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Multi-mycotoxin detection and human exposure risk assessment in medicinal foods

Xiaofang Liao [a,b], Ying Li [b], Nan Long [b], Qingbin Xu [b], Peng Li [c], Jiabo Wang [a], Lidong Zhou [b], Weijun Kong [a,b,*]

[a] School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, China
[b] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, China
[c] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, 999078, Macau

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ABSTRACT

Mycotoxin contamination in medicinal foods has attracted increasing global attention. In this study, a simple and sensitive ultrasonication assisted one-step extraction based ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS) method was developed for simultaneous detection of multi-mycotoxins in five kinds of medicinal foods rich in starch. Under optimal conditions, the developed technique displayed excellent analytical performances. Limits of detection and quantitation for the six mycotoxins were 0.04–0.25 ng/mL and 0.10–0.67 ng/mL, respectively. Average recoveries at three fortified levels ranged from 75.33 % to 118.0 %. Real-world application in 103 batches of medicinal foods displayed that 58 samples were positive with one or more mycotoxins at an occurrence rate of 56.31 % (58/103). Coix seed gave the highest positive rate of 96.15 %, followed by Lily (90 %), Chinese yam (50 %), Lotus seed (34.04 %) and Malt (30 %). Zearalenone had the highest positive rate of 28.16 % with contents in 5 Coix seeds exceeding the maximum residue limit (MRL), followed by aflatoxin B_1 of 27.18 % (28/103) with contents in 7 Coix seed and 10 Lotus seeds over its MRL, and ochratoxin A (OTA) of 11.65 % with contents in 1 Lotus seed and 5 Lily samples greater than its MRL. Exposure risk assessment indicated that Coix seed and Lotus seeds that were susceptible to aflatoxins posed great threats to human health. Long-term consumption of Lily that was easily contaminated with OTA were also harmful. This work provides a robust platform for multi-mycotoxin monitoring in medicinal foods to protect the consumers from potential health risks.

1. Introduction

Among the global food safety issues, the occurrence of mycotoxins is a foremost concern (Iqbal, 2021; Jallow et al., 2021). It is estimated that approximately 60–80 % of foods produced in the world are contaminated with mycotoxins (Eskola et al., 2020). Mycotoxins are secondary metabolites with low molecular weights naturally secreted by toxigenic fungi species (Juraschek et al., 2022). At present, >300 different mycotoxins have been discovered with varying toxicities to humans and animals (Alshannaq and Yu, 2017; Alassane-Kpembi et al., 2017). Of them, aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) with strong toxic effects (Rushing and Selim, 2019; Zhu et al., 2017) have been classified as the Group 1A and 2B human carcinogens, respectively (World Health Organization, 1993); zearalenone (ZEN) can lead to numerous diseases of the reproductive system to threaten people's health (Rai et al., 2020). They are heat-stable and cannot be reduced or eliminated easily through simple processing or roasting (Martins et al., 2017). Consumption of mycotoxin contaminated foods will cause acute or chronic mycotoxicosis with varied severe health hazards (Al-Jaal et al., 2019; Ayelign and De Saeger, 2020). Once foods are contaminated with mycotoxins exceeding the regulatory limits, they are refused into the international market and condemned for destruction, resulting in enormous economic burdens and losses (Kebede et al., 2020; Mitchell et al., 2016).To ensure food safety s and prevent economic losses from mycotoxin contamination, it is vital and urgent to introduce reliable technologies for accurate monitoring of different mycotoxins. The officially-approved classical methods are classified into screening and chromatographic categories (Iqbal, 2021). Of them, the screening methods like enzyme-linked immunosorbent assay are limited for wide use owing to their high dependence on matrices and false-positive results for structurally similar

* Corresponding author at: School of Traditional Chinese Medicine, Capital Medical University, Beijing 100069, China.
E-mail address: kongwj302@126.com (W. Kong).

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Scheme 1. Multi-mycotoxin detection and human exposure risk assessment in medicinal foods.

mycotoxins. Chromatographic techniques are commonly accepted, of which, high-performance liquid chromatography with fluorescence detector is considered suitable especially for precise detection. Regrettably, the required immunoaffinity columns are costly (Liao et al., 2020). Given the myriad of challenges associated with these existing methods, ultra-fast liquid chromatography-tandem mass spectrometry (UFLC-MS/MS) has become the most reliable and widely used choice due to its excellent separation capacity, improved analytical speed, high sensitivity and selectivity (Iqbal, 2021). It was introduced for simultaneous detection of multiple mycotoxins at trace level in complex matrices and their exposure analysis. Regarding risk assessment, it is necessary to design reasonable methods considering the different ways of food consumption. The aqueous extracts of some medicinal foods or tea are analyzed since the ingestion is carried out during cooking (El Jai et al., 2021a, b). Organic solvents are also used for extracting mycotoxins as completely as possible in other foods, such as grains and fruits, to get a more realistic result, because they are almost all eaten by human (Fan et al., 2022; Juan et al., 2017). On the other hand, if the exposure risk is low or can be ignored in the case of complete extraction, then, no safety issue will be considered when the aqueous extracts were consumed.

