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Simultaneous determination of bisphenol A, aflatoxin B1, ochratoxin A, and patulin in food matrices by liquid chromatography/mass spectrometry

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RATIONALE: Bisphenol A has been widely used in plastic containers and this has raised safety concerns for fetuses, infants, and young children. Aflatoxin B1, ochratoxin A, and patulin are among the most toxic regulated mycotoxins found as contaminants in agricultural crops and animal products. To facilitate the analysis of these chemicals for regulatory purposes, we have developed an analytical method enabling their simultaneous detection in beverages and food products.

METHODS: Analytes were extracted from food matrices such as cereal, peanut butter, cereal-based baby formula and fruit juices, and enriched by solid-phase extraction (SPE). Samples were analyzed by liquid chromatography/mass spectrometry using negative electrospray ionization with selected reaction monitoring, and matrix-matched external calibration was used for quantitation.

RESULTS: The method was validated by analysis of five types of food and beverage samples fortified with different levels of these analytes. The SPE clean-up and matrix-matched external calibration were critical for the success of this method. The quantitation limits for these analytes ranged from 0.08 to 2.0 ppb, and the overall recoveries of the analytical method were within 66 to 127%.

CONCLUSIONS: This quantitative method provided several advantages including minimal sample pretreatment, rapid and simultaneous analyte determination, high sensitivity and confirmatory identification. This method could be applied to a variety of food and beverages matrices where bisphenol A and these three mycotoxins may be present in suspect food products. Published 2013. This article is a US Government work and is in the public domain in the USA.

Bisphenol A (BPA) is a key monomer in the production of epoxy resins and polycarbonate plastics. Epoxy resins are used as coatings on the inside of almost all food and beverage cans. Known to be estrogenic since the 1930s, concerns about the use of BPA in consumer products were regularly reported.[1] A 2010 report from the United States Food and Drug Administration (FDA) raised further concerns about the potential effects of BPA on the brain, behavior, and prostate gland in fetuses, infants, and young children.[2] In the European Union and Canada, BPA use is banned in baby bottles.[3] In the United States, Connecticut was the first state to ban BPA from infant formula and baby food containers, as well as reusable food or beverage containers;[4] the California Legislature also passed AB 1319 (Butler, Chapter 467, Statutes of 2011) to ban BPA usage in baby bottles starting in 2013.[5] The maximum acceptable dose/tolerable daily intake for BPA were established at 50, 50, and 25 μg/kg body weight per day by the United States Environmental Protection Agency (US EPA),[1] European Food Safety Authority,[6] and Health Canada,[7] respectively.

Mycotoxins are toxic secondary metabolites produced by about 200 identified filamentous fungi, growing under a wide range of climatic conditions on agricultural commodities (grains, spices, fruits, coffee, nuts, etc.) in the field and during storage.[8,9] Their occurrence in food, beverages and feed has been recognized as a potential threat to human and animal health. Contamination is caused by contaminated plant materials and plant material products, or by carry-over of mycotoxins and their metabolites into animal tissues, milk and eggs after ingestion of contaminated feed. The toxic properties of mycotoxins in humans and animals include severe nephrotoxic, neurotoxic, carcinogenic, immuno-suppressive and estrogenic effects. Less critical compounds and chronic intake of small amounts of mycotoxins may reduce feed intake and weight gain in animals and cause diarrhea in human.[10]

Aflatoxin B1 (AFB1), ochratoxin A (OTA), and patulin are among the most toxic and common mycotoxins. They also represent a wide range of different physicochemical properties including differing molecular structures, molecular weights, pK_a, and solubilities. For AFB1, FDA has established action levels in food or feed at sub-ppm levels.[11] European Union (EU) legislation European Commission (EC) has set maximum allowable levels for AFB1, OTA, and patulin in foodstuffs.[12]

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BPA has been analyzed mainly by high-performance liquid chromatography (HPLC) with fluorescence and mass spectrometric (MS) detection,[13–16] and gas chromatography (GC) in combination with MS.[17–20] A trend towards the use of liquid chromatography/tandem mass spectrometry (LC/MS/MS) for mycotoxin analyses has been reported.[21–25] Compared to conventional HPLC with UV/fluorescence detection, and the screening methods by thin layer chromatography or enzyme-linked immunosorbent assays,[26–28] LC/MS/MS has the advantage of unambiguous analyte confirmation and high sensitivity. In addition, using the precursor ion-product ion multiple reaction monitoring mode (MRM) or selected reaction monitoring (SRM) of LC/MS/MS, base-to-base peak separation is no longer necessary for good quantification, and the application of a single analytical method for simultaneous determination of concurrent analytes is technically possible and has become a direction.[29,30]

The majority of the publications have adopted a clean-up of the samples for mycotoxins analysis, either by solid-phase extraction (SPE) using Mycosep columns,[31,32] C18-materials,[33] strong anion-exchange resins,[34] or by extraction with hexane for de-fatting and protein precipitation.[35] For BPA samples, liquid-liquid extraction using halogenated or other organic solvents, or SPE using a C18 column, have been reported.[20,36–38] The clean-up procedures generally take several steps or multiple SPE columns and require various organic solvents or chemicals.

Given the potential hazardous characteristics, the regulation for monitoring BPA and these mycotoxins, and the widespread usage of BPA in production of packaging materials for food and beverage, it is essential to develop a cost-effective analytical method which could detect both representative mycotoxins originated from agricultural commodities and BPA leached from packaging material in food/beverages. However, to our best knowledge, no reported analytical method has aimed at detecting these two types of contaminants simultaneously. Our work here is an effort to fill this gap.

