fruits, wine and grape juice. OTA has been reported to have nephrotoxic, carcinogenic, genotoxic and immunotoxic effects. This mycotoxin is classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen (Group 2B) [1].

The European Union (EU) enacted regulatory limits for its level depending on the food: roasted coffee (5 μg kg^{-1}) or instant coffee (10 μg kg^{-1}), grape juice (2 μg L^{-1}), cereals (5 μg kg^{-1}), all products derived from cereals (3 μg kg^{-1}) and processed cereal-based foods and baby-foods (0.5 μg kg^{-1}) [2]. OTA has an unusually long serum half-life (35 days in human), due to its binding to plasma proteins, its enterohepatic circulation, and its re-absorption from urine [3]. Given its nephrotoxicity, OTA is considered to be involved in severe kidney pathology (the Balkan endemic nephropathy) and possibly in urinary tract tumors [4]. Urine is a good biomarker for OTA exposure [5]. Therefore, there is a need for fast, reliable and low-cost analytical methods for the monitoring of OTA in food and urine samples.

Enzyme-linked immunosorbent assay (ELISA) [6] was used for the determination of OTA as it is rapid, simple, specific, sensitive and portable. However, false positives because of the cross-reactions and interferences from components in the complex matrices are sometimes encountered. Several methods for the analysis of OTA in biological fluids and food have been reported, including gas chromatography (GC) [7], thin-layer chromatography (TLC) [8], capillary electrophoresis (CE) [9] and liquid chromatography (LC). To date, the most commonly used method is HPLC with fluorescence detection as low detection limits can be reached [10].

Before the chromatographic separation, sample preparation step involving extraction, purification and concentration of the extract must be carried out to remove the major interferences present in the sample and to preconcentrate the analytes in order to achieve the desired sensitivity. Clean-up and preconcentration methods for OTA including liquid–liquid extraction (LLE) or solid-phase extraction (SPE) are currently the most widely used. A number of SPE columns (e.g., C18 [11], OASIS HLB [12], ion-exchange [13], immunoaffinity [14]) are commercially available. Of these, the immunoaffinity columns (IAC) are the most commonly used. Basically the IAC procedure is based on percolating the sample or the extract onto a column filled with sorbents of immobilized antibodies against the specific mycotoxin. These columns are popular due to their high specificity. However these columns present several problems such as the rather high cost, cannot be reused, limited lifetime and in some cases lack of specificity was observed due to cross-reaction with ochratoxin C [15].

LLE provides clean extracts but in many cases, low recoveries, formation of emulsion, time consuming procedures involving

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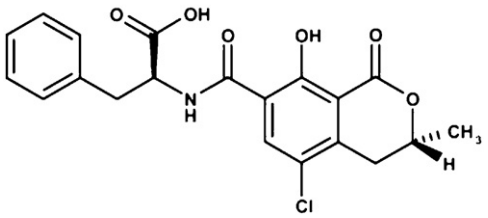

Fig. 1. Chemical structure of ochratoxin A (OTA).

large volumes of hazardous and expensive organic solvents and the difficulty to automate limit its success. Both LLE and SPE involve multistep extraction and require solvent evaporation steps where loss and/or deterioration of target analytes can be anticipated. Although the SPE technique requires moderately small volumes of organic solvents, the manual version is tedious and time consuming. Furthermore, SPE techniques rely on relatively nonselective interactions, the resultant clean-up may be insufficient for complex matrices.

The development of analytical methods that are simple, rapid and using reduced amount of solvents is of great importance and will allow more widespread mycotoxin monitoring. Solid phase microextraction (SPME) method [16,17] was proposed for the extraction of ochratoxin A. SPME allows the integration of sampling and sample extraction in a single step which eliminates or minimizes the amount of organic solvents. However, it has several disadvantages such as sample carry-over and a decline in performance with time. Other alternatives such as the hollow fiber liquid-phase microextraction (HF-LPME) [18,19] have been used. HF-LPME is effective, simple, low cost, uses minimum solvent (μL), allows the concentration of the analytes and provides excellent sample clean-up. Other methods such as the supramolecule liquid-phase miroextraction (SM-LPME) [20] and dispersive liquid–liquid microextraction (DLLME) [21] had been reported for the analysis of OTA in food.

