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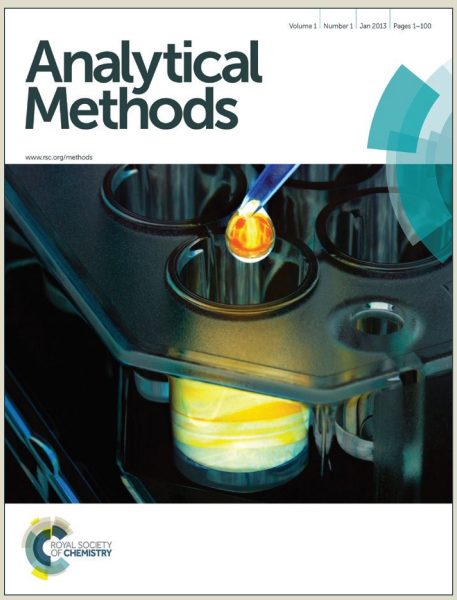

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Determination of ochratoxin A and citrinin in fruits using ultrasound-assisted solvent extraction followed by dispersive liquid-liquid microextraction with HPLC with fluorescence detection

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Abstract

A novel analytical method was developed for simultaneous determination of ochratoxin A and citrinin in fruit samples using ultrasound-assisted extraction (USAE) combined with dispersive liquid-liquid microextraction (DLLME), followed by high-performance liquid chromatography with fluorescence detection (FLD). Fruit samples were first extracted with 1% acetic acid in acetonitrile by USAE, and after centrifugation, the upper phase (acetonitrile) was used as the dispersant solvent in the subsequent DLLME step. $CHCl_3$ was used as the extraction solvent in the DLLME procedure. The experimental parameters controlling the performance of DLLME (sodium chloride percentage, sample pH, volume of extraction and disperser solvent), were optimized by means of an experimental design. To determine the presence of a matrix effect, calibration curves for standards and fortified fruit extracts (matrix matched calibration) were studied. Under optimum conditions, the mean recovery values of ochratoxin A and citrinin from three fruit samples were in the range of 75.0-103.0% (except for citrinin in apple) , with relative standard deviations lower than 5.3%. Limits of detections (LODs) were in the range 0.06-0.16 μg kg^{-1}. The proposed method was also applied for the analysis of ochratoxin A and citrinin in fifteen fruit samples purchased from markets in Guangzhou, China and no samples were contaminated with the two mycotoxins. The results show that UASE-DLLME combined with HPLC-FLD is a fast and simple method of determining of ochratoxin A and citrinin in fruit samples.

Keywords: Ochratoxin A; Citrinin; Ultrasound-assisted solvent extraction;

Dispersive liquid-liquid microextraction; Fruit samples; HPLC-FLD

Introduction

Ochratoxin A (OTA) is a toxic secondary metabolite produced mainly by several species of *Aspergillus* (*Aspergillus carbonarius*) and *Penicillium* (*Penicillium verrucosum*) molds.[1-3] OTA is commonly found in cereals and their products, dried fruits, spices, beer and wine [4-8] and is classified as Group 2B, possibly carcinogenic to humans. [9] Citrinin (CIT) is a secondary metabolite produced by several fungal species, including *Penicillium* and *Aspergillus.* [10,11] Toxicity studies have shown that CIT has nephrotoxic, hepatotoxic, immunotoxic, and carcinogenic properties.[12] CIT commonly contaminates grains, food and feedstuffs, such as wheat, corn, rice and fruit juices.[13-16] CIT is also frequently found in foods and feeds in combination with OTA.[10,13,17,18] Research has shown that some toxigenic fungi isolated from fresh fruits, could produce OTA and CIT.[19, 20] Therefore, an analysis and assessment of the OTA and CIT levels in fresh fruits should be conducted, and we must develop rapid and sensitive analytical methods of detecting OTA and CIT in fresh fruit samples.

To date, several sample treatment methods used for the determination of OTA or/and CIT in foods have been developed, such as liquid-liquid extraction (LLE) , [21] solid-phase extraction (SPE) ,[22] solid-phase microextraction (SPME) ,[23] matrix solid-phase dispersion (MSPD) ,[24] ultrasonic extraction [25] and QuEChERS extraction.[26] In recent years, a novel microextraction method known as dispersive liquid-liquid microextraction (DLLME) has been demonstrated by Aezaee and

co-workers.[27] The DLLME extraction is based on a ternary component solvent system (aqueous sample, dispersive solvent and extraction solvent). The appropriate mixture of extraction solvent (organic solvent) and dispersive solvent (water-organic miscible solvent) is rapidly injected into the aqueous sample by syringe. A cloudy solution is thereby formed. After centrifugation, the analytes are separated into the organic phase.[27, 28] To date, DLLME has been successfully applied for the determination of many mycotoxins (e.g., aflatoxins, ochratoxin A, citrinin, patulin) in various samples, such as cereals, wines, apple juices, beer, dried fruits, edible nuts and seeds, edible oils, and water.[29-39] Compared with conventional extraction methods, DLLME may be a wiser choice because of its many distinct advantages, such as its high enrichment ability, simple operation, low organic solvent consumption, high recovery and low cost.[27, 39, 40] Moreover, DLLME is not only a suitable sample preparation technique for a wide range of analytical instruments, but it can also be easily combine with most other sample preparation methods.