In this work, simultaneous determination of BPA, AFB1, OTA, and patulin was achieved, using the Thermo Finnigan LTQ XL linear ion trap mass spectrometer coupled with an Agilent model 1200 liquid chromatograph. For sample preparation, a simple procedure was established using a single Waters Oasis SPE cartridge for clean-up and enrichment of these analytes in typical food and beverage matrices, which are prone to contamination by mycotoxins and BPA, such as grape juice, apple juice, baby formula (cereal-based), cereal, and peanut butter.

Most published LC/MS analytical methods for mycotoxins have focused on positive ionization mode. In this work, the negative ionization mode was explored and employed for all analytes due to the high sensitivities for these compounds. The acquired MS data in negative ionization mode are useful for other mass spectrometrists working in relevant fields.

EXPERIMENTAL

Reagents and chemicals

Methanol was Optima LC/MS grade (Fisher Scientific, Pittsburgh, PA, USA). Ultrapure water was obtained from a Milli-Q Gradient system (Millipore, Billerica, MA, USA). Ammonium acetate was HPLC reagent grade (J.T. Baker, Santa Ana, CA, USA). AFB1, OTA, patulin, and BPA were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Extraction and clean-up materials

Oasis HLB and MAX SPE cartridges (6 cc/500 mg) were purchased from Waters Corp. (Milford, MA, USA). The Visiprep™ SPE vacuum manifold (12-port model) was purchased from Supelco Inc. (Bellefonte, PA, USA). The Reacti-Vap™ evaporator (9-port) was obtained from Thermo-Fisher Scientific (Rockford, IL, USA). The model 5804 centrifuge was obtained from Eppendorf International (Hauppauge, NY, USA). The shaker bath was obtained from Lab-Line Instruments Inc. (Melrose Park, IL, USA).

Apparatus

Identification and quantification were performed using a Thermo Finnigan LTQ XL linear ion trap mass spectrometer equipped with a TurboIonSpray electrospray ionization (ESI) source and an Agilent 1200 Series HPLC system. Chromatographic separation was performed at 20 °C on an Agilent XDB-C18 column (150 × 4.6 mm, 5 µm).

LC/MS/MS

Mobile phases used were distilled deionized water with 10 mM ammonium acetate (mobile phase A), and methanol (mobile phase B). The following elution gradient program was applied at a constant flow rate of 0.4 mL/min: at the original time point $t = 0$ min, the ratio of A:B was 95:5; from $t = 0$ to $t = 10$ min, the ratio of A:B was linearly changed to 25:75; from $t = 10$ to $t = 14.7$ min, the ratio of A:B was held at 25:75; from $t = 14.7$ to 16 min, the ratio of A:B was further linearly changed to 5:95; from $t = 16$ to $t = 16.2$ min, the ratio of A:B was changed back to the original ratio of 95:5 linearly; from $t = 16.2$ to $t = 20$ min, the ratio of A:B was held at 95:5.

ESI-LTQ MS was performed in selected reaction monitoring (SRM) scan type in negative polarity. The activation type was collision-induced dissociation (CID), with normalized collision energy at 35%, activation time of 30 ms, and isolation width of 1. The mass range and scan rate were in normal mode. The 20 min run was divided into three MS segments, and two tune files were used. In segment 1, from 0 to 10 min, patulin was scanned by153 → 109 and 125, the tune file for patulin is used; in segment 2, from 10 to 15.1 min, two scan events, 311 → 296 and 267 (AFB1), and 402 → 358 and 270 (OTA), were run; in segment 3, from 15.1 to 20 min, BPA was scanned by 227 → 212 and 183. For both segments 2 and 3, another tune file was used (see Table 1 for more information).

Sample preparation

SPE was performed using the Visiprep vacuum manifold at a flow rate of 0.8–1 mL/min. Oasis hydrophilic-lipophilic-balanced (HLB) (6 cc/500 mg) SPE cartridges were conditioned with 6 mL methanol and then with 6 mL water. Aqueous samples or extracts were loaded to the preconditioned HLB cartridges, and washed with 6 mL water. After the washed cartridge had been dried by applying vacuum, 5 mL of methanol was used as eluant. For trace concentration

Table 1. LTQ MS ESI-MS/MS conditions

Analyte	Precursor ion [M–H]$^-$	Product ions	Spray voltage (kV)	Capillary voltage (V)	Tube lens (V)	Sheath gas flow rate (mL/min)	Capillary temp (°C)
AFB1	311	296*, 267	5	–10	–52	60	310
BPA	227	212*, 183	5	–10	–52	60	310
OTA	402	358*, 270	5	–10	–52	60	310
Patulin	153	109*, 125	5	–15	–62	70	310

*The most abundant product ion used for quantification while the other ion used for confirmation.

levels of samples (≤5 ppb), the collected eluate was further condensed to 1 mL by nitrogen evaporation using the Reacti-Vap™ evaporator at 60 °C.

Clear grape juice (red grape juice from New York) and apple juice (kids' apple juice from Michigan) samples were directly loaded to the preconditioned SPE cartridge for sample clean-up and enrichment. Oats-based cereal samples were ground to powder using a pestle and mortar before further sample preparation. For peanut butter, cereal, and cereal-based baby formula, 10 g of sample was added to 15 mL 1:1 methanol/water in a 50 mL Nalgene centrifuge tube. It was mixed using a spatula, shaken for 60 min in a shaker bath at room temperature, and centrifuged for 10 min at 5000 rpm. A 10 mL aliquot of supernatant was taken and mixed with 20 mL water before SPE clean-up and enrichment.

