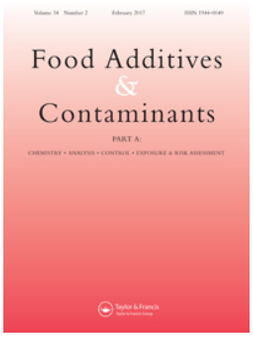

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Determination of 16 mycotoxins in vegetable oils using a QuEChERS method combined with high-performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

A simple and efficient method for determining multiple mycotoxins was developed using a QuEChERS (quick, easy, cheap, effective, rugged and safe)-based extraction procedure in vegetable oils. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used for the quantification and confirmation of 16 chemically diversified mycotoxins. Different extraction procedures were studied and optimised by spiking 16 analytes into blank matrix, and the extraction with 85% MeCN solution and C18 as cleaning sorbent allowed an efficient recovery of 72.8–105.8% with RSDs less than 7%. The limit of detection (LOD) ranged from 0.04 to 2.9 ng g^{-1}. The developed method was finally applied to screen mycotoxins in 62 vegetable oil samples. Zearalenone (ZEN), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and α-zearalenol (α-ZOL) were detected, with maximum concentrations of 0.59 (AFG1)–42.5 (ZEN) ng g^{-1}. The method developed has the advantages of high sensitivity, accuracy and selectivity, and it can be applied to the target screening of mycotoxins in real samples.

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KEYWORDS
Mycotoxins; vegetable oil; QuEChERS; HPLC-MS/MS

Introduction

Mycotoxins are a group of naturally occurring contaminants produced by different fungal species as secondary metabolites (Zachariasova et al. 2014; Saito et al. 2016). To date, more than 400 mycotoxins have been identified, but only a few of them are of major concern due to their adverse effects on human health (Chang et al. 2011; Kovacs 2012; Weidner et al. 2013; Fromme et al. 2016). These mycotoxins include aflatoxins, trichothecenes, ochratoxins, zearalenone, deoxgnivalenol, T-2 and HT-2 toxin. Because of their great structural diversity, they can cause a variety of toxic effects in humans as well as in animals. For example, aflatoxins have been classified as group 1 human carcinogens due to their role in the aetiology of liver cancer (Anon. 1989). Ochratoxins have been reported to be capable of leading to hepatomegaly, enteritidis and lymphoma (Creppy et al. 1983; Kuiper-Goodman & Scott 1989; Heussner & Bingle 2015). Zearalenone and fumonisins have been found to interfere with the reproductive function of mammals and cause immunosuppression (Abrunhosa et al. 2016). In general, mycotoxins are mainly formed from the fungal genera of *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* either in the field or during storage (De Ruyck et al. 2015). The FAO estimates that more than 25% of all agricultural product are contaminated with mycotoxins (Marin et al. 2013).

Vegetable oils are made from agricultural products such as vegetable fruits and seeds by mechanical pressure or extraction with organic solvents, and are important to our daily life because they provide energy, nutritional components and pleasant flavours (Frankel 1989; Silva et al. 2010; Cubero-Leon et al. 2014). At present, soybean, rapeseed, sunflower seed, peanut and olive are major raw materials for the production of cooking oils in the world. During the growing process these agricultural products are easily contaminated by mycotoxins due to continuously changing environmental and temperature conditions. For example, high temperature, high rainfall and relative humidity are highly conducive for fungal proliferation and mycotoxin production (Fink-Gremmels 2008; Mahmoudi & Norian 2015; Bahrami et al. 2016). In recent years, the occurrence of aflatoxins, zearalenone, ochratoxin A,

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deoxynivalenol and other mycotoxins in vegetable oils has been reported worldwide (Cavaliere et al. 2010; Afzali et al. 2012; Escobar et al. 2013), and a maximum level for ZEA in corn oil has been regulated under by European Commission No. 1126/2007 (Commission of the European Communities 2007). Therefore, to protect consumer health, it is necessary to identify and monitor mycotoxins in different vegetable oils.

Analysis of mycotoxins is challenging as they are often present at low concentrations in complex matrices. A number of analytical methods for the determination of single and multiple mycotoxins in food include TLC, immunoaffinity chromatography, ELISA and HPLC have been reported (Plattner et al. 1996; MacDonald et al. 2005; Klaric et al. 2009; Welke et al. 2009). In recent years, triple-quadrupole tandem mass spectrometry (MS/MS) is considered the gold standard for quantitative analysis of multiple compounds, and several LC-MS/MS-based methods for multiple mycotoxin analysis have been also developed for various food and feed commodities (Belen Serrano et al. 2015; Marley et al. 2015; Fabregat-Cabello et al. 2016). However, a multi-mycotoxin method for vegetable oils is still lacking. Since the main components of vegetable oils are lipids, containing a high percentage of monounsaturated and saturated fatty acids, and pigments (Moreno-Gonzalez et al. 2014), which are difficult to clean up, the selection of a suitable sorbent is very important for an effective clean-up method to decrease matrix effects and/or interferences during chromatographic analysis. The aim of this study was to develop a simple, selective and reliable method by coupling the universal QuEChERS extraction and clean-up method with HPLC-MS/MS for the simultaneous determination of 16 predominant mycotoxins in the complex matrix of vegetable oils. Although the QuEChERS method introduced by USDA scientists in early 2003 (Anastassiades et al. 2003) has been applied for the analysis of antibiotics (Lombardo-Aguei et al. 2012), veterinary drug residues (Stubbings & Bigwood 2009), and mycotoxins (Cunha & Fernandes 2010; Rasmussen et al. 2010; Sirhan et al. 2011) in different matrices, to our knowledge this is the first publication describing a QuEChERS method with HPLC-MS/MS for the quantitative determination of multiple mycotoxins in vegetable oils.