Molecularly imprinted polymers (MIPs) have been investigated as a potential clean-up system for food analysis. The hallmark of this technique is the good selectivity feature towards a particular molecule (template). Generally, the imprinting process involves prearrangement of the functional monomers around a template molecule. The functional monomers can either be covalently linked to the template (covalent approach) or arrange themselves via non-covalent intermolecular interactions around the template molecule (non-covalent approach). Then, the spatial assembly of these interaction sites is subsequently fixed through polymerization in the presence of a cross-linking agent. After the removal of the template from the polymer, the specific binding cavities with a shape and functional groups complementary to the template are created within the polymer matrix. MIP has been extensively studied and applied as sorbent for SPE (MISPE) [22]. MIPs specifically designed for OTA have already been developed using either OTA as template or using a structural analog [23,24]. The drawbacks of these MISPE, inherent to other SPE techniques, are the multisteps that are involved [25].

Micro-solid phase extraction (μ-SPE) is an interesting alternative to the multistep SPE method for the preconcentration the analytes in complex samples [26]. The key advantages of this technique are the minimized usage of solvent, simple, inexpensive and high enrichment that can be achieved. In this paper, MIP was used as sorbent in μ-SPE to extract OTA in coffee, grape juice and urine. To the best of our knowledge, there is only one paper reported on the determination of phenolic compounds in environmental samples using this technique [27] but it has not been applied in the extraction of ochratoxins.

2. Experimental

2.1. Chemicals and materials

Ochratoxin A, sodium hydrogencarbonate (≥99.5%) were purchased from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile (99.99%), HPLC-grade methanol (≥99.96%), glacial acetic acid (≥99.5%) and hydrochloric acid (37%, w/w) were purchased from Merck (Darmstadt, Germany). Ochraprep immunoaffinity columns, phosphate buffer saline tablets and naturally ochratoxin contaminated roasted coffee were purchased from R-Bio-pharm Rhone (Glasgow, Germany). Accurel polypropylene flat sheet membrane (200 μm wall thickness, 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany). Molecularly imprinted polymer (MIP) crushed monolith (AFFINIMIP^{TM} OTA) were provided by Polyintell (Val de Reuil, France). Ultrapure water (resistivity, 18.2 MΩ cm^{-1}) was produced by a Milli-Q system (Millipore, USA), and was used throughout for the preparation of solutions.

2.2. Standard solutions

Stock solution of OTA (200 μg mL^{-1}) was prepared by dissolving crystalline standard in methanol and stored at −18 °C and protected from light. Standard solutions were prepared from appropriate dilutions of the stock solution with methanol:water (80:20, v/v).

2.3. Phosphate buffer saline

Phosphate-buffered solution (PBS) at pH 7.4 was prepared by dissolving 1 phosphate-buffered saline tablet in 100 mL water.

2.4. Samples pretreatment

2.4.1. Coffee

Coffee samples were purchased from local supermarkets and stored at ambient temperature. Samples (10 g) were mixed with 100 mL of 1% $NaHCO_3$ solution. The suspension was shaken at 200 rpm for 30 min and passed through a Whatman No. 4 paper filter. Prior to the molecularly imprinted micro-solid phase extraction (MI-μSPE) procedure, pH for 10 mL of the filtrate was adjusted to 1.5 by using 1.0 M HCl.

For the IAC clean-up procedure, the filtrate (5 mL) was diluted in phosphate buffer saline solution. The diluted extract (10 mL) was applied to an Ochraprep column under gravity.

For fortification procedure, blank ground coffee sample (10 g) was weighed into a 250 mL conical flask. The blank coffee samples were fortified with OTA working solution to achieve different concentration levels and were left in the fume cupboard overnight to allow the solvent to evaporate and proceed with MI-μSPE extraction (Section 2.6) and IAC clean-up procedure (Section 2.7).

2.4.2. Grape juice

Grape juice samples were purchased from a local supermarket. A 10 mL aliquot was adjusted to pH 1.5 by using 1.0 M HCl.

2.4.3. Urine

Human urine samples were collected from healthy individuals living in Penang, Malaysia (age 25–50). All volunteers were asked to complete a rapid questionnaire regarding their gender and age. The urine samples were diluted 1:1 with water to minimize the matrix effects. 10 mL of the diluted urine samples were adjusted to pH 1.5 by using 1.0 M HCl.

For fortification procedure for grape juice and urine samples, 10 mL of the samples were fortified with OTA working solution to achieve different concentration levels and pH was adjusted to 1.5.

2.5. Preparation of the MI-μSPE

The MI-μSPE device consists of the 15 mg of MIPs packed within an envelope made from polypropylene membrane sheet of dimension 2.0 cm × 0.5 cm (Fig. 2). The edges were heat sealed. Before use, each device was cleaned by ultrasonication in methanol for 3 min. It was then stored in methanol until use.