Method development and validation

Standard solutions and quantitative procedure

The standard stock solutions were prepared in methanol. An accurately weighed 10 mg amount of each pure compound was dissolved in 10 mL of methanol in order to prepare 1000 ppm stock solution. High concentration of stock solution was adopted to decrease the relative error in weighing. Then, 1 ppm working solution of the mixture of these four compounds was prepared by mixing and diluting these four stock solutions. The calibration solutions of mixture were further obtained at the following concentration levels by dilution of the working solution in methanol: 5 ppb, 10 ppb, 30 ppb, 50 ppb, 100 ppb, 200 ppb, 500 ppb, and 1000 ppb. Good linearity was demonstrated for these four compounds in the calibration range, with r^2 >0.999. These calibration standards in methanol were used in the recovery study of spiked water samples in the first stage of method development, optimization of MS/MS parameters and HPLC separation, check of the intra-day/inter-day precisions of the analytical method, and examination of the matrix effects without usage of SPE for sample clean-up.

For analysis of fortified food and beverage samples, the matrix-matched calibration standards were used. The eluate of analytes-free matrices, which had gone through the entire same sample preparation procedures, including SPE clean-up and nitrogen evaporation (if required), was used in making up the matrix-matched calibration standard solutions.

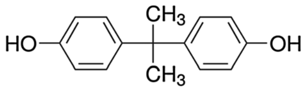
In the first stage of method development, BPA, AFB1, OTA, and patulin were spiked into water at two concentration levels (5 and 80 ppb), and Waters Oasis HLB (hydrophilic-lipophilic balanced reversed-phase sorbent) and MAX (mixed-mode anion-exchange and reversed-phase sorbent) SPE cartridges were tested for optimization of elution and recovery. An HLB cartridge and the optimized SPE procedure were used to test different matrices for limits of detection (LODs) and limits of quantification (LOQs). Two concentration levels of these analytes (5 and 250 ppb) were tested in food and beverage matrices for recovery study.

RESULTS AND DISCUSSION

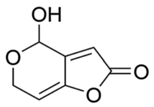
Method development

LC/MS/MS

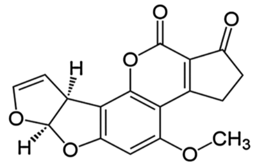
BPA, OTA, and patulin are prone to undergo negative ionization due to the stable ionization products by de-protonation (See Fig. 1 for the molecular structures). For comparison, both negative and positive ionization modes were used to tune the LTQ mass spectrometer for all the analytes. Negative ionization mode worked better for all of these target analytes including AFB1 in LTQ-MS. The precursor ions were in the form of $[M–H]^-$, 2 m/z units less than that of $[M+H]^+$. For the confirmation of precursor ions, two or more product ions should be selected when using low-resolution LC/MS analysis in accordance with relevant EU legislation.[39] Therefore, the selection of two product ions was performed in the



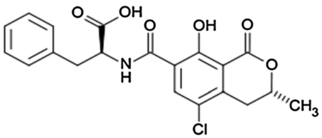
a. Bisphenol A, MW=228



b. Patulin, MW=154



c. Aflatoxin B1, MW=312



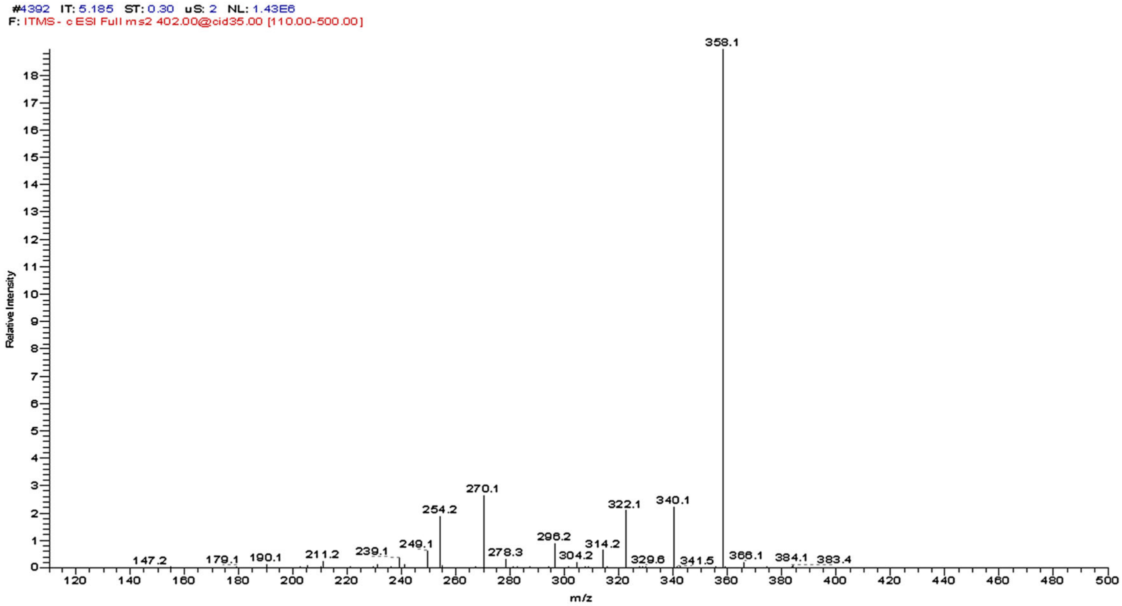
d. Ochratoxin A, MW=403

Figure 1. Molecular structures of target analytes.

