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Original Research Article

Ultra-high-pressure liquid chromatography–solid-phase clean-up for determining aflatoxins in Egyptian food commodities

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ABSTRACT

In this work, we determined the content of regulated aflatoxins (ATs) B1, B2, G1, and G2 in food commodities using solid-phase extraction (SPE) and ultra-high-pressure liquid chromatography with fluorescence detection without derivatization. We extracted ATs from the ground samples by mixing in NaCl and 80% (v/v) methanol. The sample was enriched and cleaned up by SPE technique using Bakerbond® C18 cartridges. The extract that we obtained was immediately analyzed using isocratic elution with a mobile phase consisting of acetonitrile, methanol and deionized water in a ratio of 64:18:18. Method validation was carried out by determining these ATs in a quality control material consisting of almond T02445QC and with the add-found test. The results provided satisfactory recovery within the range of 89.6–103.3%. Repeatability and intermediate precisions were assessed as RSD (%) which were found in the range of 1.1–11.3% and 1.5–12.0%, respectively. The limit of detection (S/N = 3) was 0.03, 0.02, 0.04, and 0.02 μg kg^{-1} for B1, B2, G1 and G2, respectively. Finally, the method was successfully applied to determine ATs in raw Egyptian food commodities, namely maize, popcorn, pistachio, corn, peanuts, chilli, wheat, green coffee and almond, and the corresponding RSD did not exceed 11%.

1. Introduction

Aflatoxins (ATs) are fungal secondary toxic metabolites that naturally contaminate food and feed (Fig. 1). They are produced by some *Aspergillus* moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Ali et al., 2005; Molina et al., 2009). *A. flavus* can produce only type B ATs; the other two fungi can produce type G ATs (Molina et al., 2009). These toxins are classified into B1, G1, B2 and G2 with a toxicity order of B1 > G1 > B2 > G2. Fungi can generally grow in wheat, rice, nuts, fruits and in living crops when stored for several days (Turner et al., 2009).

ATs can occur in both temperate and tropical regions around the world, depending on which fungal species are present. They can affect many food commodities including cereals, nuts, dried fruit, coffee, cocoa, spices, oil seeds, dried peas, beans, fruits, and, in particular, apples. The human food chain may contain ATs via meat or other animal products such as eggs, milk and cheese as a result of livestock eating contaminated feed. Not only are these ATs genotypically specific, but they could also be produced by one or more fungal species (Logrieco and Visconti, 2004), and in some cases, one species can form more than one AT (Frisvad, 1994).

AT compounds have a diverse range of both acute and chronic toxic effects (Chu, 1992; Betina, 1989). However, toxicology of ATs is a challenging and complex issue. Susceptibility to ATs varies across species and between persons depending largely on the fraction of the dose directed into the various possible pathways. Harmful "biological" exposure can result from the activity of the epoxide moiety which can react with proteins and DNA (Williams et al., 2004).

From a mycological perspective, there are qualitative and quantitative differences in the toxigenic abilities displayed by several strains within each aflatoxigenic species. About half of *A. flavus* strains can produce ATs of more than 106 μg kg^{-1} (Filazi and Sireli, 2013). The AT type B1 is the most well-known potent natural carcinogen, and it is usually the major product of *Aspergillus* strains (Kostarelou et al., 2014).

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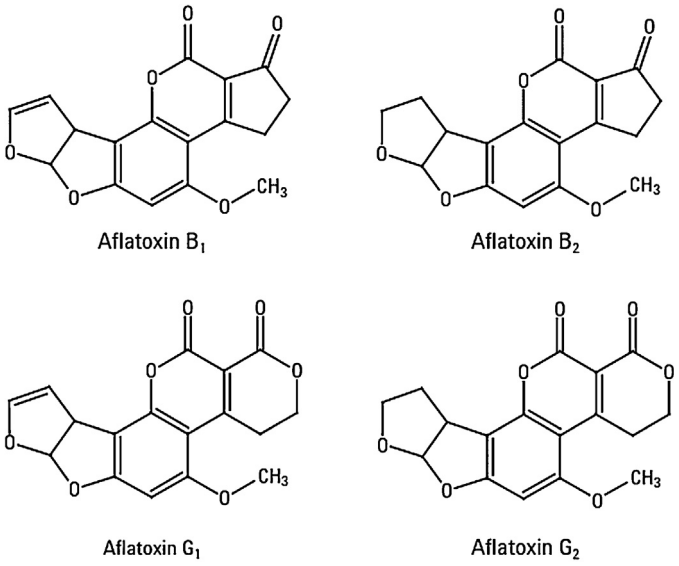

Fig. 1. Chemical structure of the studied aflatoxins B1, G1, B2 and G2.

Several techniques have been reported for the determination of Ats, such as electrochemical immune sensors (Tan et al., 2009), ion-mobility spectrometry (Sheibani et al., 2008), fluorescence spectroscopy (Nasir and Jolley, 2002), thin layer chromatography (TLC) (Stroka et al., 2000), gas chromatography (GC) (Goto et al., 1988), liquid chromatography (LC) (Sobolev and Dorner, 2002), liquid chromatography–mass spectrometry (Zhu et al., 2013), high performance liquid chromatography–fluorescence detection (Škrbić et al., 2014), and ultra-high-pressure liquid chromatography (UPLC) with UV detection (Fu et al., 2008).