Ultrasound-assisted solvent extraction (UASE) is an inexpensive, simple and efficient alternative for organic compound extraction from different solid matrices that provides more efficient contact between the solid and the solvent due to increases in both pressure (favoring penetration and transport) and temperature (improving solubility and diffusivity) .[41, 42, 43] Because of this advantage, the novel UASE technique has been widely used to extract organic compounds from different matrices.[41, 44]

The objective of the present work was to present the first attempt to combine the

advantages of USAE and DLLME to develop a new pre-treatment method for the determination of OTA and CIT in fresh fruit samples by high performance liquid chromatography-fluorescence detection (HPLC-FLD). Factors affecting the DLLME procedure were optimized via an experimental design and the methodology was then validated through calibration, precision and accuracy studies in different fruits (pear, grape and apple).

Materials and methods

Chemicals and standards

The ochratoxin A (OTA) and citrinin (CIT) standards were purchased from Pribolab (Singapore) with purities greater than 98%. HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Shanghai ANPEL Scientific Instrument Corporation (Shanghai, China). Analytical-grade carbon tetrachloride (CCl_4), chlorobenzene (C_6H_5Cl), chloroform ($CHCl_3$) and dichloromethane (CH_2Cl_2) were obtained from Tianjin Xingyue Chemical Co. (Tianjin, China). Sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$) and acetic acid (AcOH) were purchased from Chinasun Specialty Products Co. LTD (Changsha, China). Ultrapure water (UNIQUE-R20 purification system with UV+UF optional accessories, Research, China) was used throughout this work. A 0.22 μm cellulose membrane filter (Sterlitech, Kent, WA, USA) was used for filtration of the stock standard solution and fruit samples.

The stock solutions of OTA and CIT were prepared at 100 mg L^{-1} in acetonitrile and

stored in amber glass vials at -20 °C. The standard curve and spiking solutions were prepared from appropriate dilutions of stock solution with acetonitrile. Working solutions were prepared immediately before use.

Sampling and sample preparation

Pear, grape and apple samples were purchased from local markets in Guangzhou city, China. The samples were crushed homogeneously and stored at -20°C until analysis. For recovery determination, 5 g fruit samples were spiked with different volumes of standard solutions and stored at room temperature for 2 h before analysis.

The mycotoxins were extracted from the fruits using ultrasound-assisted solvent extraction, followed by DLLME. In the first step, a 5 g homogenized fruit samples was weighed into a 50 mL centrifuge tube. Then, 5 mL of acetonitrile with 50 μL of acetic acid was added and extraction proceeded in an ultrasonic bath for 5 min at room temperature. To induce phase separation, 2 g of anhydrous $MgSO_4$ and 0.5 g of NaCl were added. The tube was closed and immediately shaken vigorously on a vortex mixer for 1 min. Centrifugation was performed at 3000 rpm for 5 min, and the supernatant (ACN extract) was filtered through filter paper (Whatman No 44) and used as dispersant solvent in the subsequent DLLME step. In the DLLME step, 361 μL of $CHCl_3$ (extract solvent) was added to 788 μL of ACN extract (disperser solvent), and the mixture was rapidly injected via syringe into 5 mL of deionized water (4.9% NaCl, pH 4.5) and placed in a conical bottomed glass centrifuge tube, and the tube was vortex-shaken for 1 min. After centrifugation for 5 min at 3000 rpm, the

sedimented $CHCl_3$ phase was quantitatively transferred to a small vial and blown to dryness under a mild nitrogen stream. The dried extract was redissolved with 100 μL of ACN and transferred into a vial, and an aliquot of 20 μL was injected into the liquid chromatography via an autosampler.

