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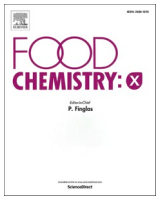

Determination of multi-mycotoxins in vegetable oil *via* liquid chromatography-high resolution mass spectrometry assisted by a complementary liquid–liquid extraction

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ABSTRACT

The simultaneous determination of multi-mycotoxins in food commodities are highly desirable due to their potential toxic effects and mass consumption of foods. Herein, liquid chromatography-quadrupole exactive orbitrap mass spectrometry was proposed to analyze multi-mycotoxins in commercial vegetable oils. Specifically, the method featured a successive liquid–liquid extraction process, in which the complementary solvents consisted of acetonitrile and water were optimized. Resultantly, matrix effects were reduced greatly. External calibration approach revealed good quantification property for each analyte. Under optimal conditions, the recovery ranging from 80.8% to 109.7%, relative standard deviation less than 11.7%, and good limit of quantification (0.35 to 45.4 ng/g) were achieved. The high accuracy of proposed method was also validated. The detection of 20 commercial vegetable oils revealed that aflatoxins B1 and B2, zearalenone were observed in 10 real samples. The as-developed method is simple and low-cost, which merits the wide applications for scanning mycotoxins in oil matrices.

1. Introduction

Mycotoxins are the low-molecular-weight secondary metabolites, which are the natural products of fungal species. However, they may cause a variety of adverse health effects on humans, and livestock (Bräse et al., 2009; Castilla-Fernández et al., 2022; Junsai et al., 2021; Zhong et al., 2021). Moreover, up to 25% of agricultural products and food matrices have been contaminated by the mycotoxins, estimated by the Food and Agricultural Organization of the United Nations (Food and Agriculture Organization of the United Nations (FAO), 2004). Recently, serious concerns have been raised from the consumers and health professionals, with respect to the presence of mycotoxins in various foods (Cui et al., 2022; Yang et al., 2020). Nowadays, vegetable oils extracted from the plant seeds, have gained immense popularity because the oils contain various nutritional components, such as, vitamin E, omega-3, omega-6 fatty acids, and monounsaturated fat (Zhou et al., 2022). The consumption of vegetable oils increases quickly. For example, the global consumption of vegetable oils amounted to 208.81 million metric tons in 2021/22, reported by the U.S. Department of agriculture. However, vegetable oils could easily be contaminated during the manufacturing process since the certain environmental conditions, i.e. high temperature and relative humidity, are favor for the fungal proliferation and mycotoxins production (Abdolmaleki et al., 2021; Bhat & Reddy, 2017; Hidalgo-Ruiz et al., 2019; Junsai et al., 2021; Lee & Ryu, 2017). The findings have revealed that a variety of mycotoxins have been identified in the oils and their seeds, including aflatoxins (AFB1, AFB2, AFG1 and AFG2), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEN), fumonisins (FB1) and T-2 toxin (T-2) (Sharmili, Jinap & Sukor, 2016). According to the classification provided by the International Agency for Research on Cancer, the four aflatoxins (AFB1, AFB2, AFG1 and AFG2) are the group 1 carcinogens, and OTA, FB1 are the group 2B carcinogens, while DON, ZEN and T2 are the group 3 carcinogen (IARC, 1993;

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IARC, 2012; Ostry et al., 2016). Therefore, it is essential to develop suitable approaches for simultaneous determination of the multi-mycotoxins in the complicated matrices, such as, vegetable oils.

The analysis of multi-mycotoxins in vegetable oils is a challenge due to the complexity of the matrices and the low concentrations of multi-mycotoxins. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been considered as a popular technique for the simultaneous determination of multi-mycotoxins in matrices because of its high efficiency, sensitivity and specificity in the structural identification and quantitative analysis (Hidalgo-Ruiz et al., 2019; Kunz et al., 2020; Li et al., 2022; Sharmili, Jinap & Sukor, 2016; Zhao et al., 2022). Nevertheless, it is well-known that the accuracy of MS detection is influenced greatly by the matrix effects (MEs) of matrices (Wang et al., 2022). Generally, the MEs originate from the co-eluting compounds of the target analytes in MS, which could lead to the suppression or enhancement in the signals of the analytes. Thus, the MEs need to be eliminated sufficiently to obtain a satisfied accuracy. As above-mentioned, there are large differences in physicochemical properties of the multi-mycotoxins, and complexity of the oil matrices (Jia et al., 2014). In this regard, several strategies have been proposed to diminish the MEs, including suitable sample pretreatment process and utilization of isotopically-labelled internal standards. The efficient sample pretreatments include liquid–liquid extraction (LLE), solid phase extraction (SPE), immunoaffinity chromatography (IAC), gel permeation chromatography, and the quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Afzali et al., 2012; Drzymala et al., 2015; Hidalgo-Ruiz et al., 2019; Qian et al., 2015; Sharmili, Jinap & Sukor, 2016; Zhou et al., 2017). The IAC can provide high selectivity. It has been regarded as the reference