Owing to the complex components and possible matrix interferences in food matrices, reasonable sample pre-processing for complete extraction of mycotoxins is essential prior to analysis. Although some techniques have been proposed for extracting diverse mycotoxins (Huertas-Pérez et al., 2017; Liu et al., 2019), they either need a cleanup step or present high consumption of reagents, time and labor. As an ideal candidate, ultrasonication assisted one-step extraction (USAE) without any further purification treatments can both simplify the operation process to reduce the cost and avoid the loss and damage of target. It is extremely suitable for highly-efficient obtainment of mycotoxins from complex samples (Wu et al., 2021).

Regarding the severe toxicity of detected mycotoxins in foods, health risk assessment of human exposure to them via daily intake is crucial for their quality and safety control. Due to the lacking of regulatory limits for mycotoxins in medicinal foods, worldwide concern has increased on their safety. Medicinal foods, especially that are rich in starch with exceptionally high edible values, are easily susceptible to mycotoxin contamination, causing great risks to human health. Therefore, this study developed a simple, rapid and sensitive UFLC-MS/MS method for multi-mycotoxin determination in 103 batches of medicinal foods rich in starch including 26 Coix seed, 47 Lotus seeds, 10 Chinese yam, 10 Lily and 10 Malt samples collected from different sources in China, the Netherlands, and Vietnam. Six mycotoxins of AFB_1, AFB_2, AFG_1, AFG_2, OTA and ZEN have got effective extraction and enrichment by using USAE without any purification operations. Method validation well verified the satisfactory specificity, sensitivity, precision, and accuracy (expressed as recovery) of the developed analytical method for real application in the 103 samples. Then, the exposure risk through intake of these mycotoxin-contaminated medicinal foods was assessed through calculating the margin of exposure (MOE), hazard quotient (HQ) and hazard index (HI) (Scheme 1). This is the first study on multi-mycotoxin analysis in complex medicinal foods and human exposure risk assessment, providing valuable references to protect the consumers from potential health risks associated with mycotoxin contamination.

2. Materials and methods

2.1. Reagents and chemicals

Acetonitrile, methanol and formic acid of HPLC grade were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Purified water from Wahaha Co., ltd (Hangzhou, China) was used.

The standard solution of aflatoxins (Product number: STD#1081 with purity ≥ 99 %, containing 2.0 μg/mL of AFB_1 and AFG_1, and 0.53 μg/mL of AFB_2 and AFG_2, dissolved in acetonitrile), OTA (Product number: STD#5012 with purity ≥ 99 %, 100.4 μg/mL in methanol) and ZEN (Product number: STD#4011 with purity ≥ 99 %; 25.0 μg/mL in acetonitrile) were all purchased from Pribolab Pte. ltd. (Singapore) and stored in a closed and shade environment at −20 ℃.

2.2. Standard solution preparation

Before use, the standard solutions were mixed in acetonitrile to obtain 200 ng/mL of AFB_1, AFG_1 and OTA, 54 ng/mL of AFB_2 and AFG_2, and 400 ng/mL of ZEN and stored at −20 ℃ in the shade. Different volumes of high-concentration of mixed standard solutions were spiked into the five kinds of medicinal food matrices of Coix seed, Lotus seed, Lily, Chinese yam, and Malt prepared to obtain gradient concentration of 0.5, 1, 2, 5, 10, 20, 50, 80 and 100 ng/ mL for AFB_1, AFG_1 and OTA, 0.135, 0.27, 0.54, 1.35, 2.7, 5.4, 13.5, 21.6 and 27 ng/mL for AFB_2 and AFG_2, 1, 2, 4, 10, 20, 40, 100, 160 and 200 ng/mL for ZEN to establish the matrix-matched calibration curves.

2.3. Sample collection and preparation

A total of 103 batches of medicinal foods rich in starch including 26 batches of Coix seed (CS1-CS26), 47 Lotus seeds (LS1-LS47), 10 Chinese yam (CY1-CY10), 10 Lily (L1-*L*10) and 10 Malt (M1-M10) were collected from different sources in China, the Netherlands, and Vietnam. All samples were smashed by using a small-size crusher and filtered through a 24-mesh sieve. Accurately 1.0 g of sample powder was weighed and placed into a 10-mL Eppendorf tube with the addition of 5 mL of 80 % acetonitrile–water solution. After vortex for 1 min, the mixture was ultrasonically extracted for 10 min, followed by centrifugation at 10000 rpm for 5 min. The supernatant was collected and filtrated through a 0.22-μm filter membrane into a 1.5 mL liquid phase vial for the subsequent UFLC-MS/MS analysis.