Materials and methods

Chemicals and reagents

Mycotoxins reference standards: α-zearalanol (α-ZAL) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Zearalenone (ZEN) was bought from O2si (Charleston, SC, USA). Deoxynivalenol (DON) was obtained from Supelco (Bellefonte, PA, USA). β-Zearalanol (β-ZAL), β-zearalenol (β-ZOL) and α-zearalenol (α-ZOL) were bought from the Australian National Measurement Institute (West Lind Field, Australia). Ochratoxin A (OTA) was purchased from Fermentek Biotechnology (Jerusalem, Israel). T-2 toxin (T2) was supplied by Toronto Research Chemicals (North York, ON, Canada). 3-Acetyldeoxynivalenol (3-Ac-DON) and 15-acetyldeoxynivalenol (15-Ac-DON) were purchased from Chiron (Trondheim, Norway). Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) were obtained from Beijing Rapid Bio Science Co., Ltd (Beijing, China). All standards were > 99% pure; their molecular structures are shown in Figure S1 in the supplemental data online.

LC-MS-grade acetonitrile (MeCN) was bought from J&K Scientific Ltd (Beijing, China). Sodium sulphate anhydrous, guaranteed reagent, was obtained from Sigma-Aldrich (Shanghai, China). Analytical-grade sodium chloride and sodium acetate were supplied by Tianjin No. 1 Chemical Reagent Factory (Tianjin, China). LC-MS-grade formic acid and ammonium acetate were purchased from Dikmapure (Beijing, China). The cleaning sorbents, neutral alumina (Al_2O_3), octadecyl (C_{18}) and primary secondary amine (PSA) silicas, were obtained from DIKMA (Beijing, China). Water for all the experiments was purified (18 MΩ) on a Milli-Q Plus apparatus from Millipore (Bedford, MA, USA).

Standard solution preparation

Solid standards of OTA and T2 were weighed and dissolved directly in acetonitrile to prepare 0.1 and 0.5 mg ml^{-1} stock solutions, respectively. Standard solutions of the other mycotoxins as purchased were diluted in acetonitrile to prepare 0.5 mg ml^{-1} stock solutions. All the stock solutions were stored in refrigerator at –18°C. An accurate volume of each standard solution was transferred into the combined solution and diluted step by step in acetonitrile to prepare a series of working standard solutions with concentrations of 0.2, 0.4, 0.8, 1.0, 2.0, 4.0, 10 and 20 ng ml^{-1} for AFs; 1.0, 2.0, 4.0, 10, 20, 40, 80 and 100 ng ml^{-1} for ZEN, α-ZAL, β-ZAL, β-ZOL, α-ZOL, T2 and OTA; 10, 20, 40, 80, 100, 160 and 200 ng ml^{-1} for DON; and 25, 50, 100, 200, 250, 400 and 500 ng ml^{-1} for 3-Ac-DON and 15-Ac-DON.

Sample extraction and clean-up

Sample of 1.0 ± 0.01 g was weighed into a 30 ml centrifuge tube and then spiked with a mycotoxin

standard mixture containing standard mycotoxins at different concentrations. After leaving the samples for 1 h for equilibration, 2 ml water were added and vortex-mixed for 1 min. Thereafter, 8 ml of the extraction solvent (MeCN) were added and after brief shaking the samples were extracted using an end-over-end shaker for 20 min. Subsequently, 4.00 ± 0.05 g of the pre-weighed Na_2SO_4 anhydrous salt and 1.00 ± 0.01 g of NaCl were added and the tube was capped immediately (a brief shaking by hand was performed immediately after the addition of salts to prevent agglomeration of the salts). The tube was then vortexed for 2 min and centrifuged for 5 min at 5000 rpm min^{-1}. A total of 8 ml of the MeCN extract (upper layer) was transferred into a 15 ml centrifuge tube containing sorbents (three sorbents (C18, PSA and neutral Al_2O_3) with different amounts (100, 150 and 200 mg) were tested for their clean-up efficiency). The tubes was shaken vigorously by hand for 5 min, and centrifuged at 8000 rpm for 5 min, and then 4 ml of the extract were transferred to a glass tube. The sample was evaporated to dryness at 40°C under a stream of N_2, and reconstituted by adding 1 ml of mobile phase A–B (1:1, v/v) for LC-MS/MS analysis.

HPLC-MS/MS condition

The LC separation was operated on an Agilent 1260 Series LC system equipped with a Agilent XDB C18 column (150 × 2.1 mm i.d., 1.8 μm) and a thermostat column compartment (column oven temperature was set at 30°C). The injection volume was 2 μl. Mobile phases A and B were deionised water and acetonitrile respectively, at flow rates of 0.4 ml min^{-1}. The gradient was performed as follows: 0–0.5 min mobile phase B 20%; 0.5–2.0 min mobile phase B 20–40%; 2.0–6.0 min mobile phase B 40–65%; 6.0–9.0 min mobile phase B 65–90%; 9.0–9.1 min mobile phase B 90–20%; 9.1–11.0 min mobile phase B 20%.