HPLC-FLD analysis

The chromatographic HPLC system (Agilent 1260 series, Germany) was equipped with a quaternary pump, an automatic sample injector, a degasser, and a fluorescence detector. HPLC separations were performed in a KR100-10 C_{18} column (5 μm, 150 mm×4.6 mm, Kromasil Limited). The mobile phases A and B were acetonitrile and 2% (v/v) acetic acid in water, respectively, operating under gradient elution. For the pear sample, the optimized program consisted of an isocratic step with a 50:50 A: B mixture for 14 min, and a linear gradient to 90% A over 2 min, which was held for 3 min. Finally, the initial conditions were re-established over 5 min and held for 7 min. For the grape and apple samples, the initial mobile phase was 45:55 A: B. The elution was isocratic for the first 12 min, and then changed to 55:45 A: B over 1 min and maintained for 4 min. Then, the composition was changed to 90:10 A: B over 2 min and maintained for 2 min, and returned to the initial composition over 5 min. The flow-rate was set at 0.5 mL/min. The temperature of the column was 30℃， and the injection volume was 20 μL. The detection wavelengths of citrinin were 331 nm (excitation) and 500 nm (emission) from start to 12 min (pear sample) or 13 min (grape and apple samples), respectively, and were changed to 333 nm (excitation) and 460 nm (emission) for OTA from 12 to 31 min (pear sample) and from 13 to 32 min

(grape and apple samples), respectively.

Results and Discussion

Optimization of extraction solvent during sonication stage

The extraction solvent during the sonication stage must also play the role of the disperser solvent during the DLLME stage. Therefore, acetonitrile, methanol and acetone, possessing this ability, were selected as extracting solvents for the sonication stage and the effect of these solvents on the extraction of OTA and CIT from fruits was investigated. Following a report by Hackbart and coworkers,[45] 1% acetic acid was added to the extract solvents in our experiments. The experiments were performed in triplicate using a pear sample spiked with OTA and CIT at a concentration of 5.0 μg kg^{-1}. A total of 5 g of spiked pear samples was extracted with 5 mL of the three extraction solvents for 5 min using an ultrasonic cleaner. Then, 2 g of anhydrous $MgSO_4$ and 0.5 g of NaCl were added and shaken vigorously by hand. After centrifugation, the upper phase was directly analyzed by HPLC-FLD to investigate the effect on the extraction recovery (ER). The results indicated that ER was higher from when using acetonitrile than when using acetone or methanol (Figure 1A). The effect of acetic acid content (0, 0.5, 1 and 1.5% in ACN) on extraction recovery was examined and the best ER was obtained with 1.0% acetic acid in acetonitrile (Figure 1B). Therefore, 1% acetic acid in acetonitrile was selected as the extraction solvent for the sonication stage and as the disperser solvent for the DLLME procedure.

DLLME optimization

In the DLLME procedure, the disperser solvent must solubilize the extraction solvent and should also be miscible in water to allow the formation of droplets of the extraction solvent in the aqueous sample. For this reason, 1% acetic acid in acetonitrile, which was used as the extraction solvent in the sonication stage, was selected as the disperser solvent. The extraction solvent during DLLME must have relatively high density, lower solubility in the aqueous phase and greater extraction capacity for the analytes. According to the literature,[27, 28, 39] halogenated solvents, including CCl_4, $CHCl_3$, CH_2Cl_2, and C_6H_5Cl, are usually selected as extraction solvents. The results showed that $CHCl_3$ provided the highest ER (recoveries of 62.6% and 56.3% for OTA and CIT, respectively). Thus, $CHCl_3$ was selected as the DLLME extraction solvent.

Preliminary experiments

Once the extraction and dispersion solvents were selected, preliminary experiments were performed in duplicate to test the effect of the individual variation in the amount of NaCl, the sample pH, and the volumes of acetonitrile as the dispersion solvent and $CHCl_3$ as the extraction solvent in the DLLME procedure, and to select the levels of the factors in the experimental design (see Figure 2). Initially, 5 mL of deionized water (5.0 μg kg^{-1} of OTA and CIT) with different NaCl amounts (0, 4, 6 and 10% (w/v), to induce salting-out effects) was extracted with a mixture of 250 μL of $CHCl_3$ (extraction solvent) and 1 mL of 1% acetic acid in acetonitrile (disperser solvent). As

seen in figure 2A, added 6% of NaCl provided better results for the two mycotoxins (recoveries of 68.2% and 61.2% for OTA and CIT, respectively). therefore, that level was used in subsequent experiments. Then, 5 mL of deionized water at different pH values (3, 4, 5, 6 and 7) with 6% NaCl was extracted in the same fashion. Figure 2B shows the effect of sample pH on the extraction efficiency of the two mycotoxins. The use of high pH values provided low recovery values. When the pH was 4, the extraction recoveries improved. Therefore, a solution of 5 mL of deionized water containing 6% (w/v) NaCl at pH 4 was subsequently extracted with 1 mL of 1% acetic acid in acetonitrile and different volumes of $CHCl_3$ (100, 150, 200, 250, 300, 350, 400 and 450 µL). In general, the recovery values increased as the volume of $CHCl_3$ increased (Figure 2C). The amount of acetonitrile was evaluated at values of 600, 800, 1000, 1200 and 1400 µL with a constant amount of $CHCl_3$ (350 µL). As seen in Figure 2D, approximately 800 µL of acetonitrile provided the best results.