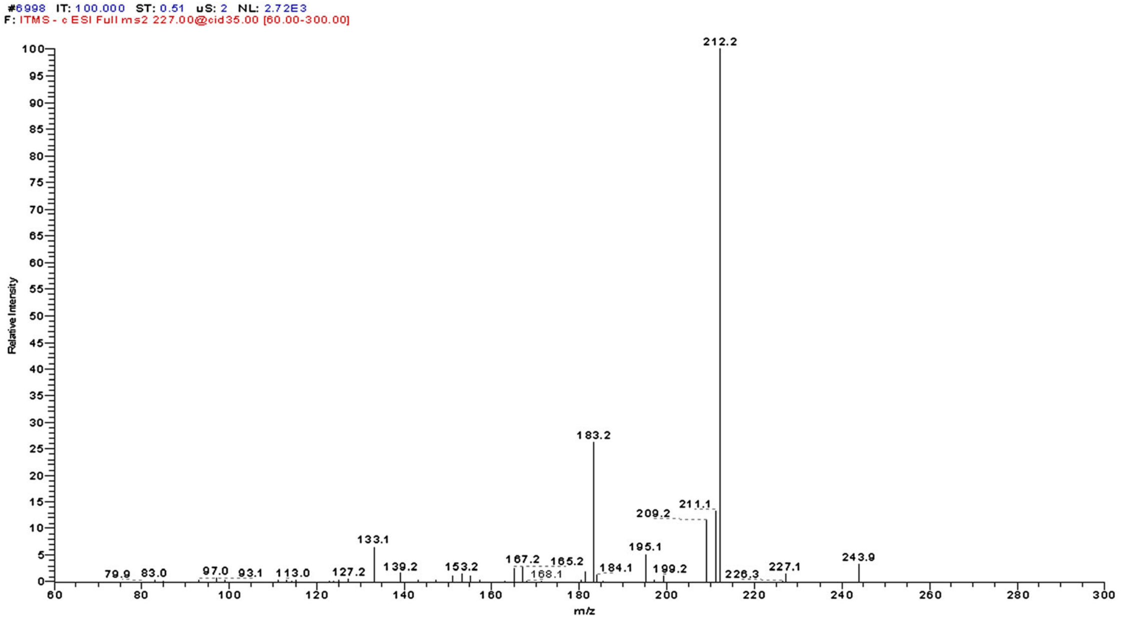
product ion scan mode (See Figs. 2 and 3 for MS/MS spectra of the target analytes). The precursor ions and corresponding product ions selected are listed in Table 1. The most abundant product ion was used for quantification, while the other was used for analyte confirmation.

Final tuning for optimization of ion source parameters was obtained by syringe pumping of standard solutions with constant infusion rate of 10 μL/min, mixed with LC solvent pumping at the flow rate of 0.4 mL/min through a Tee union. Mobile phase ratios were kept close to that used when the analyte was eluted from the LC system into the mass spectrometer. For ion source optimization of patulin, the ratio of mobile phase A to B was 40:60; for the other three compounds which shared one tuning file, a ratio of mobile phase A to B of 20:80 was used. The values for the main ion source parameters are listed in Table 1.

In order to select a good mobile phase A, several solvents were tested, including pure water, water with formic acid added, and water with ammonium acetate added. It was concluded that ammonium acetate added in water helped to get good chromatographic peak shape, and assisted in negative ionization, while formic acid did not perform well with negative ionization. Finally, 10 mM ammonium acetate in water was adopted as mobile phase A after testing with different amounts of ammonium acetate in water (See Fig. 4 for the chromatogram of a standard mixture).