For MS/MS analysis, a Q-Trap 5500 LC-MS/MS with a TurboIon Spray source was used. The MS was operated in both positive-ion (ESI+) and negative-ion modes (ESI–) with the following parameter settings: curtain gas: 35 psi; nebuliser gas: 50 psi; turbo gas: 55 psi; ion spray voltage: 5500/–4500 V; source temperature: 500°C. Nitrogen served as the nebuliser gas and collision gas. MRM mode was employed for detection. The fragment voltage and collision energy for the detection of each compound using MRM mode were optimised, as shown in Table 1.

Table 1. MS parameters of the 16 mycotoxins.

Compound	Molecular ion	Ionisation	MRM transitions (*m/z*)	CE (ev)	DP (v)	EP (v)	RT (min)
α-ZAL	$[M-H]^-$	ESI–	321.2/277.3	–30	–160	–10	7.81
			321.2/303.3	–30	–160	–10	
ZEN	$[M-H]^-$	ESI–	317.2/175	–32	–170	–10	5.85
			317.2/131.1	–40	–170	–10	
β-ZAL	$[M-H]^-$	ESI–	321.1/277.2	–32	–180	–10	6.47
			321.1/303.2	–28	–180	–10	
β-ZOL	$[M-H]^-$	ESI–	319.1/275.1	–28	–170	–10	5.96
			319.1/301.1	–28	–170	–10	
α-ZOL	$[M-H]^-$	ESI–	319.2/275.2	–28	–150	–10	6.58
			319.2/301.2	–30	–150	–10	
DON	$[M-H]^-$	ESI–	295.2/265.2	–16	–120	–10	1.45
			295.2/138.1	–27	–120	–10	
3-Ac-DON	$[M-H]^-$	ESI–	337.1/307.1	–13	–115	–10	3.97
			337.1/173.0	–15	–115	–10	
15-Ac-DON	$[M-H]^-$	ESI–	337.2/150	–23	–120	–10	3.89
			337.2/219	–14	–120	–10	
T2	$[M+H]^+$	ESI+	484.2/245.2	17	100	10	8.08
			484.2/215.1	22	100	10	
AFB1	$[M+H]^+$	ESI+	313.1/285.1	33	180	10	5.66
			313.1/241.1	52	180	10	
AFB2	$[M+H]^+$	ESI+	315.1/287.1	35	150	10	5.17
			315.1/259.1	42	150	10	
AFG1	$[M+H]^+$	ESI+	329.1/243	36	140	10	5.32
			329.1/215.1	45	140	10	
AFG2	$[M+H]^+$	ESI+	331.1/245.2	40	150	10	4.84
			331.1/257	43	150	10	
AFM1	$[M+H]^+$	ESI+	329/273.1	33	150	10	4.38
			329/259.1	34	150	10	
AFM2	$[M+H]^+$	ESI+	331.2/273.2	30	180	10	4.09
			331.2/285.2	31	180	10	
OTA	$[M+H]^+$	ESI+	404/239	30	120	10	7.77
			404/358	20	120	10	

Method validation study

Validation for the methodology presented here was conducted based on the European Commission regulation (Commission of the European Communities 2002, 2006) covering recovery, stability, precision, linearity, LOD, LOQ and specificity. Linearity was evaluated using six matrix-matched calibration points for all the target analytes in six different vegetable oil matrices. Peak area was used as analyte response. Calibration curves were constructed by plotting the peak areas (y) versus the concentration of analytes (x). The concentration ranges used for this study were: AFs (0.2–20 ng ml^{-1}), ZEN and its metabolites (2–100 ng ml^{-1}), T2 and OTA (2–80 ng ml^{-1}), and DON, 3-Ac-DON, 15-Ac-DON (10–500 ng ml^{-1}). Calibration curve equations, the determination coefficients (R^2) and RSDs for each mycotoxin were measured by calibration of the average peak areas (n = 3). Recovery and precision were assessed by spiking blank vegetable oil samples with three replicates at low, middle and high spiking levels (Table 2). After analysing and determining the concentrations for each sample, recovery rates were calculated using: % Recovery = determined concentrations/fortification level × 100 LOD and LOQ were defined as the concentrations that would result in signal-to-noise ratios of 3 and 10, and measured from peak areas of 10 independent blank vegetable oil samples spiked with mycotoxins standard mixture at a level of 1 ng g^{-1}, respectively. The matrix effect of each analyte was estimated by calculating the difference of the linear best-fit slope, obtained from the matrix-matched calibration curve and solvent-based standards calibration curve, divided by the slope of the solvent-based standards calibration curve. Selectivity was determined from t_R, ion ratios and identification points for each analyte.