2.4. UFLC-MS/MS conditions

All separations of the six mycotoxins were carried out on a Shimadzu UFLC system consisting of two LC-20ADXR pumps, a DGU20 A3 degasser, a SIL-20AC auto-sampler and CTO-20A column oven (Shimadzu, Kyoto, Japan). The six mycotoxins obtained satisfactory separation on a CORTECS C18 column (2.1 mm ID × 100 mm, 2.7 μm) at constant 35 ℃ with the mobile phase of 0.1 % formic acid in methanol (solvent A) and 0.1 % formic acid aqueous (solvent B) at the flow rate of 0.2 mL/min. The injection volume was 3 μL. The gradient procedure was as follows: 0.00 min, 80 % B; 0.50 min, 60 % B; 4.50 min, 5 % B; 6.51 min, 60 % B; 11.00 min, 60 % B.

An Applied Biosystems Sciex QTrap®5500 MS/MS system (Foster City, CA, USA) was coupled to the UFLC system with electrospray ionization (ESI) interface for qualitative and quantitative analysis of these mycotoxins. The MS/MS detection was performed in both positive and negative ESI modes under the multiple reaction monitoring (MRM) conditions. Nitrogen was used as the nebulizer (GS1), heater (GS2), curtain (CUR) gases, as well as the collision activation dissociation (CAD) gas. The ion spray (IS) voltage was set at + 5500 V and − 4500 V for the positive and negative ionization modes, respectively. The MS/MS parameters were optimized and finally set as follows: GS1 and GS2, 55 psi; CUR, 35 psi; CAD gas, medium; and capillary temperature, 550 ℃. Other MS/MS parameters regarding the six mycotoxins, including retention time (RT), precursor ion, product ions for quantification (Q) and qualitative (q), declustering potential (DP), collision energy (CE) and Collision cell exit potential (CXP) were listed in Table S1.

2.5. Matrix effect

When developing an ESI source based UFLC-MS/MS method, the matrix constituents in complex medicinal food matrices may influence the ionization efficiency of target analytes, leading to interferences on the method accuracy and reproducibility, which are called matrix effects (MEs). Matrix type, the chemical structure properties of analytes, the analyte concentration and the analytical conditions will all present varying degrees of signal enhancement or suppression effects in the actual analysis. Thus, it is of great importance to evaluate ME prior to real analysis. Standard addition is considered as a reliable way for the evaluation.

Herein, serial concentrations of the mixed mycotoxin standard solutions were added into pure solvent and the five kinds of blank medicinal food matrix extractions, respectively. ME (%) for each mycotoxin was calculated based on the slope ratio of the calibration curves according to the following equation:

$$\text{ME}\ (\%) = (Slope_{\text{matrix-matched curve}} / Slope_{\text{solvent-based curve}}) \times 100 \quad (1)$$

where $Slope_{\text{matrix-matched curve}}$ and $Slope_{\text{solvent-based curve}}$ represent the slopes of the matrix-matched and solvent-only calibration curves, respectively. In general, ME < 80 % or > 120 % indicate a signal suppression and enhancement effect, respectively, and 80 % ≤ ME ≤ 120 % is considered with no matrix interference.

2.6. Method validation

In order to obtain receivable results, the suitability and reliability of the established UFLC-MS/MS method were validated in advance regarding selectivity, sensitivity, linearity, accuracy (recovery), and precision according to the official guidance (European Commission, 2019).

2.6.1. Selectivity

In order to assess the selectivity of the method, blank sample extractions of Coix seed, Lotus seed, Chinese yam, Lily and Malt, the mixed standard solution of six mycotoxins diluted with acetonitrile–water (80:10, *v/v*), and the spiked sample solutions with 1.35, 5, 1.35, 5, 5 and 10 ng/mL of AFG_2, AFG_1, AFB_2, AFB_2, OTA and ZEN, respectively, were injected into the UFLC-MS/MS for comparable analysis.

2.6.2. Sensitivity and linearity

Method sensitivity was studied in terms of limit of detection (LOD) and quantitation (LOQ) through calculating the minimum concentration of target mycotoxins in the spiked sample solution based on signal-to-noise (S/N) ratio of approximately 3 and 10, respectively. Linearity for each mycoxotin spiked into the blank matrix was evaluated based on peak area as the dependent variable (*y*-axis) against the corresponding standard concentration as the independent variable (*x*-axis) at eight concentration points.