High-performance liquid chromatography (HPLC) combined with fluorescence detection is the most-used technique, but in many cases, it requires further derivatization (Cavaliere et al., 2006). However, quantification by LC, TLC and HPLC is the most widely used method of research and routine analysis (Vosough et al., 2010). Recently, many analytical methods have been developed for the determination of ATs in food and feed, in particular, immunochemical and liquid chromatography coupled with mass spectrometry (LC–MS/MS) methods (Berthiller et al., 2014).

Although the HPLC technique can offer better sensitivity, high dynamic range, versatility and soft ionization conditions, it is usually coupled with UV absorption, fluorescence, mass spectrometry, and amperometric detection. In case of fluorescence detection, either with pre-column or post-column, a derivatization step is always necessary to improve fluorescence properties (Elizalde-Gonzalez et al., 1998).

Furthermore, HPLC with diode array detector (DAD) and second order iterative algorithm are useful tools for quantifying ATs after cleaning up the sample with SPE (Manetta et al., 2005). In spite of the excellent sensitivity of this technique, it often requires skilled operators, extensive sample pre-treatment and expensive equipment (Sapsford et al., 2006).

UPLC involves the use of columns packed with smaller particles, lower flow rate and an increased speed in gradient separation than normal HPLC. Using columns packed with porous particles of diameter sub 2 μm increases the number of resolved peaks per unit time, and allows superior resolution and sensitivity (Singh and Singh, 2010). The resolving power of UPLC is not compromised even at high elution speed, making it suitable for fast separations, and quantitative and qualitative analyses (Du et al., 2010).

In Egypt, the level of ATs in food commodities is a critical issue, especially when exporting agricultural products. Therefore, the development of accurate, rapid and reliable methods for determination of ATs is a challenge. In this work, we validated and applied SPE with UPLC for the fast determination of ATs in several agricultural products.

2. Experimental

2.1. Chemicals and equipments

Unless otherwise stated, all chemicals and reagents used in this work were of analytical reagent grade. AT chemicals of purity greater than 99% purity were supplied by Aldrich (Aldrich Chemical Company Inc., St Louis, MO). Standard solution (2 mg mL^{-1}) was prepared by dissolving an appropriate amount from each AT in LC grade methanol (Sigma-Aldrich, Lyon, France). Then it was stored at 4 °C in the dark. Working solutions were immediately prepared by diluting from the standard solution with 100% (v/v) methanol followed by sequential dilution with 50% (v/v) methanol and 1% (v/v) acetic acid (Sigma-Aldrich Corporation, St Louis, MO, USA).

Isopropanol (Riedel-de Haen AG, Seelze, Germany) and acetonitrile (Carlo Erba Reagenti, Val de Reuil, France) were filtered through a 0.2 μm cellulose acetate membrane (Sterlitech Corporation, Kent, WA, USA). A Milli-Q water purification system (Millipore Corporation, Saint-Quentin, France) was used to get deionized water. Micropipettes with volumes from 0.1 to 1000 μL were procured from Gilson medical electronics (Villiers le Bel, France). A Sartorius electronic balance (Sartorius Corporation, Edgewood, NY, USA) was used for accurate weighing purposes. A Waring blender of a commercial type (Waring Commercial Co., Stamford, CT, USA) was employed for grinding the solid samples.

2.2. UPLC procedure

Acquity ultra-performance UPLC™ system H-class with a fluorescence (FLR) detector (Waters Company, Milford, MA, USA) was used for determination of ATs. Chromatographic separation was carried out at 30 °C using BEH C18 column (2.1 mm × 100 mm) purchased from YMC (Kyoto, Japan) with a particle size of 1.7 μm. The excitation wavelength was fixed at 365 nm. The emission wavelength was 429 nm for B1and B2 and was 455 nm for G1and G2. Empower2 software (Waters, Milford, MA, USA) was used for system control and data processing.

Isocratic elution was carried out using a filtered and degassed mobile phase, consisting of water–methanol–acetonitrile (64:18:18), flowing at a flow rate of 0.4 mL min^{-1}. The column temperature and injection volume were adapted to 30 °C and 20 μL, respectively. Before injecting the sample, the injection needle was washed with 50% (v/v) methanol.

The weak and strong washings were performed using 1000 μL solution from water–methanol–acetonitrile (3:1:1) and 500 μL acetonitrile–isopropanol–water (5:1:1), respectively. A data rate-filter of 20 points s^{-1}, time constant of 0.3 s, and total analysis time of 6.0 min were employed.

2.3. Sample preparation

Nine food commodities, namely maize, popcorn, pistachio, corn, peanuts, chilli powder, wheat, green coffee, and almond, were received as raw samples from different regions in Egypt. An accurately weighed portion of 25 g of the dry sample was ground, then mixed with 5 g of NaCl and 100 mL of 80% (v/v) methanol. The mixture was ground in the blender for 2 min. Thereafter, it was filtered through a Whatman filter paper of 1.5 μm pore size and 11 cm diameter. An aliquot of 10 mL of the supernatant solution was vortexed with 40 mL deionized water in a falcon tube for 1 min and then shaken until homogeneity. Finally, a 10 mL aliquot from

the solution was passed through the filter paper. The resulting food extract was cleaned up by SPE technique.