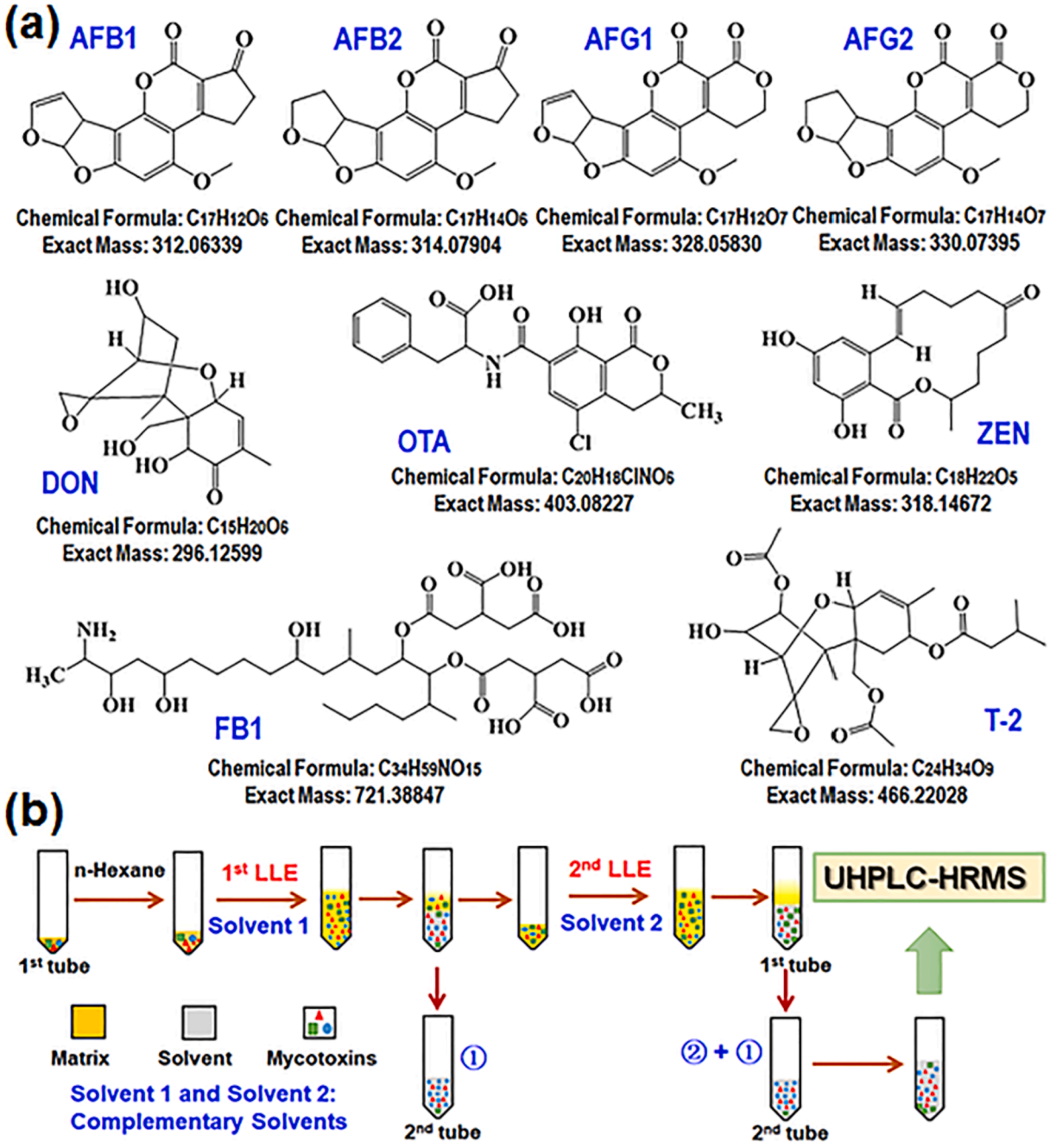

Fig. 1. (a) Chemical structure of nine mycotoxins investigated in the study, including AFB1, AFB2, AFG1, AFG2, DON, OTA, ZEN, FB1 and T-2; (b) diagrammatic representation of the complementary liquid–liquid extraction (LLE) process.

clean-up method (Ma et al., 2013). However, IAC needs the expensive cartridges, by which the matrix interferences could be eliminated effectively (Ma et al., 2013; Xie, Chen & Ying, 2016). Furthermore, the sorbents in the cartridges vary due to the different analysts. Consequently, the costs of IAC-related detection techniques are relatively high. Additionally, the MEs could also be compensated using the quantification approach (Kunz et al., 2020). Therefore, the matrix matched calibration method and isotopically-labelled internal standards approach have been widely used for the quantification analyses (Zhang et al., 2017; Zhang & Xu, 2019). Nevertheless, the insufficiency of matrix-matched matrices, high cost, and shortage of ^{13}C-isotopically-labelled internal standards for multi-mycotoxins hinder their wide applications in routine screening procedure (Slobodchikova & Vuckovic, 2018). Thus, it is highly desirable to explore cost-effective and efficient sample pretreatment approaches for simultaneous identification and quantification of multi-mycotoxins in the complicated matrices.

Herein, we report the liquid chromatography-quadrupole exactive orbitrap mass spectrometry with a complementary liquid–liquid extraction pretreatment process to analyze the multi-mycotoxins in the vegetable oils. The efficient sample pretreatment step involved a successive liquid–liquid extraction process with the complementary solvents. The matrix effects could be negligible, and good recovery was obtained through the convenient sample pretreatment. Moreover, the high accuracy was achieved using the matrix-free external calibration approach, which could be comparable with that in liquid chromatography-isotope dilution tandem mass spectrometry (LC-ID-MS/MS). The proposed approach possesses the great potential for simultaneous identification and quantification of multi-mycotoxins in the commercial vegetable oils.