2.6. MI-μSPE procedure

A 10 mL aliquot of the sample solution was added to a sample vial (12 mL). A magnetic stirring bar (15 mm × 5 mm) was placed in the solution. Next, the MI-μSPE device was placed in a 10 mL sample (pH was adjusted to 1.5) that was stirred at 1000 rpm. After the extraction (30 min), the device was removed, rinsed in water, dried with lint-free tissue and placed in a 750 μL desorption vial. 250 μL methanol:acetic acid (98:2, v/v) was added and the analytes were desorbed by ultrasonication for 20 min. After desorption, the MI-μSPE device was removed from the desorption vial and the extract was injected directly into the HPLC for analysis. The μ-SPE device could be reused after cleaning with methanol.

2.7. Immunoaffinity column (IAC) clean-up procedure

The diluted extract was passed through the IAC at a flow rate of 2–3 mL min^{-1}. The column was washed with 20 mL of PBS and OTA was eluted with 1.5 mL of methanol:acetic acid (98:2, v/v) followed by 1.5 mL of deionised water. The eluate was injected directly into the HPLC unit.

2.8. HPLC conditions

A Waters Alliance (model 2695) HPLC system (Milford, MA, USA) equipped with fluorescence detector as used. The chromatographic separation was performed on Poroshell 120 EC-C18 analytical column (100 mm × 4.6 mm × 2.7 μm) (Agilent Technologies, Wilmington, DE, USA) operated at 27 °C. The mobile phase consisted of acetonitrile, water, and acetic acid (49.5:49.5:1, v/v) with the flow rate of 1.0 mL min^{-1}. OTA exhibits natural fluorescence and the detector wavelengths were set at λ_{ex} 333 nm and λ_{em} 460 nm. The data were processed using licensed PowerChrom v2 software (EDAQ, Denistone East, Australia).

3. Results and discussion

3.1. HPLC method development

An important goal of the method development is speed of analysis. In order to reduce the retention time of OTA, the influence of variables involved in the chromatographic process were studied. As OTA is a weak acid, the mobile phase must be acidic to avoid strong tailing and unspecific adsorption to the column [28]. Mixture of acetonitrile or methanol with diluted acetic acid as reported by Aresta et al. [17] and Leitner et al. [29] were tested. Acetonitrile:water was preferred as it has lower viscosity and better separation efficiency than methanol:water. Mobile phase compositions consisting of various ratios of acetonitrile:water:acetic acid (40:59:1, 45:54:1, 48:51:1, 49.5:49.5:1, v/v) were studied. Raising the acetonitrile content resulted in shorter retention time (~3.8 min) with satisfactory peak area. Thus, this composition (49.5:49.5:1, v/v) was selected for the rest of the studies. The effect of column temperatures, flow rates and injection volumes has also been carried out. As a compromise between retention times and sensitivity, the adopted conditions for the determination of OTA: mobile phase composition acetonitrile:water:acetic acid (49.5:49.5:1, v/v); column temperature, 30 °C; flow rate, 1.0 mL min^{-1}; injection volume, 20 μL. The retention time for OTA was shorter (<4 min) compared to the report of Guillamont et al. (~12 min) who used similar HPLC conditions [30].

3.2. Optimization of MI-μSPE

μ-SPE is an equilibrium-driven process, the efficiency is dependent on the partitioning of the analyte between the aqueous phase and the sorbent. Several parameters affecting the MI-μSPE efficiency were studied and optimized (e.g., type of sorbent, pH, salt addition, extraction time, stirring speed, etc.). Optimization was carried out by triplicate analysis with 50 ng mL^{-1} OTA.

3.2.1. Mass of sorbent

The amount of MIP sorbent material was varied from 5 to 20 mg. It was found that with increasing sorbent amount, higher extraction efficiency was observed. However, when more than 15 mg of MIP was used, no additional enhancement was found. Thus, 15 mg of sorbent was used in all experiments.

3.2.2. Effect of pH

OTA is a weak acid, with pK_a values of 4.4 for the carboxylic and 7.3–7.05 for the phenolic groups [18]. Therefore, the pH of the sample solution should be adjusted to be acidic to promote its extraction, as under neutral and alkaline conditions it is present predominantly in the dissociated form. In this study, the pH of the sample solutions was varied from 1 to 3 by the addition of hydrochloric acid (1.0 M). It was found that the optimum pH for OTA extraction was pH 1.5. Therefore, further experiments were carried out at this pH.