2.4. SPE clean-up

Purification of the food extract was performed using Bakerbond® C18 (6 mL/1000 mg) cartridges (Baker, Deventer, Netherlands). A vacuum manifold (Baker, Deventer, Netherlands), provided with PTFE stopcocks and needles, was used for sample clean-up at a flow rate of 2 mL min^{-1}. Firstly, the cartridge was conditioned by successive passing of 6 mL methanol and deionized water. Secondly, a 10 mL aliquot of the food extract, corresponding to an extract from 0.5 g food sample, was percolated through the cartridge. Then, the liquid in the cartridge was completely removed by sucking air for 5 min. Next the cartridge barrel was covered with a positive pressure produced by a small motor to control the flow rate. After that, the cartridge was doubly washed by 10 mL deionized water to remove any matrices. ATs were eluted with 1 mL of methanol and collected in a Vicam cuvette (Milford, MA, USA) and then 1 mL aliquot from 1% (w/v) acetic acid was added. Prior to injecting the sample into UPLC, the extract was filtered through a 0.2 μm Acrodisc syringe filter containing GHP membrane (hydrophilic polypropylene), obtained from Pall Gellman (Pall Life Sciences, Port Washington, NY, USA). This protects the chromatographic column and instrumentation from particulates. Finally, the cleaned up extract was received in a 2 mL capacity vial and injected into UPLC.

2.5. Recovery, accuracy, precision, linearity and limit of detection

The recovery and accuracy were assessed by replicating analysis of a quality control material T02445QC of an almond matrix (water/nut slurry), obtained from the food and environment research agency FAPAS (Sand Hutton, York, UK). The standard addition method was examined for spiked coffee and chilli samples within a concentration range of 1.5–10.0 μg kg^{-1} ($n = 5$). Recovery was determined by comparing the peak area of blank food sample with that for a corresponding standard solution prepared by sequential dilution with methanol–1% (v/v) acetic acid (50:50). The mean value of detector response obtained from post-extracted sample was compared to that for blank food extract at the corresponding concentration. Results of the samples were established after five replicate measurements.

Accuracy was expressed as the deviation in recovery (%) from the expected value. The repeatability and intermediate precisions were determined using quality control samples of fortified maize, wheat and pistachio extracts within the concentration range 1.0–13.5 μg kg^{-1}. Precision was estimated as RSD % of five measured batches from each sample. Analysis was carried out on the same day and over five separate days for the repeatability and intermediate precision, respectively. Furthermore, the RSD % was assessed for the analytical application to real food samples after replicating measurements.

Linearity was examined by spiking the food extract with each AT compound to a concentration level between 0.01 and 500 μg kg^{-1}. The limit of detection (LOD) was defined by the concentration of AT in the food extract that gives a signal-to-noise ratio of 3:1. Moreover, the limit of quantification (LOQ) was estimated as the concentration of spiked AT corresponding to a signal-to-noise ratio of 10:1. Precision and accuracy of LOQ were considered acceptable within 20%, according to food and drug administration (FDA) guidelines (Williams et al., 2004).

2.6. Stability

Stability was assessed as recovery (%) of the extracted ATs from a yellow corn sample spiked to a concentration level of 1.3–5.0 μg kg^{-1}. Analysis was carried out after triplicate freeze–thaw cycles were made by freezing for 24 h at −20 °C, followed by cooling at 4 °C for 12 h, and finally thawing at 25 °C for 4 h.

2.7. Calibration curve and quantification

Standard solutions for calibration curve of individual AT were obtained by serial dilution of working solution with methanol to yield final concentrations of 4.0, 5.0, 6.0, 8.0, 10 and 20 μg L^{-1}. A 1.0 mL aliquot of the standard was mixed with 1 mL of 1% (w/v) acetic acid prior to UPLC analysis.

The external standard method was applied for quantitative analysis. The linearity of the standard curves was fitted by least-square regression. The standard curves were used to calculate the concentration of the quality control and the unknown samples. The correlation coefficients were at least 0.997.

3. Results and discussion

3.1. Chromatography

The proposed SPE-UPLC method enabled simultaneous quantification of these ATs in a single run. A chromatogram for standard sample of ATs is shown as in Fig. 2. A reversed-phase analytical column was successfully applied for isocratic elution with a tertiary mobile phase consisting of water–methanol–acetonitrile. The four analytes were rapidly separated, and the baseline was achieved after 5 min. Therefore, a run time of 6 min was found enough for separation of ATs from other interfering matrices. Sharp and symmetrical peaks of the analytes were observed, and the region of the chromatograms was free from any interfering peaks due to co-existing metabolites. The sequence of the peaks appeared according to the decrease in the degree of polarity.

Peaks eluted at retention time (t_R) of 2.7, 3.3, 3.7 and 4.6 min were identified as G2, G1, B2 and B1, respectively. Neither drift in retention nor overlapping of the peaks was observed. Both retention time and selectivity of separation were strongly affected by the composition of the mobile phase.

Typical chromatograms of control samples consisting of raw and spiked maize extracts with ATs (0.5 μg kg^{-1}) are shown in Fig. 3. The peaks were detected at relatively shorter times compared to those in the standard chromatogram, especially at low concentrations. They could not always be resolved from minor peaks due to interference with the matrix. Values of retention time of G2, G1, B2 and B1 in the spiked sample were 1.9, 2.1, 2.4 and 2.9 min, respectively. In addition, the chromatogram of the raw maize extract indicated the existence of weak signals at the corresponding retention time of each AT. Furthermore, separate analysis of six blank food samples revealed no endogenous peaks co-eluted with ATs, confirming the adequate selectivity of the applied procedure.