2. Materials and methods

2.1. Reagents and chemicals

The chemical structures of the nine mycotoxins are shown in Fig. 1a. AFB1 (GBW10172, National Institute of Metrology, China, 98.3% ±1.0%), DON (purity ≥ 99.9%) and ZEN (purity ≥ 99%) were obtained from the First standard (Worcester, MA, U.S.A.). AFB2 (purity ≥ 99%), AFG1 (purity ≥ 99%), AFG2 (purity ≥ 99 %) and FB1 (purity ≥ 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). OTA (purity ≥ 99%) and T-2 (purity ≥ 99%) were obtained from Romer (Worcester, MA, U.S.A.). Acetonitrile (ACN, HPLC grade), formic acid (HPLC grade), ammonium acetate (HPLC grade, purity > 99%) and methanol (HPLC grade) were obtained from Fisher Scientific (CA, U.S.A.). PTFE syringe filter (0.22 μm) was obtained from NovasyGen (Beijing, China). Ultrapure water system (Millipore, Bedford, MA, U.S.A.) was used in the study.

2.2. Instrument and apparatus

The separation of mycotoxins samples was performed on a Thermo Vanquish ultra-high performance liquid chromatography system equipped with an auto-injector, and a binary solvent delivery system (Thermo Fisher Scientific, U.S.A.). Chromatography separation was performed on an ACQUITY UPLC HSS T3 Column (2.1 mm × 50 mm, 1.8 μm particle size; Waters, U.S.A.). The injection volume of sample was 2.0 μL with a flow rate of 0.3 mL/min, and the column temperature was maintained at 40 °C. The mobile phase was a gradient solution, which was composed of two solutions, solution A and solution B. Solution A was aqueous solution containing 0.1% formic acid and 1 mmol/L ammonium acetate. Solution B was methanol solution containing 0.1% formic acid. Gradient elution started from the solution B with volume ratio of 28%, and then, the proportion of solution B increased linearly to 100 % within 6 min. After that, the gradient elution changed to solution B (28%) at 6.01 min, and kept isocratic for 2 min. The total operation time was 8 min.

Qualitative and quantitative profile of multi-mycotoxins were performed on a Thermo Q-Exactive Plus Orbitrap-MS detection system with an electrospray ionization (ESI) source in positive and negative ionization modes. The parameters were as follows: spray voltage: +3.70 kV (positive), and −2.70 kV (negative); capillary temperature: 320 °C; sheath gas flow rate: 5.0 μL/min; Aux gas flow rate: 10.0 μL/min; sweep gas follow rate: 0.0 μL/min; S-lens RF level: 50.0%; Aux gas heater temperature: 305 °C. The data was acquired under the Parallel Reaction Monitoring (PRM) scanning mode. The Q-Orbitrap was operated in the time-scheduled sequential PRM scanning mode with the 18 separated windows. The mode was comprised of a targeted scan (scanning range: *m/z* 100 ~ 800; resolution: 35000FWHM) and a full MS^2 scan with a fragmentation energy applied. The automatic gain control (AGC) target (the number of ions to fill C-Trap) was controlled to be $1.0e^6$ with a maximum injection time of 200 ms. The resolution window of MS/MS was 2.0 *m/z* with a resolution of 17,500 FWHM. PRM data were proceed by an Xcalibur 4.1 software (Thermo Fisher Scientific, USA), according to the optimized processing method.

2.3. Sample preparation

A complementary liquid–liquid extraction (LLE) pretreatment approach was developed for the sample preparation in the study (Fig. 1b). The mixtures of acetonitrile and water with different volume ratios were used as the complementary solvents for the LLE process. In the study, 90:10, 80:20, 70:30, 60:40 50:50, 40:60, 30:70, 20:80, and 10:90 ACN: water (v/v) were optimized accordingly. Typically, the sample (1.000 g) was initially mixed with n-hexane (5.0 mL) for 2 min in a centrifuge tube (50.0 mL). Then, 5.0 mL of 80:20 ACN: water (v/v) was added in the 1st LLE step, and the solution was mixed for another 10 min. Then, the mixture was centrifuged at 4000 rpm (1239 × g) for 5 min. After that, the clear phase at the bottom of tube was collected and transferred into another centrifuge tube (2nd tube). Subsequently, the 2nd LLE treatment was conducted, and the complementary solvent of the 1st LLE step, 5.0 mL of 20:80 ACN: water (v/v), was added into the 1st centrifuge tube. The solution was mixed for 10 min again, and centrifuged at 4000 rpm (1239×g) for 5 min. The as-obtained clear liquid at the bottom of tube was then transferred into 2nd tube, and combined with the solution obtained in the 1st LLE step. They were mixed and homogenized for a while. Prior to the UHPLC-HRMS analysis, the collected sample was filtered using a PTFE syringe filter (0.22 μm) and injected directly into the detection system.

2.4. Matrix effects

2.4.1. Calculation of the matrix effects

Firstly, the commercial peanut oil, corn oil, rapeseed oil, and blend oil were treated through the complementary liquid–liquid extraction process to yield the four blank matrices (also named as matrix-matched solvents). Then, the nine mycotoxins were added individually into the blank matrices to prepare the matrix-matched calibrants, respectively. In the meantime, the standard calibrants were prepared by adding the nine mycotoxins into the mixed solvent of ACN and water (50:50, v/v), respectively. To assess the MEs of each mycotoxin, the concentration of each analyte in the matrix-matched calibrants was equivalent to that in the standard calibrants. The ME was evaluated using UHPLC-QE MS method in the study, and the ME could be calculated according to the following Eq. (1):

$$ME = A_M/A_S \quad (1)$$

where A_M refers to the peak area of mycotoxin in the matrix-matched calibrants, and A_S refers to the peak area of mycotoxin in the standard calibrants (Zhou et al., 2017).