Results and discussion

HPLC-MS/MS optimisation

According to the ionisation properties of the 16 mycotoxins, we started by optimising analyte-specific MS/MS conditions in both positive- and negative-ion detection modes. The base peak of $[M + H]^+$ ion in the full-scan mass spectra of all analytes served as the precursor ion for monitoring in ESI+ mode. α-ZAL, ZEN and DON produced high intensities of $[M - H]^-$ ion peak with less interference and chemical noise in ESI– mode. In accordance with 2002/657/EC (Commission of the European Communities 2002), we monitored two ion transitions for each mycotoxin. Generally, the fragment ions showing the highest and the second highest intensity under the optimised collision energies were chosen as the quantitative and qualitative fragment ions, respectively, and then were tested for selectivity and sensitivity in various vegetable oil extracts. The optimised MS parameters are shown in Table 1.

In order to obtain good peak shape and high sensitivity for 16 aimed mycotoxins, the different compositions of mobile phase including MeCN/water, MeCN/0.1% formic acid solution, MeCN/0.5% formic acid and MeCN/water including 0.5 mmol l^{-1} ammonium acetate were assessed. As shown in Figure 1, MeCN/water as mobile phases at a gradient of 20% B kept for 0.5 min, rapid increasing to 40% over 1.5 min, and then linearly increasing to 65% over 4 min, followed by a slow increase to 90% over 3 min, allowed an efficient separation with good shape and sensitivity. The order of peak elution of all the examined mycotoxins is shown in chromatogram obtained blank soybean oil (Figure 1). All mycotoxins showed good peak resolution, except for 3-Ac-DON and 15-Ac-DON.

Table 2. Linear relationships, limits of detection (LODs) and limits of quantitation (LODs) of the 16 mycotoxins.

Analyte	Regression equation	R^2	Linearity (ng ml^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
AFB1	$y = 1.6729e^5x + 3476.89$	0.9994	0.2–20	0.05	0.18
AFB2	$y = 3.85487e^4x + 2343.58$	0.9995	0.2–10	0.04	0.13
AFG1	$y = 1.50281e^5x - 498.36$	0.9998	0.2–20	0.04	0.14
AFG2	$y = 1.592387e^4x + 258.19$	0.9972	0.2–8	0.05	0.18
AFM1	$y = 4.40110e^5x - 26393.55$	0.9996	0.2–8	0.04	0.13
AFM2	$y = 1.86190e^4x - 881.43$	0.9999	0.2–8	0.04	0.13
ZEN	$y = 2.44128e^5x + 2.33897e^5$	0.9994	2–100	0.04	0.12
β-ZAL	$y = 3.54508e^5x + 7.86329e^4$	0.9996	2–40	0.11	0.37
β-ZOL	$y = 1.46900e^5x + 5.75098e^4$	0.9991	2–100	0.32	1.05
α-ZOL	$y = 2.05376e^5x + 4.52442e^4$	0.9996	2–80	0.16	0.52
α-ZAL	$y = 3.76407e^5x + 5.14590e^4$	0.9998	2–40	0.10	0.32
T2	$y = 4.75036e^4x + 7411.06$	0.9998	2–80	0.11	0.36
OTA	$y = 3.55865e^4x - 5785.64$	0.9984	2–10	0.20	0.68
DON	$y = 3579.79x + 9854.24$	0.9988	10–500	1.8	6.5
3-Ac-DON	$y = 2160.10x + 14{,}950.85$	0.9988	20–500	2.3	8.3
15-Ac-DON	$y = 1670.50x - 4117.69$	0.9991	20–500	2.9	10.0

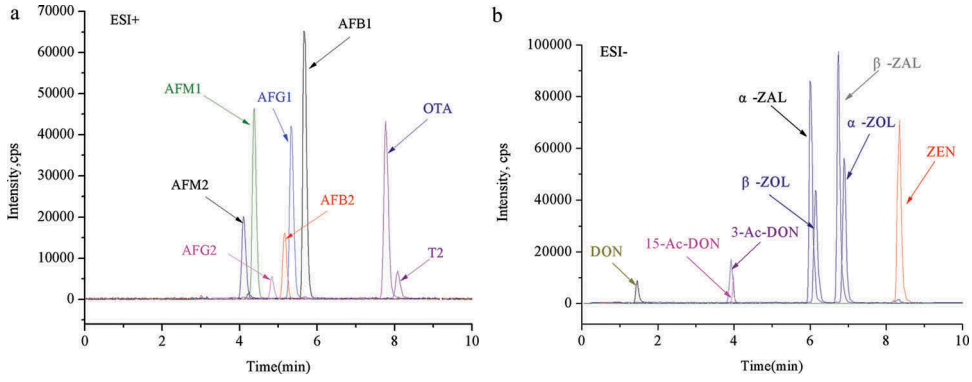

Figure 1. (colour online) (a) MRM chromatogram of 16 mycotoxins at a gradient of 20% B kept for 0.5 min, rapid increasing to 40% within 1.5 min, and then linearly increasing to 65% within 4 min, followed by a slow increase to 90% within 3 min (A: deionised water, B: acetonitrile). (b) MRM chromatogram of 16 mycotoxins at a gradient of 20% B kept for 0.5 min, rapid increasing to 40% within 1.5 min, and then linearly increasing to 65% within 4 min, followed by a slow increase to 90% within 3 min (A: deionised water, B: acetonitrile).