Experimental design

After these experiments, a central composite design (CCD) was selected to optimize the four main factors (A: sample pH, B: NaCl quantity, C: volume of acetonitrile and D: volume of $CHCl_3$) affecting the DLLME extraction yield as interactions among them may also occur. According to the design, each of the four factors (A, B, C and D) was studied at five levels. For each of the four studied variables, high and low set points were constructed in an orthogonal design (Table 1). The design included six replicates of the central point. The resulting 30 experiments, in which 5 mL of deionized water was spiked with 5.0 µg kg^{-1} of OTA and CIT and submitted to the

DLLME procedure, were randomly performed. Individual recovery of the two mycotoxins and the mean recoveries were introduced separately as the response by statistical software. Eq. (1) shows the response surface methodology (RSM) model in terms of the coded values for the mean recoveries of CIT and OTA.

$$R=88.90-1.71A-0.86B+2.14C+2.07D-0.49AB-0.29AC+0.36AD+0.59BC+0.97BD+0.12CD-3.22A^2-2.84B^2-2.11C^2-1.38D^2 \quad (1)$$

where R is the mean recovery of CIT and OTA as a function of A (salt amount), B (pH), C (disperser solvent volume) and D (extraction solvent volume).

In addition to describing the linear effects of the factors on the response, CCD explains the interaction and quadratic effects of the variables. Analysis of variance (ANOVA) was used to evaluate the significance of each factor and interaction term (Table 2). The quality of the model equation was shown by the cooperation of determination (R^2, adjusted R^2). An R^2 of 0.9519 and adjusted R^2 of 0.9069 showed a good relationship between the experimental data and the fitted model, as well as the high predictive potential of the model. The ANOVA summary showed that the model was significant, with a p-value less than 0.0001 and an F-value of 21.19. A lack-of-fit p-value of 0.0001 implies that the lack-of-fit is not significantly associated with the pure error.

In general, when the response was studied for each mycotoxin, high volumes of $CHCl_3$ and intermediate amounts of NaCl, as well as low volumes of acetonitrile, provided high recovery values. With respect to sample pH, higher recoveries were

obtained when this parameter increased from 3 to 4. However, a compromise value must be fixed when simultaneously extracting the two mycotoxins, which is also the aim of the experimental design. Figure 3 shows the response surfaces of the extraction of the two mycotoxins, choosing mean recovery percentage as response. From figure 3, it can be clearly deduced that higher volumes of acetonitrile and $CHCl_3$, as well as intermediate-to-high-NaCl percentages and pH values, provided the highest response. In fact, the final optimum DLLME conditions were predicted as follows: 4.9 % NaCl (w/v), pH 4.5, 361 μL of $CHCl_3$ and 788 μL of acetonitrile. Several experiments were then developed under these optimum conditions, obtaining the highest extraction for the two mycotoxins. Furthermore, additional extractions were carried out by slightly varying each factor at its optimum value and it was observed that recoveries did not increase.

Finally, to test the repeatability of this procedure, five extractions of deionized water at three different concentration levels were developed at optimum conditions. The results showed that the mean recoveries ranged between 78.6% and 102.3%, with an RSD less than 2.2% for the two mycotoxins (data not shown).

Method validation

To validate the proposed method, the linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) were evaluated for pear, grape and apple samples. First, the matrix-matched calibration standards were run to compensate for the signal suppression/enhancement of the two mycotoxins in matrix solution compared with

their response in pure acetonitrile solvent. According to the literature,[46] pear, grape and apple samples have the medium or mild matrix effects for the two mycotoxins (table 3). For OTA and CIT, the calibration curves were linear, with square of regression coefficients better than 0.9987 in the concentration range of 1-40 ng mL^{-1} (table 3).

The recovery and repeatability validation experiments were conducted in pear, grape and apple matrices, at three spiking levels for OTA and CIT. These experiments were performed five times according to the sample preparation described above. The results are presented in Table 4. Mean recovery values in the range of 75.5-103.0% (except CIT in apple samples) were obtained (RSD<5.3% in all cases). The limits of detection (LODs) of the proposed method were determined at a signal-to-noise ratio of 3:1 for OTA and CIT. The LODs were between 0.06 and 0.16 μg kg^{-1}. The lowest fortified level of 0.5 μg kg^{-1} was used as the LOQ for the two mycotoxins in the three fruit samples (Table 4).