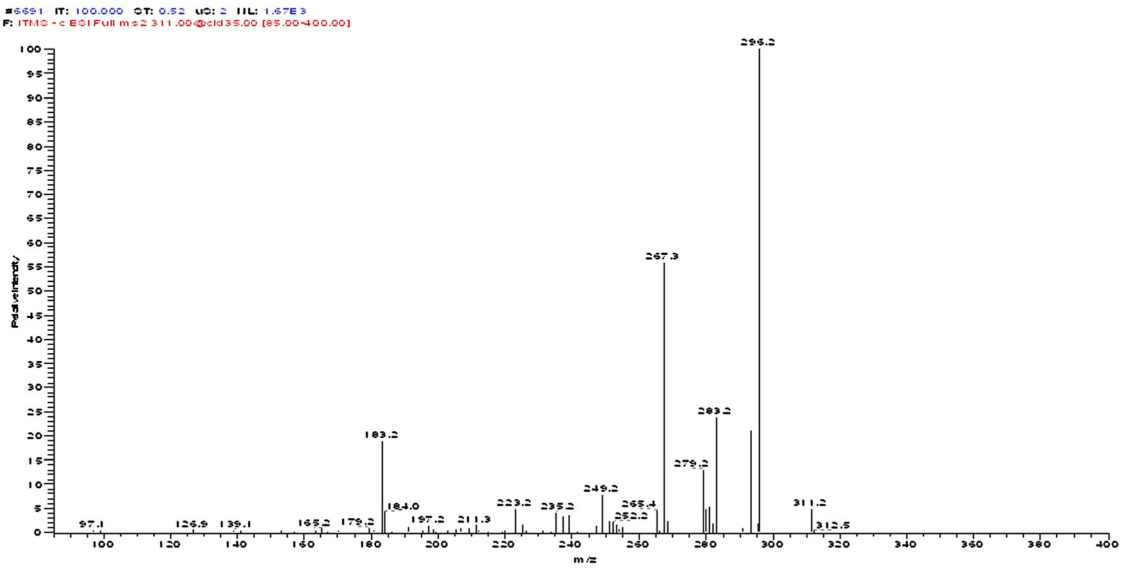


a. Ochratoxin A

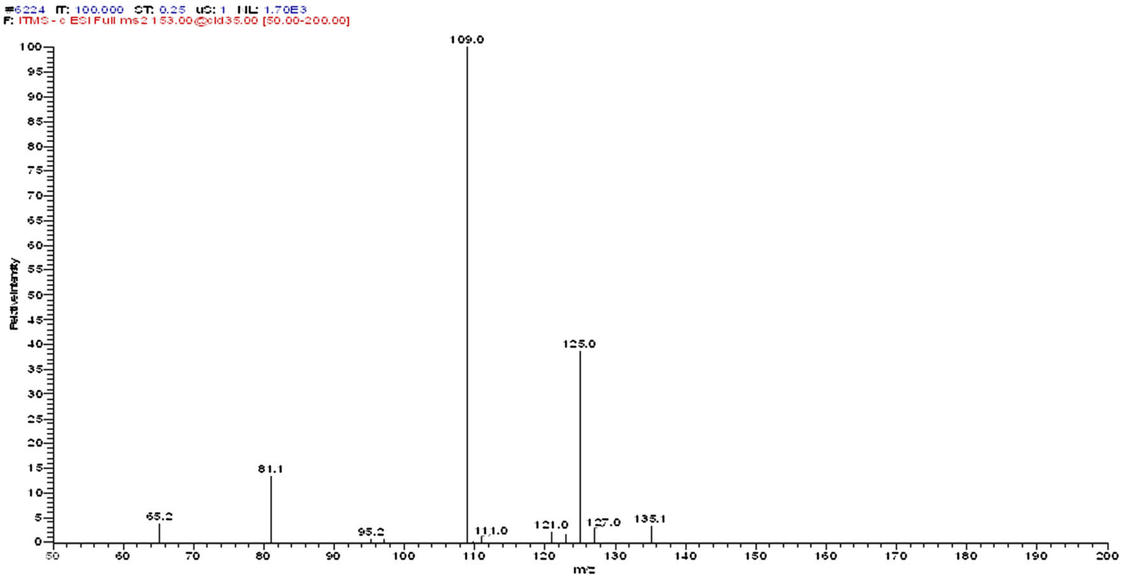


b. Bisphenol A

Figure 2. MS/MS spectra of (a) ochratoxin A and (b) bisphenol A.



a. Aflatoxin B1



b. Patulin

Figure 3. MS/MS spectra of (a) aflatoxin B1 and (b) patulin.

Sample preparation and clean-up

To test the sample matrix effects of the beverage and food matrices without SPE clean-up, the mixed work standard solution was spiked at 30 ng/mL into analytes-free liquid samples such as grape juice and apple juice, into dissolved liquid baby formula, and into peanut butter sample extracts. Then, the MS signal responses in peak areas were compared with the same concentration level of these chemicals in methanol and the results are listed in Table 2.

The compounds have different matrix effects in different matrices compared to methanol. Significant enhancement in the MS signal was observed for BPA even in water (>150%). For patulin, no corresponding MS signal could be detected in both apple juice and grape juice due to MS signal suppression in these matrices. The most significant MS signal enhancement was observed for BPA in peanut butter, which was 196% of response in methanol. This preliminary test indicated that serious matrix effects exist for the target analytes in the beverage/food matrices studied, and further sample clean-up is essential for successful method development.

Compared to silica-based C18 SPE columns which could not tolerate dry-up, Oasis sorbents are water-wettable, and maintain high retention and capacity even if the cartridge is dried. Therefore, even though C18 SPE columns have been reported in sample preparations for mycotoxins and BPA, Waters Oasis SPE cartridges were tested. In this study, two types of Waters Oasis SPE cartridges, HLB (hydrophilic-lipophilic-balanced reversed-phase sorbent) and MAX