It has been observed that the addition of formic acid in water and MeCN could enhance the responses of the $[M + H]^+$ ions, thereby improving the detection sensitivity. Thus, 0.1% and 0.5% formic acid were added to the mobile phase. The results showed this was especially true for OTA. After adding formic acid, the response was improved. However, $[H]^+$ also significantly depressed the intensity responses of the $[M - H]^-$ ions for DON, 3-Ac-DON, 15-Ac-DON, β-ZAL, β-ZOL, etc. Furthermore, the addition of the widely used volatile salt, ammonium acetate (0.5 mM), was examined, but it was found that the ammonium acetate did not obviously improve the ionisation efficiency and sensitivity of these target mycotoxins (Figure 2). Taken together, MeCN/H_2O was finally chosen as the elution mobile phase.

Sixteen compounds in the stock solution were diluted and spiked into the blank matrix and then extracted by different solutions. The extracts were injected directly into the HPLC-MS/MS system and their recoveries were compared. MeCN is one of the most commonly used extraction solvent in QuEChERS method. We initially investigated the extraction of different MeCN/water combinations (95%, 85%, 75% and 65%) for achieving acceptable recoveries for each analyte from vegetable oils (Figure 3). The best recovery for 16 mycotoxins was found at 85% combination ratios and in the range of 78.2–104.6% with 1.3–11.0% RSD values. Some investigators found that the recovery of acidic mycotoxins OTA and fumonisin B1 (FB1) in solid samples greatly improved with increasing acid content in MeCN (Ren et al. 2007; Liu et al. 2014). Therefore, addition of 0.1% formic acid to 85% MeCN solution was investigated on toxin extraction.

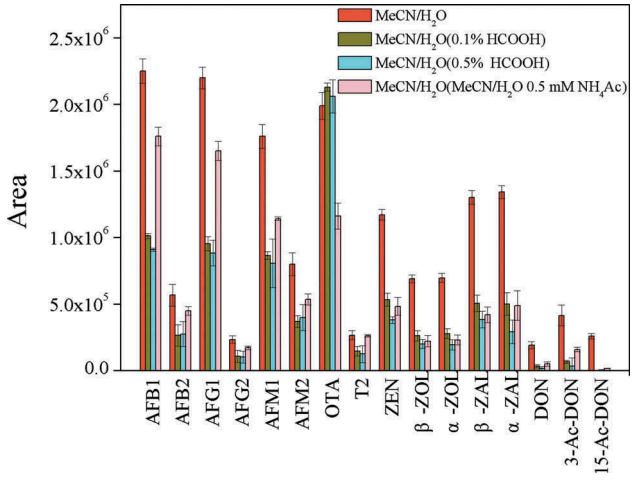

Figure 2. (colour online) Effects of different mobile phases on the response intensity of 16 mycotoxins.

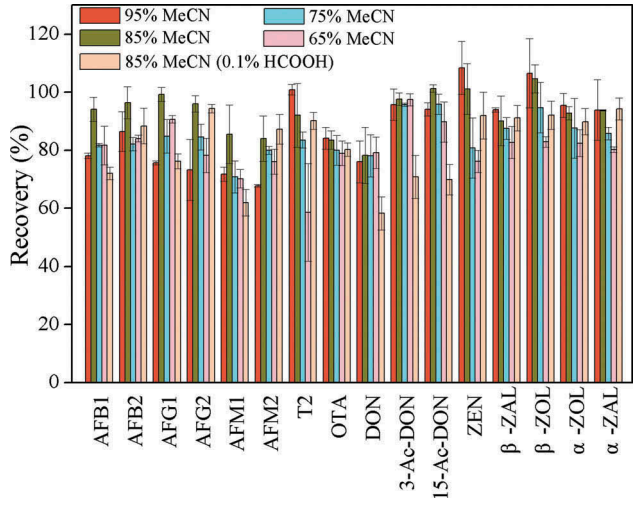

Figure 3. (colour online) Effects of different extraction solvents on the recovery of 16 mycotoxins.

Unlike previously reported results, we found that inclusion of formic acid did not obviously improve the extraction efficiency of OTA. The average recoveries of OTA in vegetable oil before and after addition of 0.1% formic acid were 83.5% and 80.2%, respectively. The remaining analytes (neutral mycotoxins) exhibited recoveries that were consistently around 80%. Taken together, we chose to use 85% MeCN solution as the extraction solvent for the following experiment.

Selection of the cleaning sorbent

The extracts from vegetable oils are generally subject to further clean-up before chromatographic analysis due to the existence of fatty acids and lipids. Here, three sorbents, C18, PSA and neutral Al_2O_3, were tested for their clean-up efficiency. The recoveries for different amounts (100, 150 and 200 mg) of three sorbents were evaluated respectively by spiking the mixed standard solution into the extracts from blank vegetable oils. The results showed that with increasing amounts of the three sorbents, the corresponding recoveries increased. The use of an optimal amount of C18 (200 mg) gave best recoveries for all analytes with the range of 77.4–117.9%, and an RSD less than 14.9% (Figure 4). Although PSA is often used to remove fatty acid in QuEChERS method, in this study we found that PSA did not give satisfactory recoveries for 16 aimed analytes and the recoveries were only in the range of 6.9–54.8%, which may be caused by PSA adsorption to most of the mycotoxin. Similar results were also reported in a previous study (Han et al. 2015). The use of neutral Al_2O_3 brought a cleaner extract, with lower fatty acid responses present in the full-scan ion GC-MS/MS chromatograms compared with those seen for C18 and PSA. However, its use resulted in the retention of a significant quantity of mycotoxin analytes. Thus, 200 mg of C18 were finally selected for sample clean-up.