Application to real samples

With the aim of demonstrating the potential of the proposed methodology for the monitoring of OTA and CIT in pear, grape and apple samples, fifteen commercial samples (five samples for each type of fruit) purchased in local markets were analyzed. All fruit samples showed no traces of the two mycotoxins. However, a more thorough and long-term investigation of fruit-derived mycotoxins is necessary to ensure consumer safety. Figure 4 shows the chromatograms of spiked and non-spiked

pear, grape and apple samples, respectively, obtained by UASE-DLLME-HPLC-FLD. A clean separation and a good chromatogram are readily achieved without the presence of sample matrix interference.

Comparison of the proposed method with other extraction methods

The efficiency of UASE combined with DLLME is comparable with conventional techniques to extract mycotoxins from different food samples. As seen in Table 5, in the proposed method, the required sample and solvent volume are smaller, and this extraction procedure is very simple and less time consuming, and the sample handling is reduced. The LODs of the proposed method are lower than or comparable with those of other methods. All of these results indicate that the UASE-DLLME method is a fast, reproducible and simple technique that can be used for the pre-concentration of OTA and CIT from fresh fruit samples.

Conclusions

A simple and reliable UASE-DLLME method combined with HPLC-FLD was developed and optimized for the quantitative determination of trace levels of OTA in pear, grape and apple samples and CIT in pear and grape samples. The combination of UASE with DLLME enables an inexpensive sample pretreatment that ensures a high enrichment factor and low detection limits. The method is simple, precise, highly sensitive, rapid and reproducible (mean recoveries were between 75.0% and 103.0%, except for CIT in apple), with LODs with the range of 0.06-0.16 μg kg^{-1}, and uses small volumes of solvents and samples, reducing the risks to human health and to the

environment. The comparison of the calibration equations of standards and fruits extracts showed the existence of a middle matrix effect for the two mycotoxins. The applicability of the entire method was tested by analyzing fifteen commercial fruit samples. All fruit samples showed no traces of OTA and CIT. This work represents the first application of UASE-DLLME-HPLC-FLD for the analysis of OTA and CIT in fresh fruits samples.

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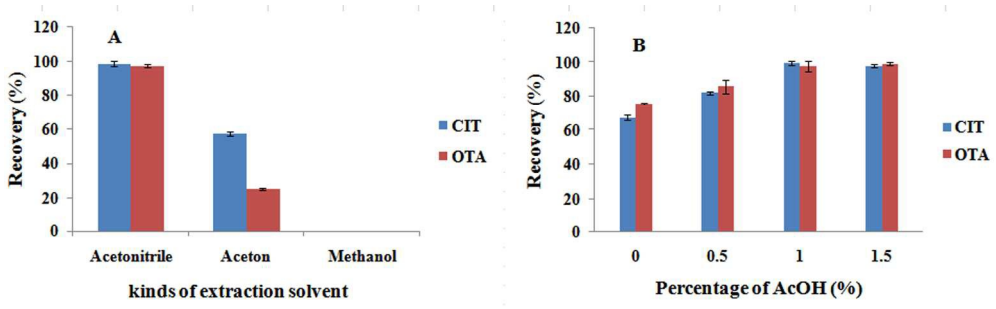
Figure captions:

Fig. 1 Effect of different types of extractant solvent (A) and percentages of acetic acid (B) on the recoveries of OTA and CIT in the sonication stage. General extraction conditions: 5 g of pear sample, sonication for 5 min. Other conditions: (A) 5 mL of 1.0 % acetic acid in every extraction solvent; (B) 5 mL of acetonitrile.