In order to quantitatively separate the analytes, an appropriate eluent should flush the UPLC column so that the matrices remain retained in the column while stripping out the analytes. Chromatograms of blank and spiked food samples have indicated that all analytes were completely separated from other detectable components in food at the selected wavelength. The contrasting peak areas recorded for the matrices during SPE step were compared with those obtained for the remaining matrices in the resulting chromatogram and it demonstrated the high efficiency of SPE cleaning up. The contrasting peaks for sample preparation were higher than those observed in the chromatograms, which conveyed good cleaning up of analyzed samples.

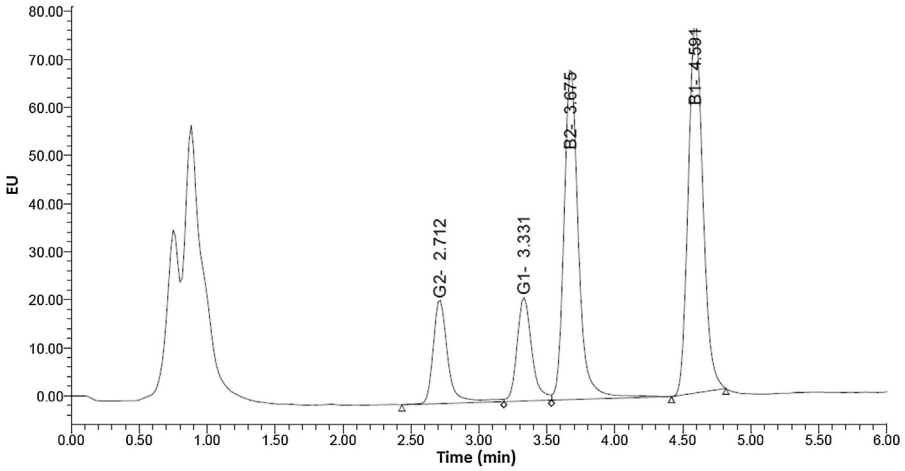

Fig. 2. Standard UPLC chromatogram of model solution containing the four ATs compounds (B1, G1, B2 and G2) at concentration level 0.4 μg kg^{-1}, injection sample volume is 10 μL, mobile phase composition: acetonitrile–methanol–water (64:18:18), flow rate 0.3 mL min^{-1}, excitation wavelength at 365 nm and emission wavelength at 455 nm.

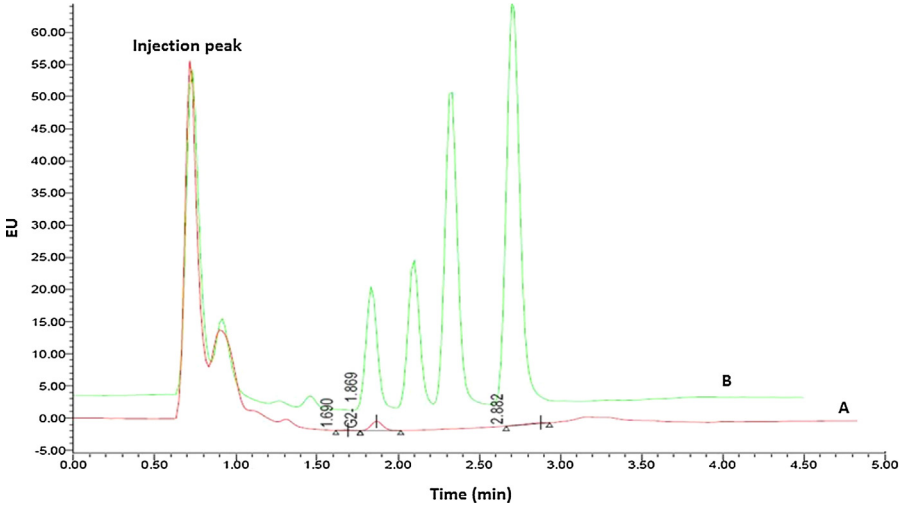

Fig. 3. Chromatograms of raw (A) and spiked (B) samples from maize extract to concentration level of 0.5 μg kg^{-1}. Mobile phase composition: acetonitrile–methanol–water (64:18:18), injected sample volume 10 μL, flow rate at 0.3 mL min^{-1}, excitation wavelength at 365 nm and emission wavelength at 455 nm.

Table 1
Recovery and accuracy results for the determination of ATs in the almond reference material and spiked coffee and chilli.

Sample	ATs	Reported (μg kg^{-1})	Found[a] (μg kg^{-1})	Recovery (%)	Total reported (μg kg^{-1})	Total found[a] (μg kg^{-1})	Total recovery (%)	Accuracy[b] (%)
T02445QC almond	B1	7.88	8.14 ± 0.05	103.3	14.9	15.1 ± 0.15	101.1	+1.1
	B2	3.57	3.51 ± 0.03	98.3				
	G1	1.82	1.76 ± 0.02	96.7				
	G2	1.70	1.65 ± 0.01	97.1				
Coffee	B1	10.0	9.72 ± 0.05	97.2	25.0	23.8 ± 0.20	95.2	−4.8
	B2	2.50	2.53 ± 0.02	101.2				
	G1	10.0	8.96 ± 0.05	89.6				
	G2	2.50	2.43 ± 0.03	97.2				
Chilli	B1	6.00	5.80 ± 0.04	96.7	15.0	14.5	96.7	−3.3
	B2	1.50	1.48 ± 0.01	98.7				
	G1	6.00	5.75 ± 0.05	95.8				
	G2	1.50	1.46 ± 0.01	97.3				

[a] The results are expressed as mean values ± S.D. based on five replicate (n = 5) determinations; confidence interval at 95% level.
[b] Accuracy (%) expressed as the deviation in recovery (%) from the expected value.