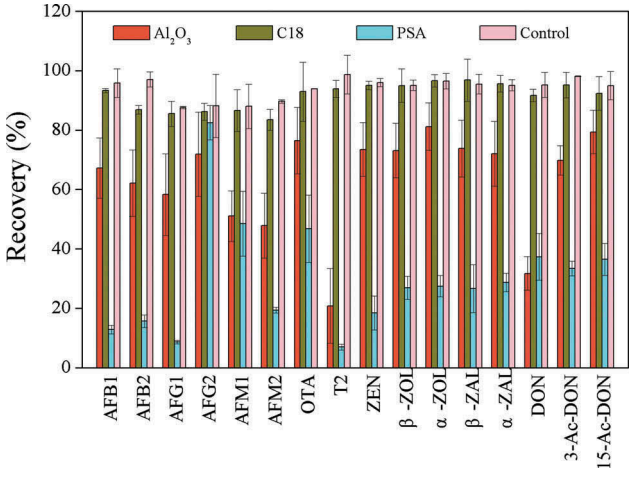

Figure 4. (colour online) Effects of different sorbents on the recovery of 16 mycotoxins.

Matrix effects (MEs)

MEs are common problems that occur when using LC-MS or MS/MS. In general, the response of the target compound can be enhanced or suppressed due to interfering matrix components. Here, by comparing the MRM response of the standard compounds prepared in blank matrix and in clean solvent, the effects of the vegetable oil matrix on 16 mycotoxins were investigated. For the selection of blank matrix, we followed the suggestion in the previous report and screened a range of vegetable oil samples (Cuadros-Rodriguez et al. 2007). One with negligible response for all the target compounds was selected as the blank matrix. As Figure 5 shows, under ESI+ mode, the OTA, AFG1 and AFG2 signals were obviously enhanced by the matrix, with average enhancement percentages higher than 50%, followed by AFM2 and AFM1 with average enhancement percentages of 10.8% and 14.3%. The other analytes under the ESI+ mode were suppressed by the matrix with suppression percentages ranging from 0.74% to 11.5%. Under ESI– mode, five out of eight compounds were suppressed and DON had the highest suppression percentage of 91.1%, followed by 3-Ac-DON and 15-Ac-DON with average suppression percentages of 72.6% and 58.9%,

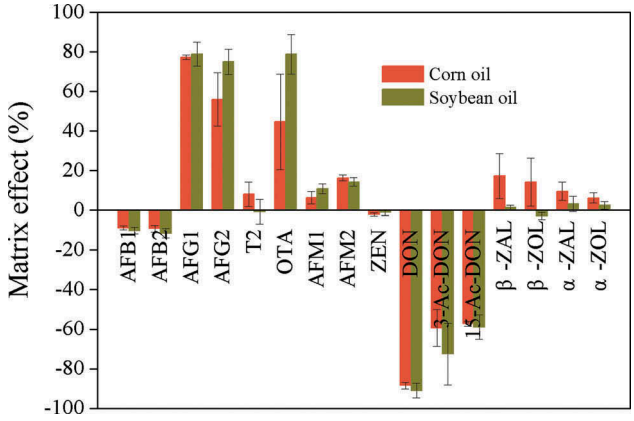

Figure 5. (colour online) Matrix effects of soybean and peanut oils on the response of each mycotoxin. The concentration range used for the matrix effect evaluation were, AFs (0.8–4 ng ml^{-1}), ZEN, α-ZAL, β-ZAL, β-ZOL, α-ZOL and T2 (4–20 ng ml^{-1}), OTA (2–10 ng ml^{-1}), DON (20–200 ng ml^{-1}), 3-Ac-DON and 15-Ac-DON (40–400 ng ml^{-1}).

respectively. Examination of the blank matrix under MRM mode demonstrated that there existed some MRM response within the entire LC elution time window. By calculating the ratio of slope (RS) between matrix standard curves and solvent standard curves, the difference of matrix effect on 16 mycotoxins was further estimated. In general, RS in the range 0.8–1.2 was considered as tolerable, and outside this range indicated severe matrix effect (Garrido Frenich et al. 2011). Among 16 compounds, 37.5% of analytes with RS values outside this range showed severe matrix effect. Therefore, in order to compensate for these matrix effects, matrix-matched external standard calibration was applied to avoid quantitation bias.

Method validation

Linearity

The linearity of response versus concentration was evaluated using six matrix-matched calibration points (not including zero concentration) over the range of 0.2–500 ng ml^{-1} for all the analytes. Good linear relationships were achieved with regression coefficients (R^2) higher than 0.997 over the examined concentration range, as shown in Table 2.

Limit of detection and limit of quantitation

LOD and LOQ were calculated as three- and 10-fold the *S/N* based on the MRM chromatograms, respectively. The obtained results are listed in Table 2. The LOD and LOQ values ranged from 0.04 to 2.9 ng g^{-1} and from 0.13 to 10 ng g^{-1}, respectively. OTA and DON gave slightly higher values than the other analytes because of low signal responses and indirect matrix effects. Nonetheless, these values still meet the requirements of the most strict European Union regulations, suggesting that this method is sensitive enough for measuring trace amounts of mycotoxins in vegetable oils.