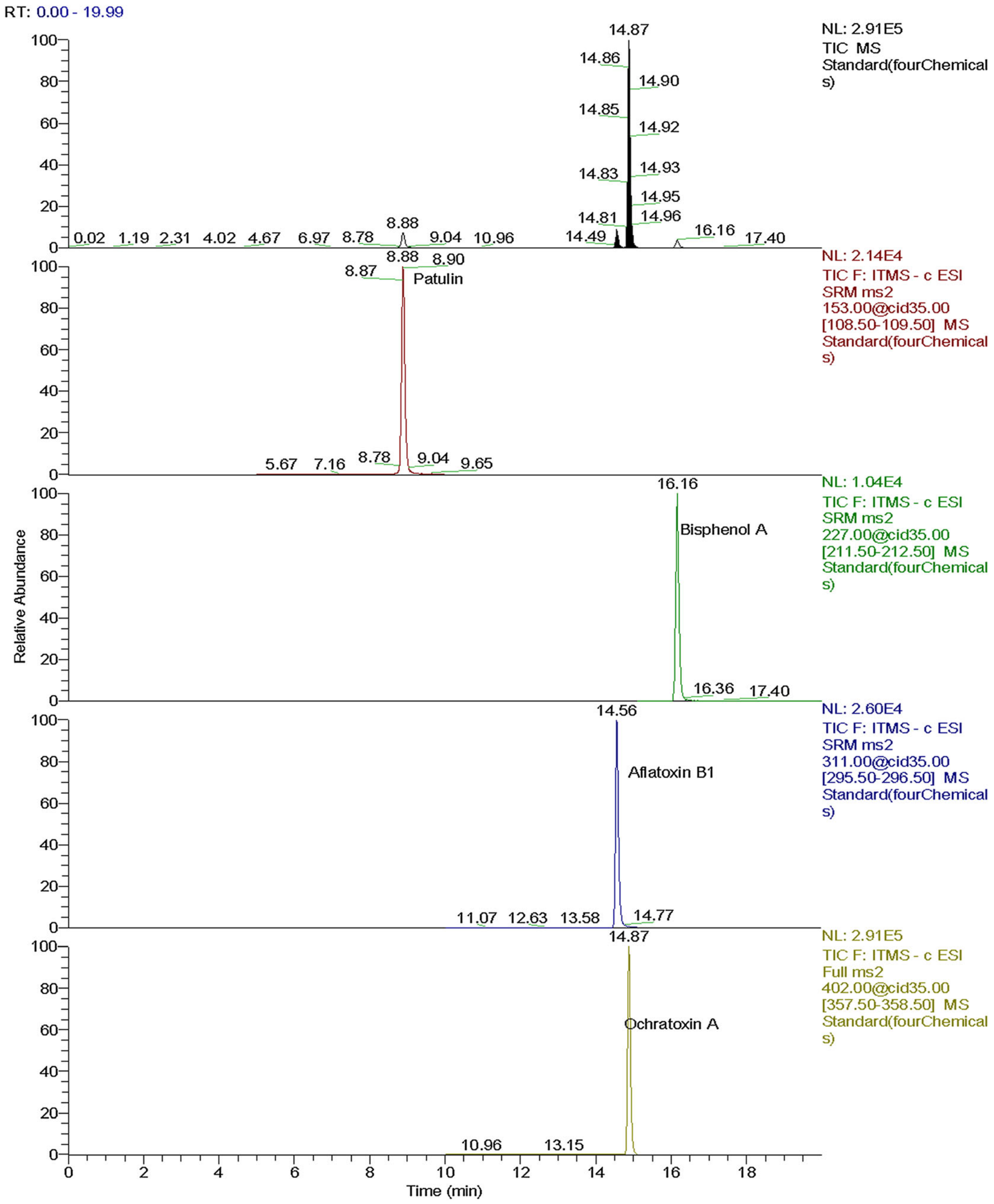


Figure 4. Chromatogram of standard mixture at 1 ppm.

(mixed-mode anion-exchange sorbent), were used on spiked water samples with respective schemes suggested by the vendor. For the Oasis MAX cartridge, it is conditioned with 6 mL acetonitrile, followed with 6 mL 5% ammonium hydroxide in water. Aqueous samples were mixed with 5% ammonium hydroxide, and loaded to pretreated MAX cartridge; washed with 3 mL 5% ammonium hydroxide, and eluted with 5 mL 4% formic acid in acetonitrile. MAX cartridges had recoveries for BPA, AFB1 and OTA comparable to HLB cartridges, but patulin could not be recovered efficiently (recovery <30%) with MAX cartridges.

Based on the testing above, Oasis HLB cartridges were used. Cartridges were conditioned with 6 mL methanol and then with 6 mL water. Aqueous samples were loaded to pretreated HLB cartridges, washed with 6 mL water, and eluted with 5 mL methanol. Elution with 5 mL 0.5% formic acid in methanol was also investigated in the stage of method development. Both elutions gave comparable recovery results, so elution with 5 mL methanol was finally adopted for the SPE procedure. The recovery results for analyzing the fortified water samples at two spiking concentration levels were obtained by using the HLB SPE clean-up procedure (Table 3). At the concentration level of 5 ng/mL, the average recoveries of three replicates were in the range of 68 to 114% for these analysts, with relative standard deviation (RSD) ranging from 3.8 to 4.7%. At the high concentration level of 80 ng/mL, the average recoveries spanned from 80.5 to 103%, with RSD ranging from 0.9 to 10.4%.