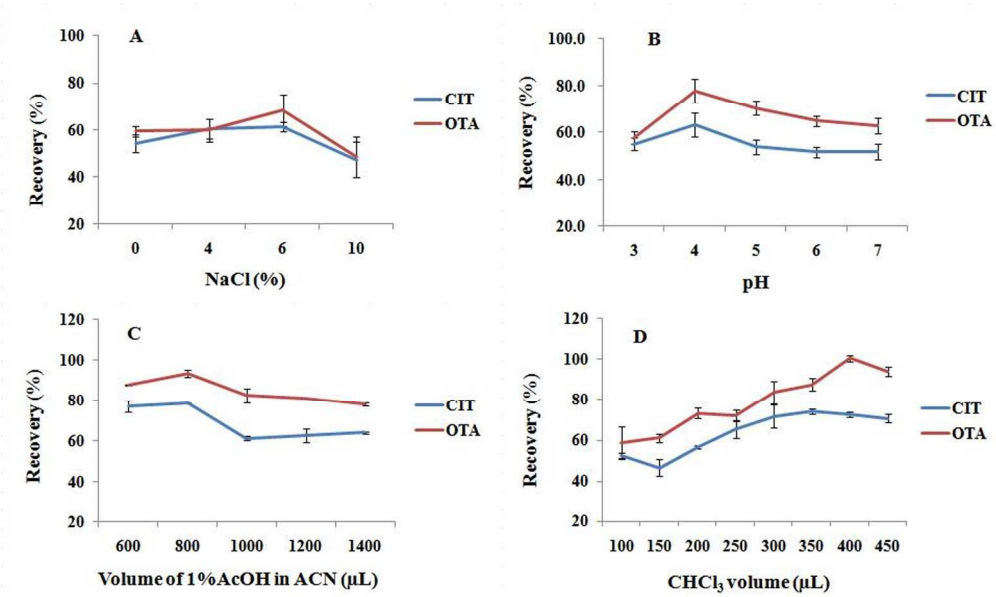
Fig. 2 Effect of variations in the DLLME procedure of (A) NaCl percentage, (B) aqueous phase pH, (C) volume of 1% acetic acid in ACN, (D) $CHCl_3$ volume on mean recoveries. General extraction conditions: 5 mL of deionized water (5.0 µg kg^{-1} of OTA and CIT) and centrifugation at 3000 rpm for 5 min. Other conditions: (A) 250 µL of $CHCl_3$ and 1000 µL of 1% acetic acid in ACN. (B) 6% (w/v) NaCl, 250 µL of $CHCl_3$ and 1000 µL of 1% acetic acid in ACN. (C) Aqueous phase pH 4, 6% (w/v) NaCl and 1000 µL of 1% acetic acid in ACN. (D) Aqueous phase pH 4, 6% (w/v) NaCl and 350 µL of $CHCl_3$.

Fig. 3 Response using the central composite design obtained by plotting: (A) pH *vs.* NaCl percentage and (B) ACN volume *vs.* $CHCl_3$ volume

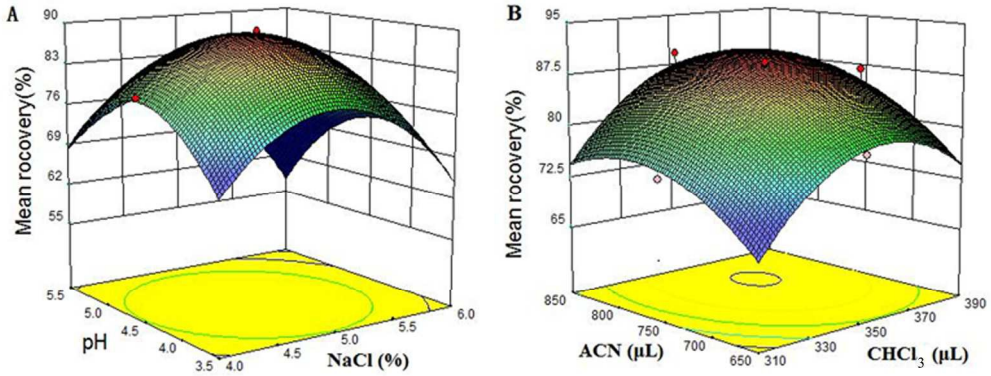
Fig. 4 The chromatogram obtained by UASE-DLLME-HPLC-FLD for pear (A), grape (B) and apple (C) under optimum conditions. Blue line: Non-spiked; Red line: spiked with 5.0 µg kg^{-1} of OTA and CIT.