Method accuracy, precision and specificity

The accuracy and precision of the method were evaluated based on the average recovery and the RSD of the recoveries respectively in the spiking recovery test. Three levels of mycotoxins were spiked into six kinds of blank vegetable oils including sunflower seed oil, peanut oil, soybean oil, corn oil, linseed oil and olive oil, and each level contained three replicates. The spiked samples were subjected to extraction and clean-up before analysis using HPLC-MS/MS method. As shown in Table 3, most analytes spiked into six kinds of blank vegetable oils showed high recoveries in the range of 81.7–105.8%, and only DON, 3-Ac-DON and 15-Ac-DON had relative low recovery ranging from 72.8% to 86.9%, but still falling within an acceptable concentration range (Commission of the European Communities 2002, 2006). For within-laboratory reproducibility, all analytes gave excellent RSD values of ≤ 6.4%, which were lower than the acceptable limits of 23% for spiking levels of ≤ 100 μg kg^{-1}, and 16% for 1000 μg kg^{-1} spiking level (Commission of the European Communities 2002). These results demonstrated that the method presented herein is highly accurate and reliable.

In accordance with EU Regulation 2002/657/EC (Commission of the European Communities 2002), the method was developed with compound confirmation using two MRM transitions (one precursor ion and two daughter ions), as discussed above. All 16 mycotoxins can be identified exclusively based on retention time and/or MRM transitions. Although matrix effects can enhance or suppress the analyte responses, no interference compounds have both the MRM transition and retention time similar to those for the target analytes under the optimised conditions. Thus, the developed method has the advantage of high detection specificity.

Application of the method on real samples

Using the developed method, six kinds of representative vegetable oils including 12 sunflower seed oil samples, 10 peanut oil samples, 12 soybean oil samples, eight corn oil samples, 10 linseed oil samples and 10 olive oil from local markets were analysed according to the above procedures. Of the 62 samples tested, six were positive for mycotoxin contamination. The detected mycotoxins were ZEN (n.d.–42.5 ng g^{-1}), AFB1 (n.d.–11.0 ng g^{-1}), AFB2 (n.d.–4.56 ng g^{-1}), AFG1 (n.d.–0.59 ng g^{-1}) and α-ZOL (n.d.–1.43 ng g^{-1}), and they were mainly found in soybean oil and peanut oil, as shown in Figure S2 and Table S1 in the supplemental data online. Although the contamination levels were below the MLs prescribed by the European Union for typical foods, these findings demonstrate the present state of vegetable oil contamination, and thus to ensure consumer health, more attention needs to be paid towards them.

Table 3. Recoveries and relative standard deviations (RSDs) of the 16 mycotoxins in six kinds of vegetable oils.