3.2.3. Effect of salt addition

Generally, the addition of sodium chloride decreases the solubility of analytes in the sample solution by increasing the ionic strength (salting out effect) and subsequently enhances the extraction efficiency. The effect of salt was determined by adding sodium chloride (NaCl) from 5 to 30% (w/v) to the sample solutions. It was found that the extraction efficiency decreased as the salt concentration is increased (salt addition of 0, 5 and 10% resulted in extraction efficiencies of 28, 17 and 5%, respectively), probably due to the increased in viscosity of the aqueous sample, thereby impeding the mass-transfer process. The amount of time required to attain equilibrium increased due to the rate of mass transfer of the analyte from the aqueous phase to the solid sorbent [31]. Thus, all the subsequent experiments were performed without adding NaCl.

3.2.4. Effect of extraction time

The effect of extraction time was investigated since mass transfer is a time-dependent process. Extraction times between 10 and 60 min at a stirring rate of 1000 rpm were evaluated. The extraction efficiency was determined by the mass transfer of analyte from the sample solution to the sorbent during extraction [27]. The extraction efficiency increased with exposure time, and equilibrium was attained after about 30 min. Thus, the extraction time for all subsequent experiments was conducted for 30 min.

3.2.5. Effect of stirring

Fast stirring was employed to enhance the extraction efficiency since stirring permits the continuous exposure of the sorbent surface to fresh aqueous sample. The effect of stirring speeds (250–1200 rpm) was investigated. Higher extraction efficiency was obtained when the solution was stirred at 1000 rpm, so it was selected for the rest of the studies.

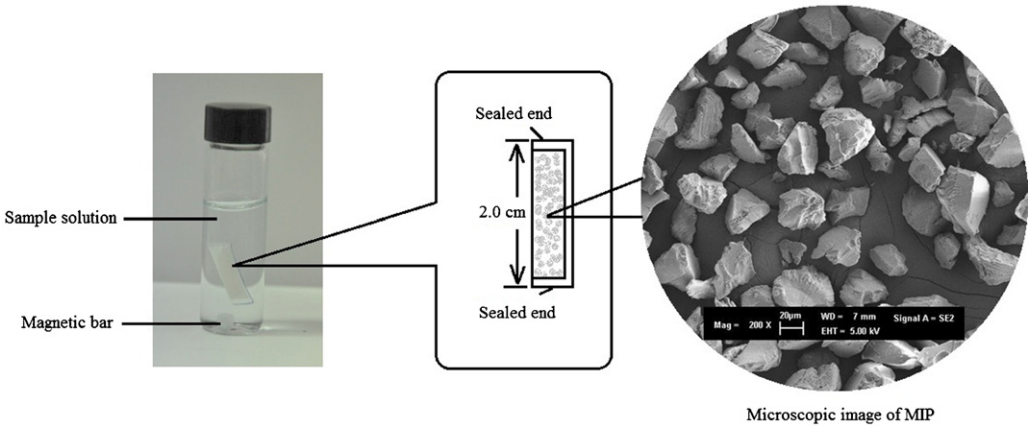

Fig. 2. Schematic diagram and microscopic image of MIP sorbent used.

3.2.6. Effect of desorption solvent, volume and time

Six desorption solvents were investigated, i.e., methanol, methanol:water (90:10, v/v), methanol:acetic acid (98:2, v/v), acetonitrile, acetonitrile:water (90:10, v/v) and acetonitrile:acetic acid (98:2, v/v). Methanol:acetic acid (98:2, v/v) gave the best peak area compared to the others and hence it was chosen as the desorption solvent for the subsequent experiments. Acetic acid is required to ensure the total elution of OTA from the MIP by disrupting the electrostatic interactions between the analyte and the monomers [32]. In fact, by using 100% methanol, lower extraction efficiency was found, thus demonstrating the necessity to add acetic acid in the desorption solvent.

Desorption experiments were carried out by using different volumes (250–500 μL) of methanol:acetic acid mixture (98:2, v/v). As expected, lower volume of solvent gave higher peak areas. Desorption volume less than 250 μL was not sufficient to immerse the μ-SPE devices during the ultrasonication but higher volume of methanol (<500 μL) caused a decrease in the peak area resulting from dilution of the analyte. Thus, 250 μL was chosen for subsequent desorptions.

The effect of desorption (ultrasonication) time (5–30 min) was investigated with methanol:acetic acid mixture (98:2, v/v). It was found that 20 min was suitable. After 20 min, there was a slight decrease in the desorption probably due to the analytes being re-adsorbed by the sorbent material. No carryover of analytes was observed under the above conditions.