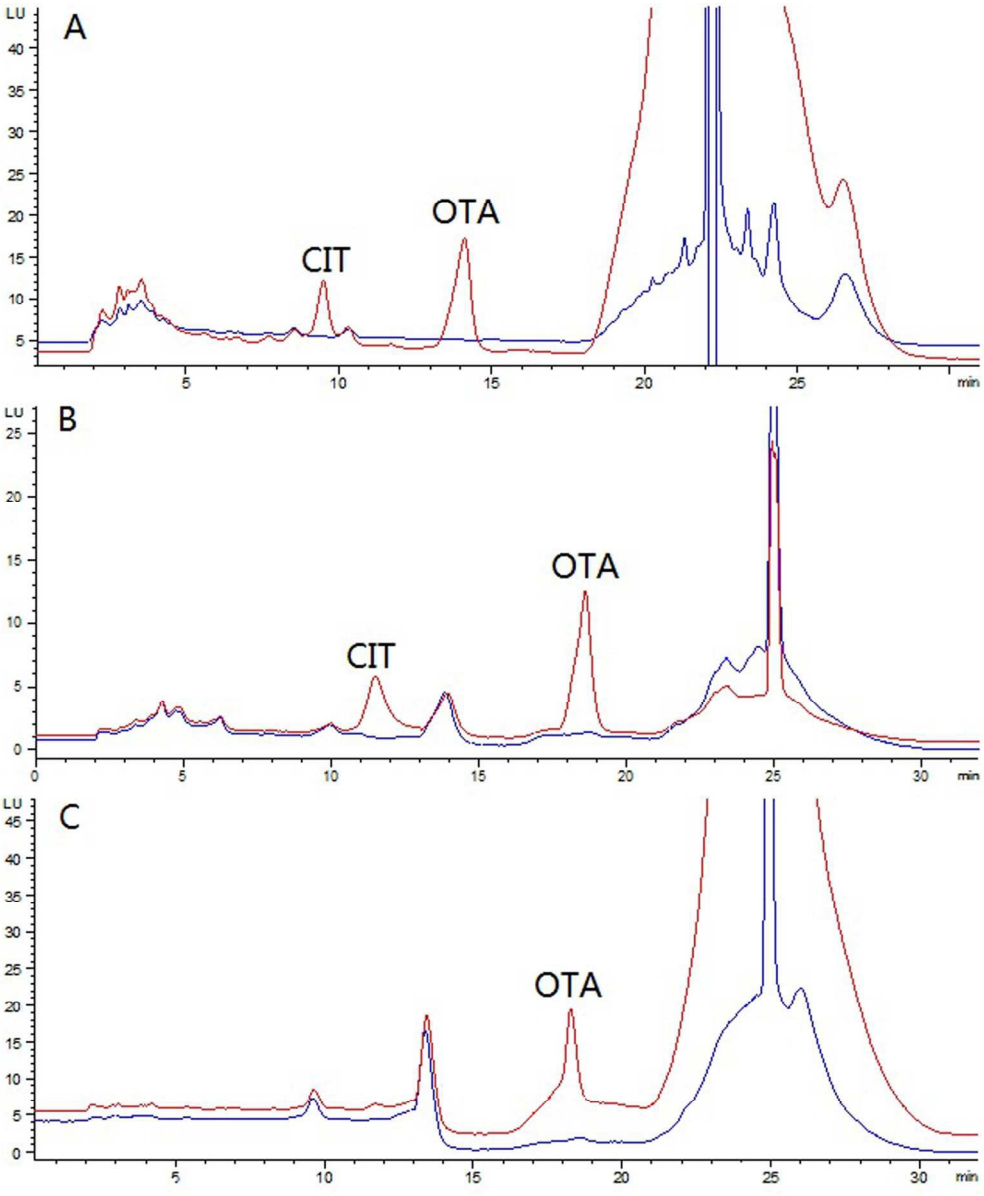
253x76mm (300 x 300 DPI)



254x152mm (300 x 300 DPI)



252x98mm (300 x 300 DPI)



170x203mm (300 x 300 DPI)

Table 1 The experimental range and levels of the variables in the CCD

Variable	Parameter	Variable levels				
		-α (low)	-1	0	+1	+α (high)
A	Salt addition (%)	4.0	4.5	5.0	5.5	6.0
B	pH	3.5	4.0	4.5	5.0	5.5
C	Volume of ACN (μL)	650	700	750	800	850
D	Volume of $CHCl_3$ (μL)	310	330	350	370	390

Table 2 Analysis of variance (ANOVA) for response surface quadratic model (OTA and CIT)

Source	Sum of squares	d.f. [a]	Mean Square	F-Value [b]	p-value [c]	Prob> F
Model	829.2315	14	59.2308	21.19	0.0001	(significant)
A- NaCl	70.2126	1	70.2126	25.11	0.0002	
B- pH	17.5959	1	17.5959	6.29	0.0241	
C-Vf	109.4401	1	109.4401	39.14	0.0001	
D-Vc	102.7134	1	102.7134	36.74	0.0001	
AB	3.8514	1	3.8514	1.38	0.2588	
AC	1.3514	1	1.3514	0.48	0.4975	
AD	2.0664	1	2.0664	0.74	0.4035	
BC	5.5814	1	5.5814	2.00	0.1781	
BD	14.9189	1	14.9189	5.34	0.0355	
CD	0.2139	1	0.2139	0.08	0.7859	
A^2	284.0777	1	284.0777	101.61	0.0001	
B^2	221.7313	1	221.7313	79.31	0.0001	
C^2	121.6209	1	121.6209	43.50	0.0001	
D^2	52.2902	1	52.2902	18.70	0.0006	
Residual	41.936875	15	2.7957917			
Lack of Fit	41.416875	10	4.1416875	39.82391827	0.0004	(significant)
Pure Error	0.5200	5	0.1040			
Cor Total	871.1684	29				

[a] Degrees of freedom.