		Recoveries (%)											
		Sunflower seed oil		Peanut oil		Soybean oil		Corn oil		Linseed oil		Olive oil	
Analyte	Spiked (ng g^{-1})	Mean	RSD[a]	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD	Mean	RSD
AFB1	0.5	93.8	2.6	94.9	1.9	98.6	1.8	93.2	6.4	91.0	4.7	96.2	3.8
	2	92.1	1.8	90.8	3.1	95.1	3.0	91.4	1.0	88.7	2.1	91.1	3.4
	5	94.5	2.4	94.9	1.7	96.6	1.0	93.3	1.8	87.8	1.7	92.2	1.6
AFB2	0.5	91.6	3.8	96.6	2.3	98.1	2.9	90.6	5.9	83.0	3.2	89.6	5.1
	2	91.5	1.1	97.2	2.2	94.8	3.5	92.6	2.7	85.2	2.7	91.2	2.8
	5	93.0	2.2	95.1	2.3	100.3	1.4	94.4	3.2	85.9	1.4	94.4	2.0
AFG1	0.5	93.1	1.9	93.0	3.6	91.9	3.8	92.5	4.7	84.2	2.6	89.8	3.1
	2	91.1	2.0	95.1	5.9	101.0	3.3	92.4	5.3	86.1	2.1	94.6	1.4
	5	94.7	2.2	96.1	2.3	101.6	4.1	95.6	1.4	86.2	0.4	93.5	2.6
AFG2	0.5	91.2	2.3	95.0	2.4	105.8	6.5	92.2	5.0	83.5	1.2	90.9	3.9
	2	92.5	2.3	91.1	2.4	96.9	1.8	92.3	3.0	85.0	1.1	91.7	1.6
	5	92.5	1.7	94.3	0.8	98.1	1.8	94.7	2.4	85.2	2.5	93.1	1.9
AFM1	0.5	92.0	4.2	95.0	5.9	99.5	2.6	94.2	1.7	88.1	2.8	90.5	3.6
	2	91.6	2.7	94.0	6.0	99.2	1.3	93.0	1.7	86.6	1.7	92.4	3.1
	5	94.6	1.9	93.6	1.0	99.6	4.0	93.2	1.8	85.5	1.5	92.9	2.4
AFM2	0.5	90.3	3.6	94.3	2.5	97.4	3.8	89.3	5.2	91.9	3.0	91.3	3.8
	2	92.1	1.6	92.6	3.5	99.5	5.3	90.1	5.1	89.9	2.0	88.6	3.9
	5	94.4	0.7	93.0	2.2	101.0	5.1	91.5	2.9	91.3	2.4	93.1	1.4
ZEN	2	91.3	1.3	92.2	5.5	89.2	1.6	86.3	2.3	84.2	4.3	84.6	4.1
	8	91.7	2.0	93.6	4.2	90.0	2.8	84.4	2.4	82.8	3.0	82.7	2.3
	20	93.2	1.2	96.4	0.8	92.2	2.1	87.6	3.3	82.5	3.6	85.7	3.4
β-ZAL	2	90.9	1.7	96.5	4.1	89.0	4.0	92.4	2.3	86.5	3.0	88.8	3.5
	8	92.0	0.9	93.1	4.2	91.6	1.1	91.4	1.8	84.4	4.8	85.0	0.9
	20	94.6	1.0	93.9	1.3	90.3	4.0	92.5	2.3	87.4	1.6	87.1	1.4
β-ZOL	2	90.1	6.0	94.8	1.7	90.2	2.6	89.5	5.9	90.0	2.8	85.9	5.1
	8	93.6	1.8	92.1	2.5	88.8	4.8	91.3	2.6	86.1	6.2	85.2	1.0
	20	91.6	2.0	95.6	1.8	92.4	2.0	94.7	2.6	87.6	0.7	86.8	1.2
α-ZOL	2	95.2	1.8	92.4	4.8	90.0	2.8	87.7	3.1	79.9	4.1	81.7	3.8
	8	94.0	1.4	93.7	4.7	91.0	1.1	85.3	0.9	84.8	2.1	83.6	1.6
	20	96.6	3.4	94.6	1.5	90.7	3.3	86.7	1.9	81.8	3.5	85.2	2.9
α-ZAL	2	92.1	2.9	94.5	2.1	91.9	2.9	87.4	2.1	82.3	3.8	83.2	2.0
	8	93.2	2.5	90.8	3.4	91.1	1.1	88.9	1.2	80.1	4.0	83.4	2.6
	20	95.2	4.5	95.0	1.1	93.9	4.2	92.1	1.2	83.0	3.3	82.9	2.1
T2	2	91.6	2.0	91.5	1.9	96.9	1.0	91.7	1.6	91.7	3.4	87.8	5.7
	8	89.5	3.7	94.2	2.8	94.6	3.0	93.1	4.1	89.9	1.7	89.3	1.2
	20	88.8	0.2	91.7	3.0	96.3	1.3	92.1	2.3	89.2	1.8	89.7	2.9
OTA	2	82.2	1.9	94.3	6.3	92.4	2.6	85.3	3.1	83.4	2.8	90.4	4.7
	8	83.8	0.9	92.9	3.8	91.6	3.3	89.3	1.0	84.5	3.4	90.9	2.7
	20	88.4	3.1	91.1	4.1	92.3	1.8	88.8	1.9	84.8	2.7	91.6	1.0
DON	20	72.8	3.0	74.6	3.4	77.5	4.0	75.1	1.4	73.2	1.4	80.1	1.4
	80	77.4	3.4	76.1	1.6	78.7	2.0	73.4	1.8	75.7	3.8	75.4	2.9
	200	75.8	1.9	75.6	1.0	78.8	2.6	74.7	1.3	73.2	1.7	78.5	2.0
3 – Ac – DON	50	79.4	5.4	83.9	4.7	91.7	3.0	79.8	5.1	82.7	2.2	84.9	1.9
	200	82.4	2.1	82.4	2.5	92.8	1.3	80.2	1.6	82.4	3.0	86.4	2.9
	500	81.3	1.2	85.3	0.9	90.2	2.3	82.2	2.7	83.5	3.7	86.9	3.2
15-Ac-DON	50	82.7	2.7	79.5	2.1	86.5	4.3	75.8	3.9	80.3	2.9	73.6	1.4
	200	80.6	1.2	81.7	1.0	87.0	1.5	76.6	2.2	78.3	2.7	75.6	2.8
	500	81.2	2.5	80.4	2.9	85.7	2.9	77.6	3.6	79.1	1.4	80.1	1.3

Note: [a]RSD was calculated based on $n_{\text{sample number}} = 3$.

Conclusions

A modified QuEChERS method in combination with optimised HPLC-MS/MS for the simultaneous analysis of 16 common mycotoxins in vegetable oils was developed. These analytes can be separated on a C18 column and analysed within 30 min. The LODs of the sample for these analytes ranged from 0.04 to 2.9 ng ml^{-1}, which are lower than the available maximum tolerance levels in various foodstuffs regulated by the European Union, United States, China and other countries. Spiking recoveries at the different ranges (AFs, 0.5–5 ng g^{-1}; α-ZAL, ZEN, β-ZAL, β-ZOL, α-ZOL, T2 and OTA, 2–20 ng g^{-1}; DON, 20–200 ng g^{-1}; 3-Ac-DON and 15-Ac-DON, 50–500 ng g^{-1}) were within 72.8–105.8% ($n = 3$, RSD < 7%). This method has the advantages of high sensitivity, selectivity, accuracy and throughout, and is thus ideal for applications in target screening of mycotoxins in real vegetable oil samples.

Disclosure statement

No potential conflict of interest was reported by the authors.

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