2.4.2. Post-column infusion system

A post-column infusion system was used to check the MEs of the UHPLC-QE MS (Gab-Allah, Choi & Kim, 2021). In the system, blank peanut oil matrix was obtained from the commercial peanut oil sample, which was treated through the complementary liquid–liquid extraction process. Then, the blank matrix was injected into the LC column. In the meanwhile, the standard solution containing nine target analytes (20 ng/g) was also infused at a constant flow (10 μL/min) into LC eluent via a T-connector (Gab-Allah, Choi & Kim, 2021). T-connector was installed between the LC column and the MS ion source. The MS was operated in the normal PRM mode to obtain the matrix effect profiles.

2.5. Quantification of mycotoxins in vegetable oils

The quantification analysis of each mycotoxin was checked via five-point external calibration curve approach in the study. The calibration curve ($y = ax + b$) was plotted, on the basis of concentration of mycotoxins as the x-axis, and the corresponding peak area as the y-axis. Then, the concentration (M) was calculated according to the following Eq. (2).

$$M = \frac{(A_x - b)}{a} \times R \quad (2)$$

Where M is the concentration of mycotoxins in vegetable oil sample (ng/g); a is the slope of the calibration curve; A_x is peak area of the target mycotoxins in vegetable oil sample; b is the intercept of the calibration curve and R refers to the dilution ratio.

2.6. Method validation

The typical parameters of method validation, including linearity, recovery, intra-day and inter-day precision, limit of detection (LOD), and limit of quantification (LOQ) were evaluated in detail. Product ions were utilized for the LOQ evaluation. A calibration curve was performed to assess the linearity, with the concentration ranging from 0.04 to 8232 ng/g. The recoveries were investigated by the spiked samples at three levels (LOQ, 10LOQ and 100LOQ), with the peanut oil, corn oil, rapeseed oil and blend oil as the representative matrices. Precision, repeatability (intra-day precision), and reproducibility (inter-day precision) were also evaluated, and the results were expressed with a relative standard deviation (RSD, %). The limit of detection (LOD) was defined as the lowest concentration, at which the analyte could be detected (mass error <5 ppm). The limit of quantification (LOQ) was calculated on the basis of signal-to-noise ratio at 10.

3. Results and discussion

3.1. Optimization of UHPLC/QE MS parameters

The UHPLC QE-MS was utilized to detect the multi-mycotoxins, and the parameters for both mass spectrometry and chromatography were optimized accordingly. The target compounds with concentration of 500 ng/g were infused directly into the QE-MS system. Full mass scanning was carried out in the range between 100 and 800 (m/z) with both positive and negative ionization full scan mode. Additionally, the formic acid or ammonium acetate was also added into the mobile phase to promote the formation of adducts. Thus, $[M + H]^+$ and $[M + NH_4]^+$ could be found in the MS (Lim et al., 2015). The ions with a high intensity threshold were then fragmented to produce the MS^2 ions. The precursors and corresponding product ions of the nine mycotoxins with theoretical mass and experimental mass are summarized in Table 1. Eight mycotoxins, including, AFB1, AFB2, AFG1, AFG2, DON, OTA, FB1 and T-2, were performed in the positive ESI^+ mode, whereas ZEN was conducted in a negative ESI^- (Rodríguez-Carrasco et al., 2019). More importantly, all the accuracy for the mycotoxins were less than 3.5 ppm in the study (Table 1). Consequently, the utilization of Orbitrap MS system enabled a highly confident identification of analytes because of its high mass accuracy.

Chromatographic conditions were also adjusted to achieve the satisfied separation and retention time for the nine mycotoxins. The separation was optimized using different mobile phases with an UPLC HSS T3 (2.1 mm × 50 mm, 1.8 μm) column. Specially, acetonitrile and methanol were the organic phases, and aqueous solution was the polar phase containing different concentrations of formic acid or ammonium acetate (Sharmili, Jinap & Sukor, 2016; Mao et al., 2018). At the same time, the concentrations of formic acid (FA, 0.01–0.5%) and ammonium acetate (AA, 1–5 mmol/L) were also optimized. The typical chromatogram for the nine analysts is shown in Fig. 2a. The optimal mobile phase was methanol and water, and both of them contained formic acid (0.1%) and ammonium acetate (1.0 mmol/L).

3.2. Complementary LLE process

It is well-known that the nine mycotoxins are complicated compounds. Their acidity ranges from pK_a of 3.2 to 17.8, and their polarity changes from logP of −1.0 to 4.6 (HMDB; PubChem; Slobodchikova & Vuckovic, 2018). To obtain high recovery and good selectivity, the MEs of oil matrices need to be minimized during the measurement. The extraction solvents for the LLE steps were optimized in detail. Peanut oil was used during the pretreatment optimization. In the study, hexane was applied to remove the lipophilic components of the oil samples, as shown in Fig. 1b. Acetonitrile and methanol are commonly organic solvents for the sample pretreatment step because of their suitable polarities and the high extraction yields for both polar and medium polar compounds. In addition, water is as a green solvent for purification and extraction process due to its dipole moment and hydrogen bond. Thus, the three solvents and their mixtures were applied in the LLE process. The volume of extraction solvent was 5 mL, and the corresponding recoveries are shown in Fig. 3a. Nevertheless, single component, methanol, is unsuitable for the oil extraction because it is completely soluble with the oil matrices. The recoveries for multi-mycotoxins, including AFB1, AFB2, AFG1, AFG2, DON and T-2, were in the range from 10% to 108% when seven different extraction solvents were utilized (Fig. 3a). However, as for the OTA, ZEN and FB1, the typical recovery was very low (~20%), in case of single acetonitrile as the extraction solvent. When a certain amount of water was mixed with acetonitrile, the recoveries enhanced largely. Moreover, as for the OTA and FB1, the mixed

Table 1
UHPLC-HRMS parameters for the determination of multi-mycotoxins.