3.2.7. Adopted extraction conditions

The adopted conditions were: 15 mg of MIP as the extraction sorbent; pH 1.5; without addition of salt; extraction time, 30 min at room temperature; stirring speed, 1000 rpm; desorption solvent, methanol:acetic acid mixture (98:2, v/v); desorption volume, 250 μL; desorption time, 20 min. Under these conditions, the extraction efficiency of 31% and enrichment factor of 13 was obtained. Extraction efficiency (E_e) was evaluated by the following equation [33]:

$$E_e = \frac{C_s V_s}{C_w V_w} \times 100\% \quad (1)$$

where C_s and C_w are the concentrations of analyte found in the final extract (desorbed analyte) and present in the original sample solution, respectively. V_s is the volume of the concentrated extract, and V_w is the volume of the original sample solution. Enrichment factor was calculated based on the following equation:

$$E_e = \frac{C_s}{C_w} \quad (2)$$

where C_s is the concentration of analyte in the final extract and C_w is the initial concentration of analyte in the sample solution before the extraction.

3.3. Method validation

3.3.1. Linearity, LOD and LOQ

Calibration curve of MI-μSPE for OTA standards were done by diluting appropriate volumes of the working standard solution with water into seven different concentrations (0.5–50 ng mL^{-1}). All samples were run in triplicate. The regression equations and correlation coefficients were $y = 685949x + 435015$ ($R^2 = 0.9994$). The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the equations:

$$\text{LOD} = \frac{3.3 s_a}{b}$$

$$\text{LOQ} = \frac{10 s_a}{b}$$

where s_a is the standard deviation of the intercept and b is the slope of the regression line obtained from the calibration graph. The LOD and LOQ for OTA standards were 0.02 and 0.06 ng mL^{-1}, respectively.

Matrix match calibrations were performed by spiking known amount of OTA into coffee, grape juice and urine samples that were originally free from OTA. This approach enables the assessment of possible matrix effects on the detector response. It was found that the linear range, LOD and LOQ values are similar between the matrices (Table 1), indicating that matrix components were eliminated during the MI-μSPE procedure.

3.3.2. Recovery, intra-day and inter-day precision

Recovery studies were carried out by spiking OTA to the non-contaminated coffee, grape juice and urine at different concentrations of OTA. Five replicate samples were studied at each concentration. Good recoveries were found for all samples (Table 2).

Intra-day precision (repeatability) was estimated at three concentration levels of OTA that were spiked to the samples. Inter-day precision (reproducibility) was performed by spiking to the matrix with three concentration levels of OTA and all samples were analyzed on five different days. Intra-day and inter-day precisions for peak areas, expressed as the percentage relative standard deviation, RSD, were 0.2–2.3% and 0.8–4.8%, respectively, indicating the good precision of the developed method.

Table 1
Method validation parameters obtained from the matrix match calibration.

Sample	Linear range (ng g^{-1})	Regression equation	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})
Coffee	0.5–50	$y = 198206x - 259319$	0.9987	0.06	0.19
Grape juice	0.5–50	$y = 605339x - 157685$	0.9985	0.02[a]	0.06[a]
Urine	0.5–50	$y = 654603x - 42022$	0.9984	0.02[a]	0.08[a]

[a] Expressed as ng mL^{-1}.

Table 2
Recoveries, intra-day and inter-day data of spiked coffee, grape juice and urine samples.

Spiked sample	Recovery (%) ± %RSD ($n = 9$)	Intra-day (%RSD, $n = 5$)	Inter-day (%RSD, $n = 25$)
Coffee (ng g^{-1})			
1	90.6 ± 5.7	2.3	4.8
25	97.9 ± 1.6	0.3	2.4
50	99.4 ± 2.8	0.6	1.7
Grape juice (ng mL^{-1})			
1	100.5 ± 0.9	1.0	1.1
25	101.5 ± 0.2	0.2	0.8
50	99.2 ± 3.0	0.2	3.8
Urine (ng mL^{-1})			
1	99.3 ± 2.7	0.6	1.4
25	98.6 ± 3.6	0.3	1.8
50	98.7 ± 5.2	0.9	3.0

RSD: Relative standard deviation.

Table 3
Comparison between MI-μSPE with IAC in spiked coffee samples and naturally OTA contaminated coffee sample.

	MI-μSPE	IAC
Linearity		
Linear range(ng g^{-1})	0.5–50	0.5–50
Regression equation	$y = 198206x + 259319$	$y = 248632x + 38267$
R^2	0.9987	0.9989
LOD (ng g^{-1})	0.06	0.15
LOQ (ng g^{-1})	0.19	0.45
Naturally OTA contaminated roasted coffee sample (7.1 ng g^{-1})		
Concentration obtained (ng g^{-1}) ± %RSD ($n = 6$)	7.4 ± 0.9	6.8 ± 2.5

RSD: Relative standard deviation.