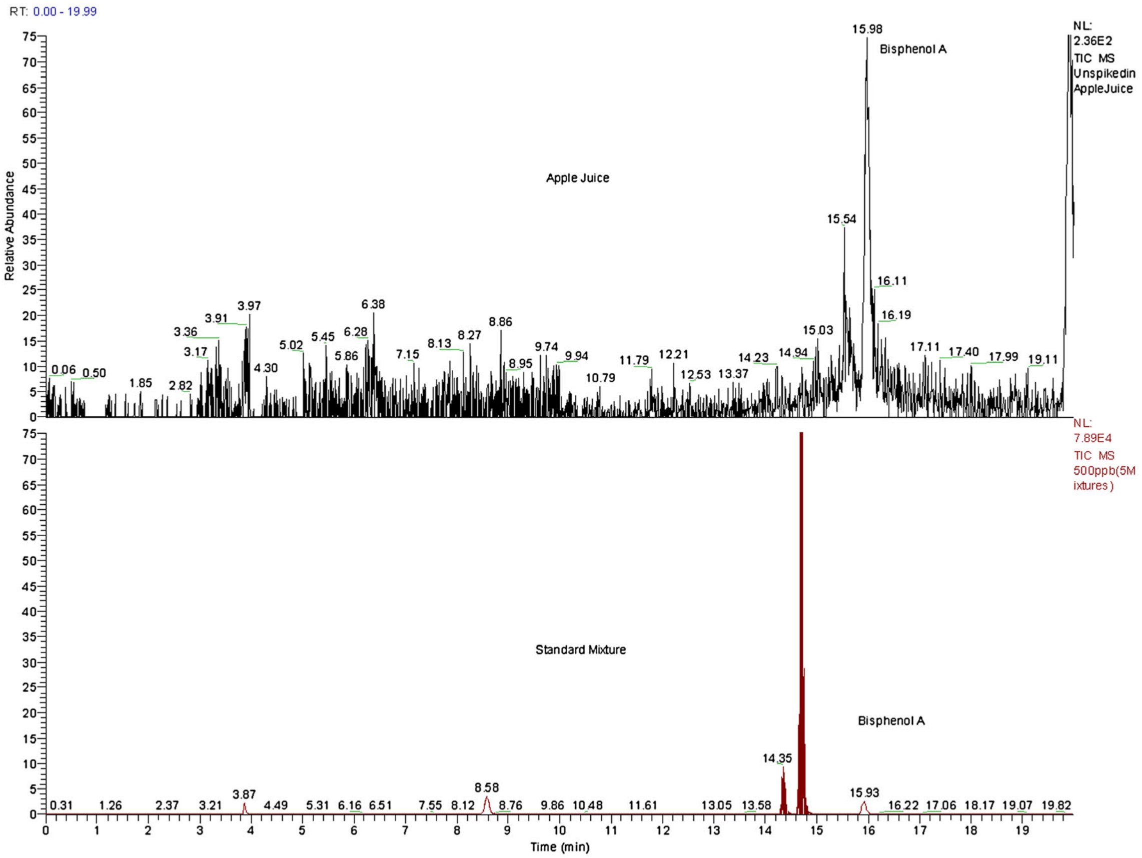


Figure 5. Chromatogram of an apple juice sample in which bisphenol A was detected at 2 ng/mL compared with the standard mixture run in the same sequence.

Table 2. Matrix effects to MS response without SPE clean-up

	Percentage of response in matrix/matrix extract compared to in pure methanol					
	Apple juice	Grape juice	Cereal-based baby formula	Peanut butter	Oats-based cereal	Water
BPA	85	102	129	196	72	174
AFB1	105	150	110	134	67	141
OTA	93	92	121	119	113	122
Patulin	0	0	87	135	81	119

Table 3. Recoveries of four compounds at two concentration levels in water

	5 ng/mL (n = 3)		80 ng/mL (n = 3)	
	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
BPA	85.9	3.8	80.5	0.9
AFB1	68.0	4.4	82.5	6.0
OTA	94.3	4.7	88.5	10.4
Patulin	114.0	4.6	103	1.4

Evaluation of matrix effects and validation of the analytical method

Several approaches can be found in the literature to reduce or compensate for matrix effects. These include sample pretreatment and clean-up, application of internal standards, and use of matrix-matched calibration. Closely related compounds, which do not occur in naturally contaminated samples and show similar chemical structures to the target analytes, ideally stable-isotope-labeled mycotoxins, are the best choice as internal standards for compensating matrix effects due to their identical chemical and chromatographic properties compared to the target toxins. However, for simultaneous

Table 4. Calibration equations between concentration level (X, ng/mL) in different matrix extracts and MS response (Y, peak area)

	Calibration equation ($Y = aX + b$, r^2)					
	Methanol	Apple juice	Cereal-based baby formula	Grape juice	Oats-based cereal	Peanut butter
AFB1	$a = 109.253$ $b = 0$ $r^2 = 0.9997$	$a = 31.588$ $b = -115.959$ $r^2 = 0.9951$	$a = 81.472$ $b = 0$ $r^2 = 0.9982$	$a = 31.631$ $b = -126.515$ $r^2 = 0.9975$	$a = 64.077$ $b = -70.204$ $r^2 = 0.9940$	$a = 21.153$ $b = 0$ $r^2 = 0.9985$
BPA	$a = 51.1631$ $b = 0$ $r^2 = 0.9994$	$a = 18.414$ $b = -84.991$ $r^2 = 0.9981$	$a = 40.704$ $b = -229.142$ $r^2 = 0.9986$	$a = 19.263$ $b = -88.779$ $r^2 = 0.9979$	$a = 32.139$ $b = 85.599$ $r^2 = 0.9950$	$a = 7.374$ $b = 0$ $r^2 = 0.9990$
OTA	$a = 1438.72$ $b = 0$ $r^2 = 0.9994$	$a = 671.973$ $b = 0$ $r^2 = 0.9996$	$a = 1081.66$ $b = 0$ $r^2 = 0.9978$	$a = 671.279$ $b = 0$ $r^2 = 0.9978$	$a = 1207.86$ $b = 679.842$ $r^2 = 0.9970$	$a = 576.332$ $b = 0$ $r^2 = 0.9995$
Patulin	$a = 209.649$ $b = 0$ $r^2 = 0.9994$	$a = 17.871$ $b = 0$ $r^2 = 0.9956$	$a = 136.426$ $b = 0$ $r^2 = 0.9990$	$a = 15.296$ $b = 216.895$ $r^2 = 0.9972$	$a = 176.169$ $b = 152.43$ $r^2 = 0.9952$	$a = 25.273$ $b = 0$ $r^2 = 0.9972$

Table 5. Recoveries of four compounds in different matrices by SPE (determined by matrix-matched calibration standards)