3.2. Recovery, accuracy and precision

The recovery was calculated as the ratio of the average peak area of analyte in the fortified food extract to that of a reference peak at the same concentration. The SPE enabled achievement of good recovery and enhancement of LOD since it provided appreciable matrix suppression. Results for recovery and accuracy are summarized in Table 1. The recovery values for the reference

Table 2
Precision data of spiked food extract expressed as repeatability and intermediate relative standard deviation RSD (%) calculated for five replicate analyses over 5 days.

Food sample	ATs	Fortified ($\mu g\,kg^{-1}$)	Repeatability precision ($n=5$)			Intermediate precision ($n=5$)		
			Found[a] ($\mu g\,kg^{-1}$)	Recovery (%)	RSD (%)	Found[a] ($\mu g\,kg^{-1}$)	Recovery (%)	RSD (%)
Maize	B1	13.5	14.3 ± 0.16	106	1.1	12.5 ± 0.23	93	1.8
	B2	13.5	13.0 ± 0.45	92	3.5	12.0 ± 0.50	89	4.2
	G1	10.0	9.9 ± 0.25	99	2.5	9.2 ± 0.33	92	3.6
	G2	8.5	8.6 ± 0.74	101	8.6	8.1 ± 0.12	95	1.5
Wheat	B1	1.7	1.6 ± 0.18	94	11.3	1.5 ± 0.18	88	12.0
	B2	3.8	3.3 ± 0.06	89	1.8	3.5 ± 0.28	92	8.0
	G1	2.1	2.0 ± 0.14	95	7.0	1.8 ± 0.10	86	4.3
	G2	5.0	5.2 ± 0.47	104	9.0	4.8 ± 0.32	96	6.7
Pistachio	B1	1.0	0.9 ± 0.05	90	5.6	1.0 ± 0.08	100	8.0
	B2	1.4	1.2 ± 0.07	86	5.8	1.3 ± 0.12	93	8.0
	G2	2.0	1.8 ± 0.14	90	7.8	1.7 ± 0.08	90	3.8
	G1	1.5	1.4 ± 0.09	93	6.4	1.6 ± 0.13	93	7.9

[a] The results are expressed as mean values ± standard deviation based on five replicate ($n=5$) determinations; confidence interval at 95% level.

material ranged from 96.7% to 103.3%, and the total recovery was 101.1%. The spiked coffee and chilli afforded recovery values in the range 89.6–101.2% and 95.8–98.7% and total recovery was 95.2% and 96.7%, respectively. This confirmed good accuracy of the proposed analytical method.

Accuracy measurements showed that the amounts of ATs in the reference material T02445QC, and spiked coffee and chilli were in the acceptable range. There was no significant difference between the achieved results and the reported values (Table 1). The accuracy of the total recovery ranged between −4.2% and +1.1%.

The precision of the developed method was determined in the fortified food extracts of maize, wheat and pistachio. The results obtained are shown in Table 2. The relative repeatability standard deviation for all the estimated levels was below 11.3%. Also, the intermediate precision was in the range 1.5–12.0% which is considered as satisfactory results. Higher values of standard deviation reflected the effect of the heterogeneous composition of the studied food samples. Furthermore, satisfactory recovery results for spiked maize, wheat and pistachio samples were obtained over the range 86–106%. The mean value of recovery was found to be independent on the level of fortification but it mainly varied with the type of food sample. For example, maximum recovery was observed for B1 in maize; the lowest recovery was observed for B2 in pistachio, which indicated strong matrix interference with the stationary phase in the separating column. Although the value of recovery for all ATs was consistent, precise, and reproducible; nevertheless, it was low in case of spiked food samples, which might be due to adsorption of the matrices onto the SPE stationary phase.

3.3. Stability

Stability of ATs in spiked raw yellow corn extract was investigated. A blank food extract was spiked with ATs to concentration levels of 5.0, 1.3, 5.0 and 1.3 $\mu g\,kg^{-1}$ of B1, B2, G1 and G2, respectively. The obtained recovery values are compiled in Table 3. Results showed that the recovery percentage varied over the range 92.3–98.2%. The corresponding RSD varied between 0.8% and 2.4%, which are considered acceptable results. This confirmed that no significant deterioration of ATs has occurred under the studied conditions. Also, a minor change in the retention time by an interval of ±0.2 min was observed, which conveyed good chemical stability of the examined samples.