3.4. Comparison of MI-μSPE with immunoaffinity column

The analytical characteristics of the newly developed method were compared to the immunoaffinity column (IAC) clean-up procedure. The methods were found to have similar sensitivity and linear range (Table 3). A naturally OTA contaminated coffee (7.1 ng g^{-1}) reference material was analyzed using both sample preparation methods before the HPLC analysis (Fig. 3). The developed method was verified against IAC method using paired *t*-test method at 95% confidence limit. The calculation showed no significant difference between the two methods. The MI-μSPE device can be reused up to fifteen times without any significant reduction in peak area. Although the IACs offer high selectivity and reproducibility and very low limits of detection for LC, the single-use immunoaffinity columns are relatively costly and the manual processing of SPE is time-consuming, entailing higher personnel costs.

3.5. Comparison with previously reported methods

The analytical characteristics of the newly developed methods were compared with the other reported methods (Table 4). The LOD and LOQ values obtained are lower compared to an earlier HPLC work that was developed for coffee and wine [15,18] using SPME and LPME as sample preparation. It is also interesting to note that the sensitivity of the proposed method (reflected in LOD and LOQ) is at least comparable [21] or even better [5] than the more expensive LC–MS/MS methods.

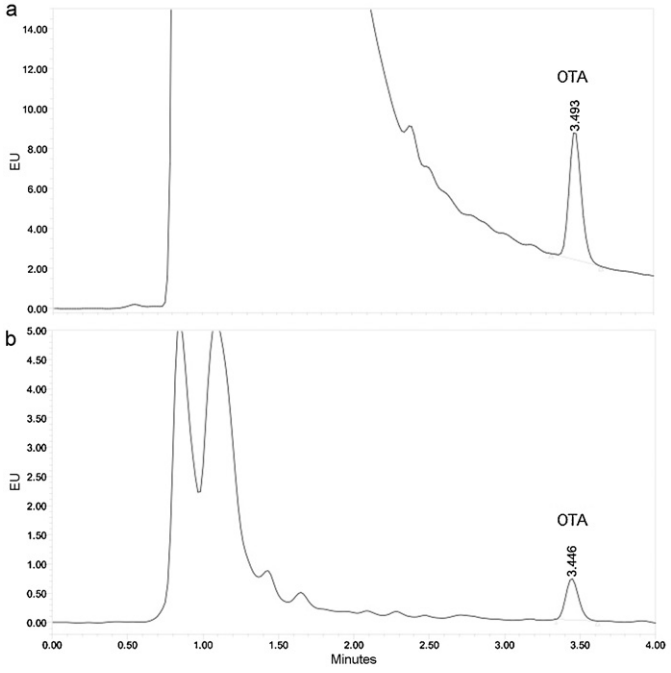

Fig. 3. Comparison of the elution fractions obtained with the (a) MI-μSPE and (b) IAC after the extraction of naturally OTA contaminated coffee (7.1 ng g^{-1}).

Table 4
Comparison of the developed method with the previous study for the determination of OTA.

Instrument	Sample preparation	Type of sample	Linear range ($ng\,mL^{-1}$/$ng\,g^{-1}$)	LOD ($ng\,mL^{-1}$)	LOQ ($ng\,mL^{-1}$)	Repeatability (% RSD)	Recovery (%)	Ref
HPLC-FL[a]	MI-μSPE[c]	Standard	0.5–300	0.02	0.06	–	–	Current work
		Coffee	0.5–50	0.06	0.19	<2.3 ($n=5$)	91–99	Current work
		Grape juice	0.5–50	0.02	0.06	<1.0 ($n=5$)	99–101	Current work
		Urine	0.5–50	0.02	0.08	<0.9 ($n=5$)	96–99	Current work
	SPME[d]	Coffee	2–32	0.30	2.00	<3.3 ($n=5$)	–	[15]
	HF-LPME[e]	Wine	0.25–10	0.20	0.25	<7 ($n=3$)	74–79	[18]
LC–MS/MS[b]	SPME	Urine	0.7–50	0.30	0.70	<14.3 ($n=5$)	91–109	[5]
	DLLME[f]	Wine	0.0025–400	0.005	0.015	<5.8 ($n=6$)	97–102	[21]

[a] High performance liquid chromatography coupled with fluorescence detector.
[b] Liquid chromatography–mass spectrometry.
[c] Molecular imprinted polymer micro-solid phase extraction.
[d] Solid phase microextraction.
[e] Hollow fiber liquid-phase microextraction.
[f] Dispersive liquid–liquid microextraction.