					Recovery (average ± RSD, %)					
	Apple juice		Grape juice		Cereal-based baby formula		Oats-based cereal		Peanut butter	
	250 ppb	5 ppb	250 ppb	5 ppb	250 ppb	5 ppb	250 ppb	5 ppb	250 ppb	5 ppb
BPA	83 ± 9	104 ± 14	111 ± 5	103 ± 2	92 ± 4	66 ± 3	84 ± 4	124 ± 11	75 ± 3	78 ± 5
AFB1	73 ± 12	117 ± 7	106 ± 3	80 ± 11	83 ± 12	70 ± 2	74 ± 5	85 ± 8	93 ± 13	75 ± 0
OTA	66 ± 13	91 ± 1	89 ± 3	116 ± 4	103 ± 3	118 ± 13	86 ± 8	90 ± 4	105 ± 6	87 ± 1
Patulin	100 ± 7	112 ± 1	107 ± 3	103 ± 7	95 ± 3	127 ± 5	112 ± 12	119 ± 6	100 ± 5	102 ± 3

analysis of multiple classes of analytes which cover a variety of physicochemical properties such as those in this work, this approach is not proper due to the non-availability as well as the cost of obtaining multiple internal standards. The matrix-matched calibration has been widely used even with the application of clean-up procedures as a certain degree of matrix effects may still exist even after multiple steps of sample clean-up procedures.[23,33]

To evaluate the impact of SPE clean-up procedures on matrix effects, matrix-matched calibration standards were prepared by adding the working solution to the eluates of analytes-free matrices which were obtained by following the same SPE clean-up procedures, and the calibration equations were derived for these different matrices, including grape juice, apple juice, cereal, cereal-based baby formula, and peanut butter (Table 4).

By comparison to the calibration equations among different matrices as well as methanol, it could be concluded that matrix-matched calibration are still required even after SPE clean-up, for accurate quantification of all of these four analytes. The ’a’ value (slope of the calibration curve) in the calibration equation for each analyte in methanol was significantly greater than those of other matrices, indicating that all the four matrices had suppression effects on MS signals, with BPA, patulin and AFB1 affected more than OTA in general. Each analyte demonstrated a specific calibration correlation which varied with different matrices. However, good linearities for these calibrations were obtained, with no coefficient (r^2) below 0.994. These results justify the application of matrix-matched calibration for quantitation of these chemicals in the matrices chosen for this study. The chromatogram for an apple juice sample is given in Fig. 5, with detection of BPA at 2 ng/mL.

Two concentration levels of these analytes (5 and 250 ppb) were spiked in duplicates to these matrices, and the recoveries were calculated based on the matrix-matched standard calibrations (Table 5). It could be seen that the average recoveries ranged within 70 to 120% for most of analytes. The intra-

Table 6. Intra-day and inter-day precision (n = 3)

Analyte	Concentration (ppb)	RSDr (%)	RSDR (%)
AFB1	100	3.3	4.9
BPA	100	5.7	6.0
OTA	100	2.3	5.0
Patulin	100	2.1	7.4

RSDr = Relative standard deviation intra-day precision.
RSDR = Relative standard deviation inter-day precision.

Table 7. Spiked concentration range used in assessment of LOD/LOQ and LOQ estimations in different matrices

		LOQ				
	Concentration range (ng/mL)	Apple juice (ng/mL)	Grape juice (ng/mL)	Cereal-based formula (ng/g)	Oats-based cereal (ng/g)	Peanut butter (ng/g)
AFB1	5–50	0.5	0.5	1	1.5	0.75
BPA	10–100	1	1	2	1	1.5
OTA	1–10	0.1	0.1	0.08	0.08	0.15
Patulin	10–100	1	1	0.8	1	1.5

day and inter-day precision study indicated that this method performed reliably and stably, with the RSD of intra-day precision below 6%, and the RSDn of inter-day precision below 8% for all target analytes (Table 6).

LOD/LOQ

Matrix extracts obtained from the SPE procedure were used to measure the detection limits of these analytes. Based on the sensitivities of analytes, the concentration ratio of 1:5:10:10 for OTA, AFB1, patulin, and BPA, respectively, was used in mixture for spiking into the matrix extracts to obtain similar MS responses. The limits of detection (LODs) of the target analytes were defined as the level which has a signal-to-noise (S/N) ratio of 3:1 while limits of quantitation (LOQs) as S/N ratio of 10:1 on mass spectrometric platforms.

For liquid samples, the calculation was based on 10 mL of sample being loaded onto the SPE cartridge and the final eluate being condensed from 5 mL to 1 mL prior to LC/MS injection. For cereal, cereal-based baby formula, and peanut butter, the calculation was based on 10 g of sample being extracted in 15 mL of 1:1 methanol/water, then centrifuged and 10 mL of supernatant being mixed with 20 mL of water, and going through the SPE procedure, with the final eluate being condensed from 5 mL to 1 mL prior to LC/MS injection. The LOQs of these analytes were in the range of 0.08 to 2 ppb (Table 7) meeting the method performance criteria required by the Commission Regulation No 165/2010, 1881/2006 and 123/2005, for monitoring the maximum levels of AFB1, patulin, and OTA in corresponding food matrices, except AFB1 in processed cereal-based baby formula. This analytical method has the potential to detect even lower levels of target toxins by increasing the volume of sample extracts loaded to the HLB cartridge or further condensation of the final eluate volume prior to LC/MS injection.

CONCLUSIONS

An analytical method, which uses a single Oasis HLB SPE cartridge for sample clean-up and LC/MS in negative electrospray ionization mode for detection of target analytes, was developed to analyze BPA and three typical mycotoxins (aflatoxin B1, patulin, and ochratoxin A) in grape juice, apple juice, cereal, cereal-based baby formula, and peanut butter. By using the simple clean-up procedure and matrix-matched external calibration, good recoveries and sensitivities were obtained for the determination of these target analytes. This cost-effective method has the advantages of simple sample pretreatment, rapid and simultaneous determination, and can be expanded to cover aflatoxin and ochratoxin derivatives. Moreover, it can be improved to accommodate more strict legal demands in the future.

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