3.4. Linearity and limit of detection

A linear correlation was obtained for spiked food with ATs within a concentration range of 0.04–500 $\mu g\,kg^{-1}$. Regression analysis between peak area (A) and AT concentration (C, $\mu g\,kg^{-1}$) revealed good linear equations, which can be represented as: $A = 2.5 \times 10^6\,C - 2.6 \times 10^5$ ($R^2 = 0.941$), $A = 7.4 \times 10^6\,C - 1.9 \times 10^5$ ($R^2 = 0.921$), $A = 8.4 \times 10^5\,C - 1.25 \times 10^5$ ($R^2 = 0.929$) and $A = 2.5 \times 10^6\,C - 7.5 \times 10^4$ ($R^2 = 0.915$) for B1, B2, G1 and G1, respectively. The obtained correlation coefficients indicated good linearity in the analytical response over the specified concentration range. The LOD was 0.03, 0.02, 0.04 and 0.02 $\mu g\,kg^{-1}$ and the LOQ was 0.10, 0.05, 0.12 and 0.05 $\mu g\,kg^{-1}$for B1, B2, G1 and G2, respectively. Therefore, coupling of SPE with UPLC technique has improved the sensitivity of the method to an adequate limit so that it allowed accurate determination of ATs in real samples.

3.5. Analysis of raw food commodities

The SPE-UPLC method described in this paper has successfully been applied to the determination of these ATs in raw Egyptian agricultural food commodities. The obtained chromatograms for raw food extracts are shown in Fig. 4. In the majority of samples, the analytes were detected at the specified retention times of standard samples. For maize sample depicted in Fig. 4B, there was no significant shift in the retention time from the reference value. Sometimes, it was slightly shifted to shorter

Table 3
Stability evaluation for aflatoxins B1, B2, G2 and G2 from fortified yellow corn samples ($n=5$) over a period of 24 h freeze-release cycles.

Food sample	ATs	Fortified ($\mu g\,kg^{-1}$)	Found[a] ($\mu g\,kg^{-1}$)	Recovery (%)	RSD (%)
Yellow corn	B1	5.0	4.90 ± 0.07	98.0	1.4
	B2	1.3	1.20 ± 0.01	92.3	0.8
	G1	5.0	4.91 ± 0.06	98.2	1.2
	G2	1.3	1.23 ± 0.03	94.6	2.4

[a] The results are expressed as mean values ± standard deviation based on five replicate ($n=5$) determinations; confidence interval at 95% level.