Table 5
OTA concentrations in samples analyzed.

Sample analyzed	No. of positive sample	OTA found[b] ($ng\,g^{-1}$)
Coffee		
Roasted coffee (8)[a]	3	nd–4.43
Instant coffee (5)	5	1.58–9.82
Grape Juice		
White grape juice (5)	5	0.35–0.59[c]
Red grape juice (6)	5	nd–1.05[c]
Urine		
Male urine (7)	0	nd
Female urine (7)	0	nd

nd: not detected.
[a] Number of sample.
[b] Range of OTA detected.
[c] Expressed as $ng\,mL^{-1}$.

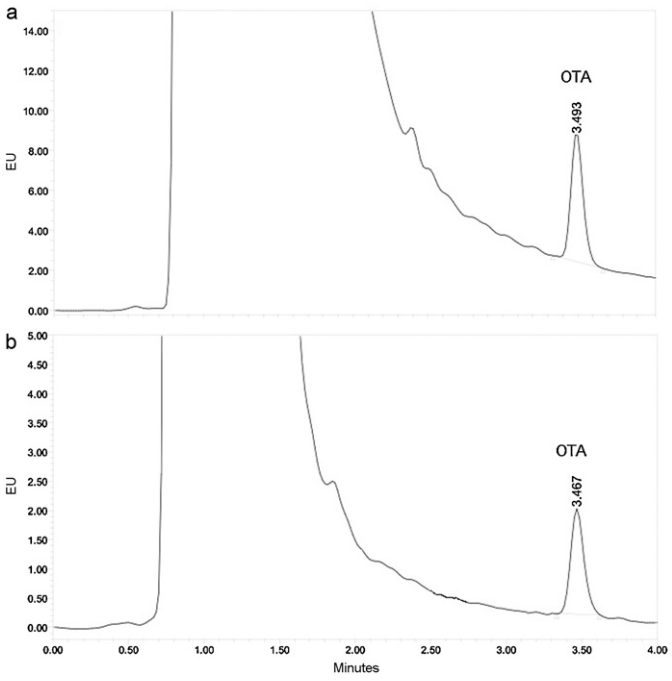

Fig. 4. Typical chromatograms of (a) coffee and (b) grape juice subjected to the MI-μSPE.

3.6. Analysis of real samples

The developed method was applied to the determination of OTA in coffee, grape juice and urine samples. Among the thirteen coffee samples analyzed, three roasted coffee samples and five instant coffee samples were contaminated with OTA (62%). The levels of OTA in roasted coffee ranged from 0.71 to 4.43 $ng\,g^{-1}$ (Table 5) and in instant coffee ranged from 1.58 to 9.82 $ng\,g^{-1}$.

OTA was detected in all the white grape juice samples, ranging from 0.35 to 0.59 $ng\,mL^{-1}$, while five red grape juice samples were detected with OTA ranging from 0.35 to 1.05 $ng\,mL^{-1}$. No OTA were detected in all the fourteen urine samples analyzed. The levels of OTA for the contaminated samples did not exceed the legal limits established by the European Union (5 $ng\,g^{-1}$ for roasted coffee, 10 $ng\,g^{-1}$ for instant coffee and 2 $ng\,mL^{-1}$ for grape juice). The chromatograms obtained (Fig. 4) indicate that the OTA peaks are not interfered by other matrix components.

4. Conclusions

MI-μSPE method for the extraction of OTA has been developed for the first time and was used in the analysis of coffee, grape juice and urine. The method exploits the high specificity of the MIP and the simplicity of the μ-SPE and shows considerable promise as it offers at least comparable or better sensitivity than the LC–MS/MS methods [5,21]. In view of its simplicity, reusability and minimal usage of solvent, the proposed MI-μSPE method can be an interesting alternative sample preparation over the commonly used IAC procedure.