Mycotoxins	Adduct ions	Theoretical mass (m/z)	Experimental mass (m/z)	Accuracy (Δppm)	Product ion (m/z)	Collison energy (CE)
AFB1	$[M+H]^+$	313.07066	313.06976	2.87	285.074	25
AFB2	$[M+H]^+$	315.08631	315.08530	3.21	287.091	27
AFG1	$[M+H]^+$	329.06558	329.06470	2.67	243.064	30
AFG2	$[M+H]^+$	331.08123	331.08020	3.11	313.070	32
DON	$[M+H]^+$	297.13326	297.13278	1.62	249.112	11
OTA	$[M+H]^+$	404.08954	404.08853	2.50	257.020	27
ZEN	$[M-H]^-$	317.13945	317.13934	0.35	175.039	28
FB1	$[M+H]^+$	722.39575	722.39624	−0.68	334.310	48
T-2	$[M+NH_4]^+$	484.25411	484.25430	−0.39	305.138	10

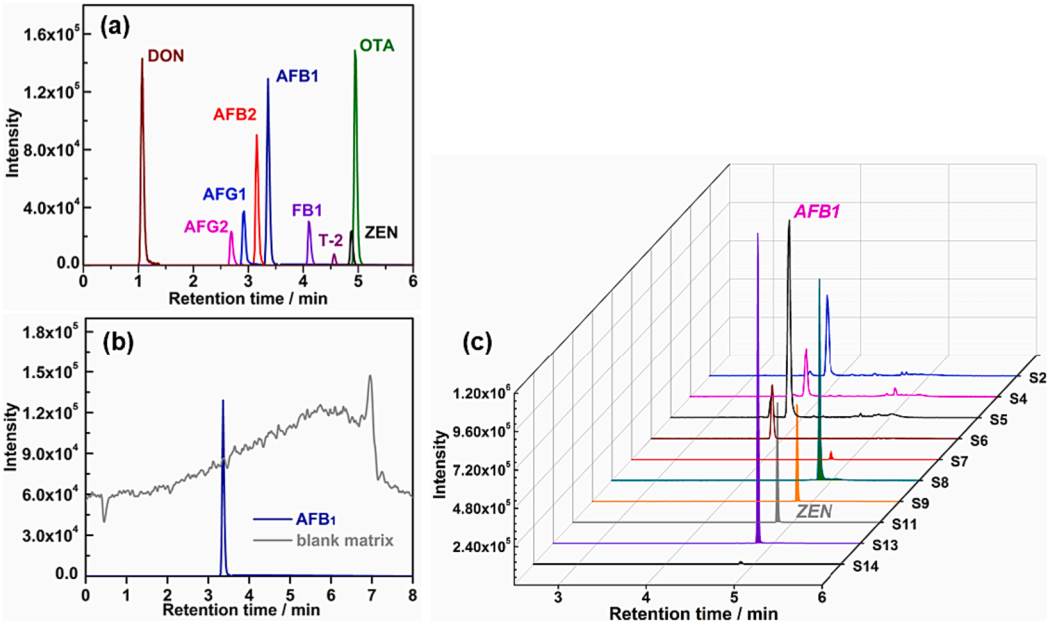

Fig. 2. (a) The typical parallel reaction monitoring (PRM) chromatograms of the nine mycotoxins under the optimal condition, including AFB1, AFB2, AFG1, AFG2, DON, OTA, ZEN, FB1 and T-2; (b) the PRM chromatogram representing matrix effect profile obtained from the post-column infusion of the AFB1 standard solution at a level of 20 ng/g and LC run of extract of blank oil sample after the complementary LLE process; overlay is PRM chromatogram obtained from a normal LC run of blank oil sample fortified with AFB1 standard solution; (c) the typical chromatogram of multi-mycotoxins in ten real oil samples obtained from supermarkets in Beijing, detected by UHPLC QE-MS method assisted by a complementary liquid–liquid extraction process.