[b] Test for comparing model variance with residual (error) variance.

[c] Probability of seeing the observed F-value if the null phyothesis is true.

Table 3 Calibration data of the UASE-DLLME-HPLC-FLD procedure for OTA and CIT in pear, grape and apple samples

Fruit	mycotoxin	Linearity (ng mL^{-1})	*S(Sa)* [a]	$R^2(Ra^2)$ [b]	Ratio (%)	Matrix effect
Pear	OTA	1-40	8.41(11.14)	0.9994(0.9994)	32.4	Medium
	CIT	1-40	8.52(5.72)	0.9993(0.9996)	-32.9	Medium
Grape	OTA	1-40	13.18(19.54)	0.9999(0.9999)	48.3	Medium
	CIT	1-40	12.46(9.30)	0.9971(0.9995)	-25.4	Medium
Apple	OTA	1-40	13.18(17.14)	0.9999(0.9987)	30	Medium
	CIT	1-40	12.46(10.35)	0.9971(0.9999)	-16.9	Mild

[a] S and R^2, slope and determination coefficient of the calibration curves obtained from ACN solution;

[b] S_a and R_a^2, slope and determination coefficient of the calibration curves obtained from matrix matched standard solutions;

Table 4 Mean recoveries, RSD values, LODs and LOQs of OTA and CIT in pear, grape and apple samples after USAE-DLLME-HPLC-FLD

Mycotoxin	Fruits	Spiked level (μg/kg)	Mean recovery	RSD (%)	LOD[a] (μg/kg)	LOQ[b] (μg/kg)	Mycotoxin	Fruits	Spiked level (μg/kg)	Mean recovery	RSD (%)	LOD (μg/kg)	LOQ (μg/kg)
OTA	Pear	0.5	94.7	1.3	0.08	0.5	CIT	Pear	0.5	75.7	0.6	0.16	0.5
		2.0	96.4	1.2					2.0	75.0	1.8		
		5.0	98.8	0.5					5.0	77.0	2.5		
	Grape	0.5	98.5	2.8	0.06	0.5		Grape	0.5	75.5	3.3	0.16	0.5
		2.0	98.3	0.6					2.0	80.1	1.9		
		5.0	95.7	1.0					5.0	80.2	0.9		
	Apple	0.5	103.0	5.3	0.10	0.5		Apple	0.5	23.1	2.2	---	---
		2.0	101.4	0.6					2.0	1.27	1.2		
		5.0	98.3	0.9					5.0	17.0	2.2		

[a] LOD, limit of detection for S/N=3.

[b] LOQ, limit of quantification for the lowest fortified level.

Table 5 Comparison of the proposed method with other methods for the determination of OTA and CIT in foods.

Methods	Mycotoxins	Matrix	Amount of samples (g)	Solvents	LOD[a] ($\mu g\ kg^{-1}$)	Recoveries (%)	Ref.
LLE-HPLC-FLD[b]	OTA CIT	Rice	20	90 mL acetonitrile，150 mL hexane，25 mL $CHCl_3$	0.08 0.11	84.1 103.0	18
IAC-HPLC-FLD[c]	OTA CIT	Olive	25	180 mL acetonitrile，55 mL hexane	0.05 0.05	88.9-95.6 92.7-96.8	47
SPME-LC-FLD[d]	OTA	Green coffee bean	0.5	9 mL $CHCl_3$	0.3	---[g]	48
QuEChERS-HPLC-FLD[e]	OTA CIT	Rice	10	20 mL acetonitrile	1.0 0.7	75.9-77.8 76.8-105.3	45
UASE-DLLME-HPLC-FLD[f]	OTA CIT	Fruits	5	5 mL acetonitrile，360 μL $CHCl_3$	0.06-0.10 0.16	94.7-103.0 75.0-80.2	Proposed method

[a] Limit of detections.

[b] Liquid liquid extraction-high performance liquid chromatography-fluorescence detector.

[c] Immunoaffinity column-high performance liquid chromatography-fluorescence detector.

[d] Solid phase microextraction-high performance liquid chromatography-fluorescence detector.

[e] The “Quick Easy Cheap Effective Rugged and Safe”-high performance liquid chromatography-fluorescence detector.

[f] Ultrasound solvent extraction-dispersive liquid-liquid micrextraction-high performance liquid chromatography-fluorescence detector.

[g] Not specified.