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References

[1] IARC, Monograph on the Evaluation of Carcinogenic Risks to Humans, Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins; International Agency for Research on Cancer, Lyon, France, vol. 56, 1993, p. 489.
[2] Commission Regulation (EC) 105/2010 of 5 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A. Off. J. Eur. Communities, L 35/8.
[3] A. Pfohl-Leszkowicz, R.A. Manderville, Nutr. Food Res. 51 (2007) 61.
[4] A. Pfohl-Leszkowicz, T. Petkova-Bocharova, I.N. Chernozemsky, M. Castegnaro, Food Addit. Contam. 19 (2002) 282.
[5] R. Vatinno, D. Vuckovic, C.G. Zambonin, J. Pawliszyn, J. Chromatogr. A 1201 (2008) 215.
[6] D. Flajs, A.M. Domijan, D. Ivic, B. Cvjetkovic, M. Peraica, Food Control 20 (2009) 590.
[7] G.J. Soleas, J. Yan, D.M. Goldberg, J. Agric. Food Chem. 49 (2001) 2733.
[8] A. Pittet, D. Royer, J. Agric. Food Chem. 50 (2002) 243.
[9] S. Almeda, L. Arce, F. Benavente, V. Sanz-Nebot, J. Barbosa, M. Valcárcel, Anal. Bioanal. Chem. 394 (2009) 609.
[10] S. Quintela, M.C. Villarán, I.L.D. Armentia, E. Elejalde, Food Chem. 126 (2011) 302.
[11] J.M. Sáez, A. Medina, J.V. Gimeno-Adelantado, R. Mateo, M. Jiménez, J. Chromatogr. A 1029 (2004) 125.
[12] L.K. Sørensen, T.H. Elbæk, J. Chromatogr. B 820 (2005) 183.
[13] M. Reinsch, A. Töpfer, Á. Lehmann, I. Nehls, Anal. Bioanal. Chem. 381 (2005) 1592.
[14] J. Feizy, H.R. Beheshti, S.F. Janati, N.K. Fahim, Food Addit. Contam.: Part B 4 (2011) 67.
[15] R. Vatinno, A. Aresta, C.G. Zambonin, F. Palmisano, J. Chromatogr. A 1187 (2008) 145.
[16] R. Vatinno, A. Aresta, C.G. Zambonin, F. Palmisano, J. Pharm. Biomed. Anal. 44 (2007) 1014.
[17] A. Aresta, R. Vatinno, F. Palmisano, C.G. Zambonin, J. Chromatogr. A 1115 (2006) 196.
[18] E. González-Peñas, C. Leache, M. Viscarret, A. Pérezde Obanos, C. Araguás, A. López de Cerain, J. Chromatogr. A 1025 (2004) 163.
[19] R. Romero-González, A.G. Frenich, J.L.M. Vidal, M.M. Aguilera-Luiz, Talanta 82 (2010) 171.
[20] S. García-Fonseca, A. Ballesteros-Gómez, S. Rubio, D. Pérez-Bendito, J. Chromatogr. A 1217 (2010) 2376.
[21] L. Campone, A. Piccinelli, L. Rastrelli, Anal. Bioanal. Chem. 399 (2011) 1279.
[22] F.G. Tamayo, E. Turiel, A. Martín-Esteban, J. Chromatogr. A 1152 (2007) 32.
[23] J. Jodlbauer, N.M. Maier, W. Lindner, J. Chromatogr. A 945 (2002) 45.
[24] J.C.C. Yu, E.P.C. Lai, Toxins 2 (2010) 1536.
[25] N.M. Maier, G. Buttinger, S. Welhartizki, E. Gavioli, W. Lindner, J. Chromatogr. B 804 (2004) 103.
[26] S. Kanimozhi, C. Basheer, K. Narasimhan, L. Liu, S. Koh, F. Xue, M. Choolani, H.K. Lee, Anal. Chim. Acta 687 (2010) 56.
[27] Q. Feng, L. Zhao, J.M. Lin, Anal Chim. Acta 650 (2009) 70–76.
[28] H. Valenta, J. Chromatogr. A 815 (1998) 75.
[29] A. Leitner, P. Zöllner, A. Paolillo, J. Stroka, A. Papadopoulou-Bouraoui, S. Jaborek, E. Anklam, W. Lindner, Anal. Chim. Acta 453 (2002) 33.
[30] E.M. Guillamont, C.M. Lino, M.L. Baeta, A.S. Pena, M.I.N. Silveira, J.M. Vinuesa, Anal. Bioanal. Chem. 383 (2005) 570.
[31] C. Basheer, G.C. Han, M.H. Toh, K.L. Hian, Anal. Chem. 79 (2007) 6845.
[32] W.H. Ali, D. Derrien, F. Alix, C. Pérollier, O. Lépine, S. Bayoudh, F. Chapuis-Hugon, V. Pichon, J. Chromatogr. A 1217 (2010) 6668.
[33] O. Sae-Khow, S. Mitra, J. Chromatogr. A 1216 (2009) 2270.