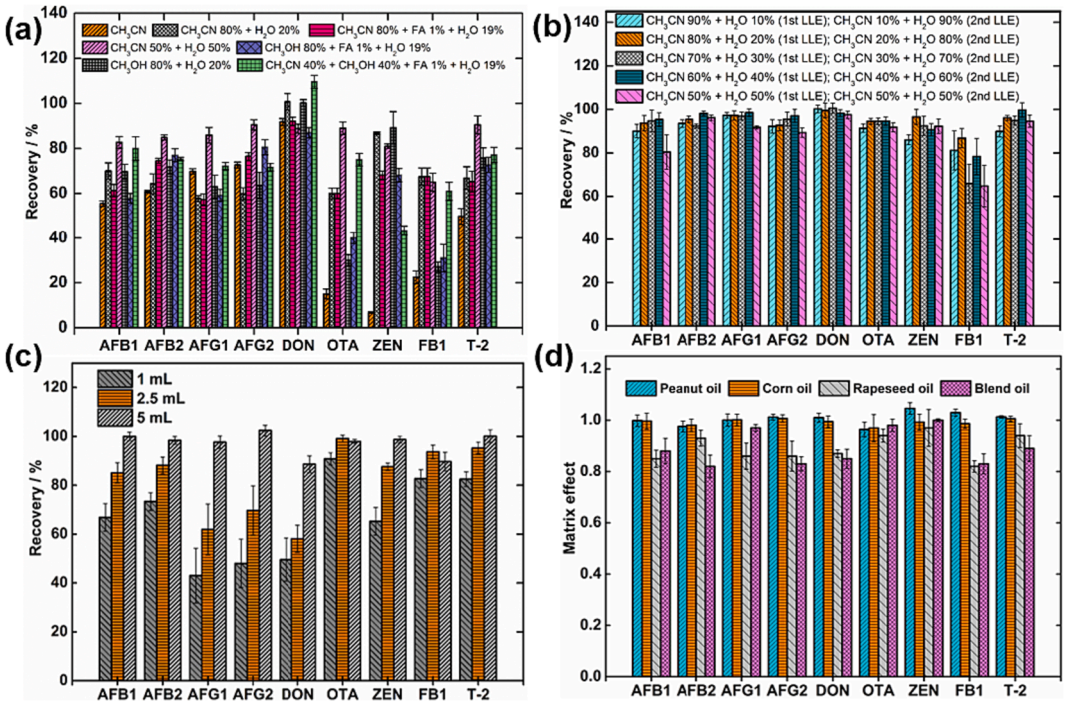

Fig. 3. (a) Effect of the type of the extraction solvents (5 mL) on the recoveries of multi-mycotoxins; (b) effect of the complementary extraction solvents (5 mL) used in the 1st LLE and 2nd LLE processes on the recoveries of multi-mycotoxins; (c) effect of the volume of extraction solvent on the recoveries of multi-mycotoxins; (d) matrix effects of multi-mycotoxins in the four types of oil matrices. Five sample replicates (n = 5) were conducted in the study.

solvents of acetonitrile and water showed higher recovery than those in the mixed solvents of methanol and water. The recoveries of nine analysts in the mixed solvents (acetonitrile and water) were up to 60%. Interestingly, most of the mycotoxins reached the highest recoveries when extracted using the mixed solvents of acetonitrile and water (50:50, v/v). Additionally, the recoveries changed negligibly upon increasing the acidity of the mixed solvents, i.e. adding FA (1%) into the solvent. When the seven solvents were used in the 1st LLE step, the average recovery for each mycotoxin was merely 60%. Obviously, the value was far lower than the expectation value (85%), which meets the basic requirement for the accurate quantification (Hidalgo-Ruiz et al., 2019).

In this regard, a complementary LLE approach was proposed in the study (Fig. 1b), in which the 2nd LLE process was conducted using the complementary solvents of the 1st LLE step, and the solvent volume was 5 mL in the two extraction steps. For example, the complementary solvent for the mixture of acetonitrile and water (90:10, v/v) was the mixture of acetonitrile and water (10:90, v/v). Mixed solvents of acetonitrile and water with various volume ratios, including 80:20, 70:30, 60:40 and 50:50, and their complementary solvents, were prepared and utilized as the extraction solvents in the LLE process. The corresponding recovery is shown in Fig. 3b, as for the multi-mycotoxins extracted by different complementary solvents. Obviously, upon the complementary liquid–liquid extraction pretreatment process, the recoveries for all analytes enhanced dramatically, and most of recoveries were over 85% (Fig. 3b). The optimal extraction solvents for the LLE process were the mixture of acetonitrile and water (80:20, v/v) and the mixture of acetonitrile and water (20:80, v/v) since the recovery of FB1 was over 85%. Furthermore, different volumes of extraction solvents (1.0, 2.5 and 5.0 mL) in each extraction process were further investigated. As shown in Fig. 3c, the recoveries reached the peak values when the volume was 5.0 mL. The corresponding recovery for the multi-mycotoxins ranged from 96% to 105%. Thus, the optimal volume of extraction solvent was 5.0 mL for each LLE step in the study.

3.3. Matrix effects (MEs)

Since it is the major source of substantial losses in the detection sensitivity and precision, the MEs of as-proposed technique are evaluated. Four oil matrices, including peanut oil, corn oil, rapeseed oil and blend oil, were chose for the matrix effect evaluations. As shown in Fig. 3d, the values of ME changed from 0.96 to 1.05 in peanut oil, 0.98 to 1.06 in corn oil, 0.85 to 0.97 in rapeseed oil, and 0.82 to 1.00 in blend oil, respectively. The results demonstrated that there were no obvious ion enhancement or depression effect for the nine targets in the various oil matrices. Moreover, the post-column infusion technique was applied to evaluate the MEs of the proposed method (Gab-Allah, Choi & Kim, 2021). The MEs of the nine mycotoxins were measured individually. The typical PRM chromatogram represented ME profile obtained from post-column infusion of AFB1 (Fig. 2b). The proposed complementary LLE approach exhibited a steady and stable ionization profile, suggesting no substantial signal suppression or enhancement regions from co-eluting sample matrices in the LC run. The same phenomena were also observed for other eight mycotoxins (Fig. S1). The results also confirmed that the complementary LLE could effectively eliminate sample matrices during the multi-mycotoxins detection. The negligible matrix effects could result from the successive liquid–liquid extraction pretreatment using the complementary solvents as the extraction solvents. More importantly, the as-obtained MEs could be comparable to those in the previous approaches, i.e. SPE cleanup, QuEChERS, gel permeation chromatography and IAC cleanup (Afzali et al., 2012; Desmarchelier et al., 2014; Qian et al., 2015; Sharmili, Jinap & Sukor, 2016).