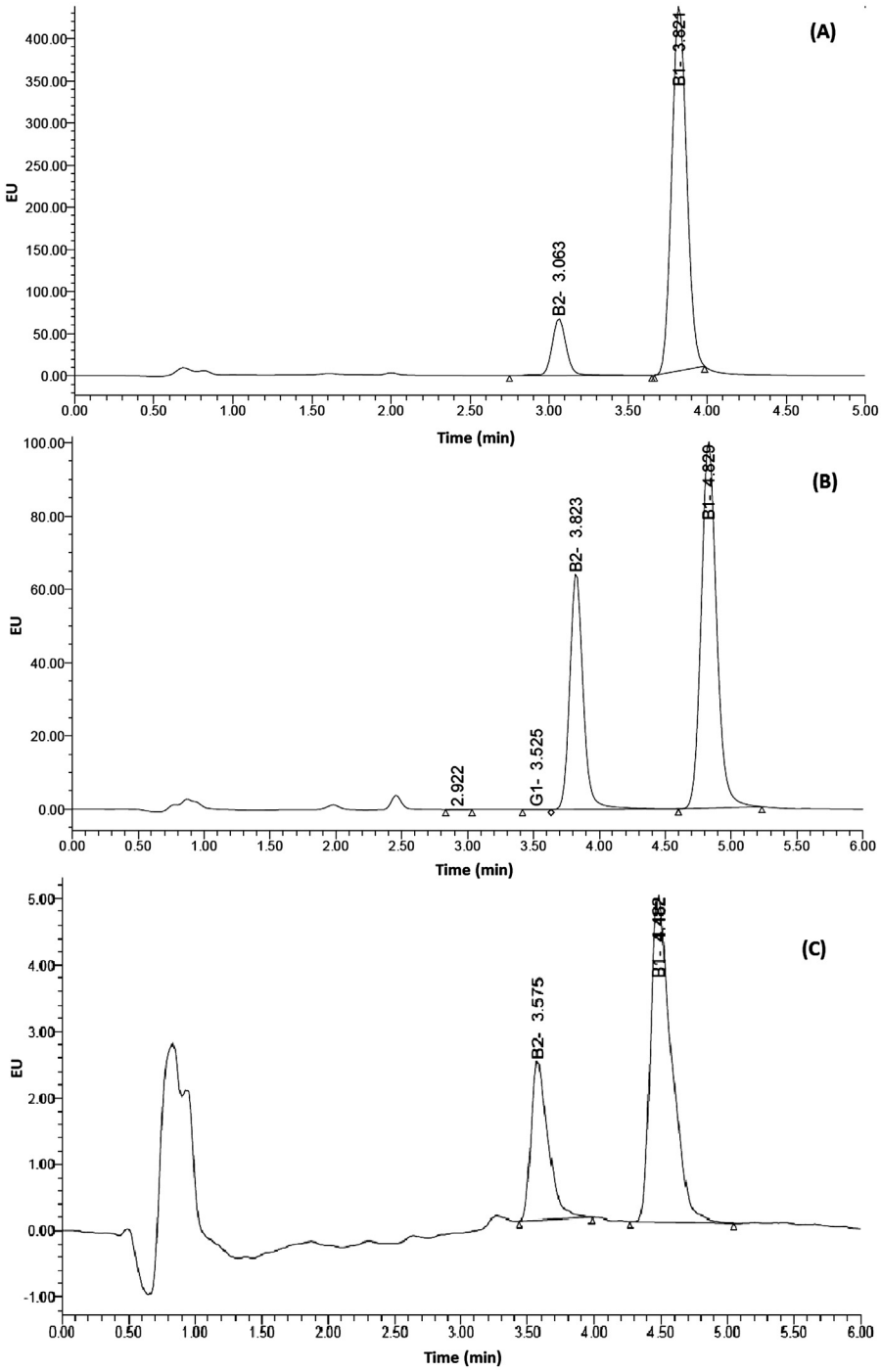

Fig. 4. Chromatograms for analysis of raw chilli (A), maize (B) and pistachio (C) samples by the proposed UPLC method.

intervals such as in case of maize, chilli and corn. For example, in the analysis of chilli (Fig. 4A), it was 3.06 and 3.82 min for B2 and B1, respectively, with a deviation from the standard chromatogram by −0.7 min. However, the strongest shift was found with pistachio (Fig. 4C). The peaks appeared about 1 min earlier than those in the reference chromatogram. The peak resolution for B1 and B2 was 0.9 min and for G1 and G2 it was 0.6 min. The sensitivity of the method towards B1 and G1 was higher than for B2 and G2.

In Table 4, the results for determination of individual and the total amounts of ATs in various food commodities are compiled. The total amount of ATs was calculated as the sum of the individual concentration of each AT. The accuracy of the results was confirmed by the calculated relative standard deviation. Most analyzed samples contain ATs type B1 and B2 while the aflatoxins G1 and G2 were detected only in maize, chilli and wheat. This explained why the fungus *A. flavus*, which produces ATs B1 and B2, is common in Egypt, but *A. parasiticus*, which produces the four types of ATs, is uncommon. The accuracy of determination was not decreased in case of real samples since there was no derivatization step involved. Finally, it was possible for a well-trained analyst to obtain up to 12 h^{-1} sample throughputs within a working day.

Table 4
Analysis of several raw Egyptian food commodities by the proposed SPE-UPLC method.

Food sample	Found (mean ± S.D)[a] (μg kg^{-1})				Total amount (mean ± S.D) (μg kg^{-1})	RSD (%)
	B1	B2	G1	G2		
Maize	1.33 ± 0.01	1.34 ± 0.04	0.99 ± 0.04	0.87 ± 0.1	3.14 ± 0.04	1.3
Popcorn	0.30 ± 0.01	0.04 ± 0.003	ND[b]	ND	0.37 ± 0.02	5.4
Pistachio	0.26 ± 0.02	0.05 ± 0.002	ND	ND	0.35 ± 0.03	8.6
Corn	307.4 ± 1.3	20.7 ± 0.092	ND	ND	333.5 ± 9.6	2.9
Peanut	2.6 ± 0.04	0.39 ± 0.033	ND	ND	2.98 ± 0.22	7.4
Chilli	2.03 ± 0.046	0.11 ± 0.013	0.12 ± 0.010	ND	2.7 ± 0.03	11.0
Wheat (I)	0.20 ± 0.011	0.07 ± 0.005	0.24 ± 0.016	0.06 ± 0.006	0.47 ± 0.04	8.5
Wheat (II)	0.26 ± 0.022	0.05 ± 0.003	0.21 ± 0.018	0.05 ± 0.003	0.29 ± 0.02	7.0
Green Coffee	0.04 ± 0.002	ND	ND	ND	0.05 ± 0.003	6.0
Almond	8.68 ± 0.03	0.51 ± 0.005	1.14 ± 0.007	0.50 ± 0.01	9.98 ± 0.17	1.7

[a] The results are expressed as mean values ± standard deviation based on five replicate ($n = 5$) determinations; confidence interval at 95% level.
[b] ND: Not detected. Value of LOD: B2, G1, and G2 is 0.02, 0.04, and 0.02 μg kg^{-1}, respectively.

4. Conclusions

We have developed a simple, rapid, and sensitive SPE-UPLC method for the simultaneous determination of Ats, namely B1, B2, G1 and G2, in several food commodities. The procedure was fully validated according to guidelines. ATs in the samples were extracted and cleaned up by the SPE cartridge and then quantified without derivatization. The LOD was suitable for real sample analysis. The present method afforded accuracy, precision, and sensitivity necessary for quantitative measurements and rapid monitoring of ATs in several food supplements.

References

Ali, N., Hashim, N., Saad, B., Safan, K., Nakajima, M., Yoshizawa, T., 2005. Evaluation of a method to determine the natural occurrence of aflatoxins in commercial traditional herbal medicines from Malaysia and Indonesia. Food Chem. Toxicol. 43, 1763–1772.

Berthiller, F., Burdaspal, P.A., Crews, C., Iha, M.H., Krska, R., Lattanzio, V.M.T., MacDonald, S., Malone, R.J., Maragos, C., Solfrizzo, M., Stroka, J., Whitaker, T.B., 2014. Developments in mycotoxin analysis: an update for 2012–2013. Review paper. World Mycotoxin J. 7, 3–33.

Betina, V., 1989. In: Betina, V. (Ed.), Mycotoxins: Chemical Biological and Environmental Aspects. Elsevier, Amsterdam, The Netherlands.

Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., Lagana, A., 2006. Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M1 in cow milk: comparison between electrospray and atmospheric pressure photoionization sources. J. Chromatogr. A 1101, 69–78.

Chu, F.S., 1992. Handbook of applied mycology. In: Bhatnagar, D., Lillehoj, E.B., Arora, D.K. (Eds.), Mycotoxins in Ecological Systems. Marcel Dekker, New York, p. 87.

Du, G., Zhao, H.Y., Zhang, Q.W., Li, G.H., Li, Y.C., Wang, X.Y., 2010. A rapid method for simultaneous determination of 14 phenolic compounds in Radix Puerariae using microwave assisted extraction and UHPLC coupled with diode array detection and time of flight mass spectrometry. J. Chromatogr. A 1217, 705–714.

Elizalde-Gonzalez, M., Mattusch, J., Wennrich, R., 1998. Stability and determination of aflatoxins by high-performance liquid chromatography with amperometric detection. J. Chromatogr. A 828, 439–444.

Filazi, A., Sireli, U.T., 2013. Occurrence of aflatoxins in food, license, InTech, pp. 143, http://dx.doi.org/10.5772/51031 (Chapter 7).

Frisvad, J.C., 1994. Classification of organisms by secondary metabolites. In: Hawksworth, D.L. (Ed.), The Identification and Characterization of Pest Organisms. CAB International, Wallingford, United Kingdom, pp. 303–321.

Fu, Z., Huang, X., Min, S., 2008. Rapid determination of aflatoxins in corn and peanuts. J. Chromatogr. A 1209, 271–274.

Goto, T., Matsui, M., Kitsuwa, T., 1988. Determination of aflatoxins by capillary column gas chromatography. J. Chromatogr. 447, 410–414.

Kostarelou, P., Kanapitsas, A., Pyrri, I., Kapsanaki-Gotsi, E., Markaki, P., 2014. Aflatoxin B1 production by *Aspergillus parasiticus* and strains of *Aspergillus* section *Nigri* in currants of Greek origin. Food Control 43, 121–128.

Logrieco, A., Visconti, A., 2004. An Overview on Toxigenic Fungi and Mycotoxins in Europe. Springer Science & Business Media, , pp. 11.

Manetta, A., Di Giuseppe, L., Giammarco, M., Fusaro, I., Simonella, A., Gramenzi, A., Formigoni, A., 2005. High-performance liquid chromatography with postcolumn derivatisation and fluorescence detection for sensitive determination of aflatoxin M1 in milk and cheese. J. Chromatogr. A 1083, 219–222.

Molina, M.A., Jiménez, J.R., Granados, J.M., de Castro, M.L., 2009. High throughput aflatoxin determination in plant material by automated solid phase extraction on-line coupled to laser-induced fluorescence screening and determination by liquid chromatography–triple quadrupole mass spectrometry. J. Chromatogr. A 1216, 1115–1125.

Nasir, M.S., Jolley, M.E., 2002. Development of a fluorescence polarization assay for the determination of aflatoxins in grains. J. Agric. Food Chem. 50, 3116–3121.

Sapsford, K., Taitt, C., Fertig, S., Moore, M., Lassman, M., Maragos, C., Shriver-Lake, L., 2006. Indirect competitive immunoassay for detection of aflatoxin B1 in corn and nut products using the array biosensor. Biosens. Bioelectron. 21, 2298–2305.

Sheibani, A., Tabrizchi, M., Ghaziaskar, H.S., 2008. Determination of aflatoxins B1 and B2 using ion mobility spectrometry. Talanta 75, 233–238.

Singh, S., Singh, A.K., 2010. Application of ultra high pressure liquid chromatography to natural products. Int. J. Ther. Appl. 3, 1–6.

Škrbić, B., Živančev, J., Godula, M., 2014. Multimycotoxin analysis of crude extracts of nuts with ultra-high performance liquid chromatography/tandem mass spectrometry. J. Food Compos. Anal. 34, 171–177.

Sobolev, V.S., Dorner, J.W., 2002. Cleanup procedure for determination of aflatoxins in major agricultural commodities by liquid chromatography. J. AOAC Int. 85, 642–645.

Stroka, J., Otterdijkb, R., Anklam, E., 2000. Immunoaffinity column clean-up prior to thin-layer chromatography for the determination of aflatoxins in various food matrices. J. Chromatogr. A 904, 251–256.

Tan, Y., Chu, X., Li Shen, G., Qin Yu, R., 2009. A signal-amplified electrochemical immunosensor for aflatoxin B1 determination in rice. Anal. Biochem. 387, 82–86.

Turner, N.W., Subrahmanyam, S., Piletsky, S.A., 2009. Analytical methods for determination of mycotoxins: a review. Anal. Chim. Acta 632, 168–180.

Vosough, M., Bayat, M., Salemi, A., 2010. Matrix-free analysis of aflatoxins in pistachio nuts using parallel factor modeling of liquid chromatography diode-array detection data. Anal. Chim. Acta 663, 11–18.

Williams, L.D., Meredith, F.I., Riley, R.T., 2004. J. Chromatogr. B 806, 311.

Zhu, Z., Liu, G., Chen, Y., Cheng, J., 2013. Assessment of aflatoxins in pigmented rice using a validated immunoaffinity column method with fluorescence HPLC. J. Food Compos. Anal. 31, 252–258.