3.4. Method validation

Subsequently, the performance of the proposed approach was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision. Five sample replicates (n = 5) were conducted in each experiment. The corresponding value of each parameter is summarized in Table 2, demonstrating that the good linearity for the nine mycotoxins. The LODs of the nine mycotoxins were in the range of 0.04 ~ 9.20 ng/g, and LOQs changed from 0.35 to 45.44 ng/g. To further validate the proposed method, nine mycotoxins were spiked individually into four types of vegetable oils, including peanut oil, corn oil, rapeseed oil and blend oil, at three concentration levels (LOQ, 10LOQ and 100LOQ). The recoveries changed from 80.8% to 109.7% with the RSD lower than 11.7% (Table 3). In addition, as for the inter-day and intra-day assessments, the RSD for all analytes were lower than 11.7%.

Table 2
Validation parameters for the multi-mycotoxins detected in this study.

Mycotoxins	Linear rang (ng/g)	R^2 [a]	LOD (ng/g) [b]	LOQ (ng/g) [c]
AFB1	0.04–75.87	0.9998	0.04	0.35
AFB2	0.09–83.43	0.9997	0.09	0.46
AFG1	0.09–77.85	0.9999	0.09	0.43
AFG2	0.20–244.59	1.000	0.20	1.35
DON	8.20–8232.19	0.9999	9.20	45.44
OTA	0.40–795.26	0.9999	0.40	2.16
ZEN	0.50–390.78	0.9994	0.45	4.39
FB1	2.20–2019.88	0.9998	2.20	11.15
T-2	0.60–602.34	0.9999	0.60	3.33

[a] R^2: coefficient of determination.
[b] LOD: limit of detection.
[c] LOQ: limit of quantification.

Furthermore, liquid chromatography-isotope dilution tandem mass spectrometry (LC-ID-MS/MS) was used to validate the results measured by the proposed method. As summarized in Table S1, the values measured by the two methods were very close in case of analyzing the nine mycotoxins in the peanut oil matrix. Furthermore, the results of the two methods were evaluated using the independent sample *t*-test (ISO Guide 35, 2017). The value of *t* for each mycotoxin was smaller than the critical value of $t_{0.05}$ (2.31) (Table S1). The results demonstrate that the precisions of the proposed method and LC-ID-MS/MS method were consistent. Therefore, the proposed method had a good precision for the detection of multi-mycotoxins. In addition, the proposed method was utilized to determine the mycotoxins in the certified reference materials, GBW10175 (ZEN in corn oil) and GBW10176 (AFB1 in peanut oil). The measured values are summarized in Table S2. Obviously, the measured values by the proposed method could be comparable to the certified values (Table S2), reflecting the high accuracy of as-developed method. Therefore, all results demonstrate that high accuracy of the quantification for mycotoxins in vegetable oils could be achieved via the complementary LLE pretreatment process and an external calibration method.

For comparison, previous approaches for detection of mycotoxins in the vegetable oils are summarized in Table S3. Evidently, the complementary LLE pretreatment process could reduce the matrix effect effectively, compared with the conventional SPE and QuEChERS procedure (Hidalgo-Ruiz et al., 2019; Sharmili, Jinap, & Sukor, 2016; Zhang et al., 2017; Zhou et al, 2017). In addition, compared with LC-ID-MS/MS method or matrix-matched calibration method, the as-developed method possessed the outstanding precision and recovery for the multi-mycotoxins detection in vegetable oils (Desmarchelier et al., 2014; Hidalgo-Ruiz et al., 2019; ISO Guide 35, 2017; Qian et al., 2015; Sharmili, Jinap & Sukor, 2016; Yu et al., 2019; Zhang et al., 2017; Zhou et al., 2017). More importantly, although its sensitivity was relatively inferior to those pretreatment methods with sample enrichment processes, the proposed method could also be suitable for the worldwide MRL regulation (Qian et al., 2015; Xu et al., 2021;Yu et al., 2019; Zhou et al., 2017).

3.5. Identification and quantification of multi-mycotoxins in real vegetable oils

Finally, the proposed method was utilized to determine the multi-mycotoxins in commercial vegetable oils. Twenty brands of vegetable oils were collected from the supermarkets in Beijing. Different matrices of oils were analyzed, including peanut oil, sunflower seed oil, corn oil, blended oil, rapeseed oil, soybean oil and olive oil. The measured values for the multi-mycotoxins in oil samples are summarized in Table S4, and the typical chromatograms of ten oil samples are shown in Fig. 2c. The results showed that 10 out of 20 samples were contaminated by mycotoxins. Especially, the mycotoxins, i.e. AFB1, AFB2 and ZEN could be

Table 3
Average recoveries (%, n = 5) and relative standard deviations (RSD) of nine mycotoxins extracted from the four different matrices at three fortification levels.

Mycotoxins	Peanut oil			Corn oil			Rapeseed oil			Blend oil		
	LOQ [a]	10LOQ	100LOQ	LOQ	10LOQ	100LOQ	LOQ	10LOQ	100LOQ	LOQ	10LOQ	100LOQ
AFB1	98.5 ± 6.3	100.0 ± 3.6	99.8 ± 2.5	96.5 ± 5.3	99.0 ± 3.3	99.8 ± 3.5	89.2 ± 5.3	86.4 ± 5.8	90.8 ± 3.9	86.3 ± 4.5	90.3 ± 3.8	96.9 ± 3.1
AFB2	102.3 ± 9.7	96. 8 ± 2.2	100.4 ± 0.9	100.3 ± 7.7	97. 8 ± 4.3	99.8 ± 3.5	90.2 ± 7.8	96.4 ± 5.8	97.8 ± 3.2	88.3 ± 3.5	89.3 ± 3.9	90.8 ± 2.9
AFG1	100.2 ± 9.1	101.6 ± 2.0	99.8 ± 1.6	101.3 ± 3.1	101.6 ± 2.0	99.8 ± 1.6	94.9 ± 6.8	90.1 ± 10.8	90.6 ± 6.6	108.2 ± 8.9	90.6 ± 6.0	94.5 ± 4.7
AFG2	109.7 ± 9.5	97.2 ± 7.5	99.8 ± 2.1	105.6 ± 5.5	96.2 ± 6.5	98.9 ± 5.1	90.3 ± 7.2	90.6 ± 10.2	91.5 ± 5.8	85.8 ± 9.7	88.2 ± 7.5	90.6 ± 8.9
DON	101.4 ± 7.1	97.3 ± 2.8	100.1 ± 2.0	98.4 ± 6.1	95.3 ± 2.9	101.1 ± 2.9	89.8 ± 5.8	88.3 ± 4.8	86.6 ± 3.7	86.7 ± 5.8	87.3 ± 4.1	88.6 ± 3.6
OTA	101.1 ± 9.7	96.6 ± 3.3	98.9 ± 3.3	96.8.1 ± 7.7	94.6 ± 3.9	97.6 ± 4.8	103.6 ± 5.5	103.0 ± 3.2	98.3 ± 4.6	107.9 ± 5.7	106.2 ± 4.2	101.2 ± 2.9
ZEN	94.5 ± 11.1	102.5 ± 4.6	102.8 ± 2.3	95.5 ± 10.5	100.5 ± 5.6	103.8 ± 4.63	108.9 ± 5.2	99.4 ± 4.3	101.3 ± 6.3	102.4 ± 6.0	103.2 ± 5.2	106.0 ± 4.0
FB1	98.8 ± 10.7	98.6 ± 6.6	103.0 ± 4.0	95.8 ± 8.9	97.6 ± 9.8	102.0 ± 9.5	80.8 ± 11.7	90.6 ± 8.6	90.9 ± 8.1	108.8 ± 10.7	106.6 ± 11.2	93.4 ± 7.4
T-2	93.5 ± 7.2	96.0 ± 4.9	105.3 ± 8.4	95.5 ± 7.9	97.0 ± 5.2	104.5 ± 7.4	102.3 ± 5.9	93.3 ± 4.0	95.6 ± 3.9	88.2 ± 7.0	92.3 ± 6.1	91.0 ± 5.8

[a] LOQ: limit of quantification.

detected in the oils. The concentration of AFB1 in peanut oils ranged from 7.20 (S4) to 38.9 ng/g (S5), and the concentration of AFB2 was in the range from 1.40 to 6.79 ng/g. In addition, AFG1 and AFG2 could be detected occasionally in peanut oils. Moreover, our results revealed that AFB1 was present at the highest concentration in the contaminated peanut oil, corresponding to the previous reports (Zhou et al., 2017). However, a high concentration of ZEN could be observed in oils with a range of 5.30 ~ 209.4 ng/g, and these samples included S7, S8, S9, S11, S13 and S14 (Fig. 2c). ZEN could also be found in the blended oil, soybean oil and olive oil. ZEN was the mycotoxin which was found in most of real samples. It was often frequently reported in the previous studies (Hidalgo-Ruiz et al., 2019). Nowadays, over 80 countries and regions have established the maximum limits (MLs) of AFB1 and ZEN in the various foods and agricultural commodities. For example, the ML for ZEN is in the range between 50 and 400 ng/g, and the corresponding ML for AFB1 is 5–20 ng/g in food and feed commodities (Food and Agriculture Organization of the United Nations (FAO), 2004). Thus, as observed from Table S4, the measured values in samples S2 and S5 were very close to the ML of AFB1. The ZEN in S13 also reached the ML. All results demonstrate that the as-developed UHPLC QE-MS assisted by a complementary liquid–liquid extraction process is a facile and efficient approach for simultaneous detection of multi-mycotoxins in the real vegetable oils. The proposed method could be a reference approach for the detection of commercial oil samples.

4. Conclusions

In conclusion, liquid chromatography-quadrupole exactive orbitrap mass spectrometry employing a successive liquid–liquid extraction pretreatment process has been successfully developed for simultaneous identification and quantification of multi-mycotoxins in the vegetable oils. The matrix effects could be sufficiently eliminated by the complementary liquid–liquid extraction pretreatment. More importantly, the results of method validation confirmed that the proposed approach exhibited a satisfactory linearity, LOD, LOQ, recovery as well as reproducibility. Besides, the proposed approach also demonstrated its significant application merits for the analysis of mycotoxins in real oils samples.

CRediT authorship contribution statement

Shuangqing Li: Investigation, Formal analysis, Validation, Writing – original draft. **Siyao Zhang:** Investigation, Formal analysis. **Xiaomin Li:** Resources, Conceptualization, Methodology, Writing – review & editing. **Shukun Zhou:** Formal analysis, Validation. **Jiahui Ma:** Formal analysis, Validation. **Xiaotong Zhao:** Formal analysis, Validation. **Qinghe Zhang:** Resources, Supervision. **Xiong Yin:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

The following Figures and Tables were supplied in the Supplementary data, including, Figure S1: The PRM chromatogram obtained from post-column infusion of eight mycotoxins (AFB2, AFG1, AFG2, ZEN, DON, OTA, FB1 and T-2); Table S1: Comparison of the measured values for the nine mycotoxins; Table S2: Comparison between the certified values and the measured values in CRM; Table S3: Comparison of reported approaches for determination of mycotoxins in oil sample; Table S4: The measured values of the multi-mycotoxins in the 20 real samples. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100887.

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