For this evaluation, the six mycotoxins in the five kinds of spiked medicinal food extractions with 0.5, 1, 2, 5, 10, 20, 50, 80 and 100 ng/mL of AFB_1, $AFG_1$and OTA; 0.135, 0.27, 0.54, 1.35, 2.7, 5.4, 13.5, 21.6 and 27 ng/mL of AFB_2 and AFG_2; 1, 2, 4, 10, 20, 40, 100, 160 and 200 ng/mL of ZEN were prepared and analyzed to construct the calibration curves. Then, the linear ranges along with the corresponding correlation coefficient (R^2) for each calibration curve was obtained. LOD and LOQ for each analyte were determined by analyzing several decreasing concentrations of each analyte until the S/N ratios were reached.

2.6.3. Accuracy and precision

Method accuracy and precision were investigated via the recovery and reproducibility tests, respectively.

For each analyte, the method accuracy was evaluated by measuring the recovery of the six mycotoxins in the five kinds of spiked medicinal food samples at three (low, medium and high) concentration levels: 5, 10 and 15 μg/kg of AFB_1, AFG_1 and OTA, 1.35, 2.70 and 4.05 μg/kg of AFB_2 and AFG_2, 10, 20 and 30 μg/kg of ZEN. Then, three parallel spiked samples were extracted and tested according to the above method. The recovery (%) was calculated according to the above-obtained matrix-matched calibration curves based on the following equation:

$$\text{Recovery}\ (\%) = (\text{measured amount} / \text{spiked amount}) \times 100 \quad (2)$$

In conformation with the relevant regulations of General Rule 9101 of the *Chinese Pharmacopoeia* (Chinese Pharmacopoeia Commission,

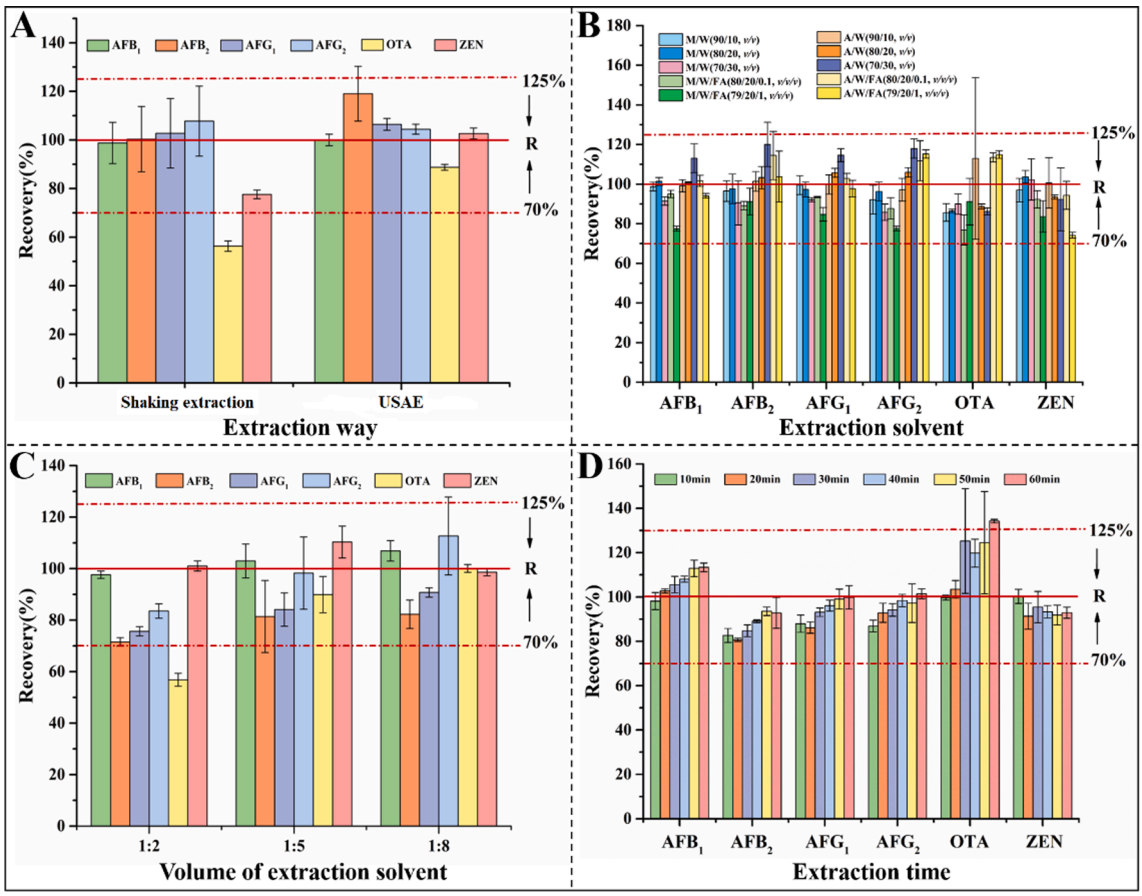

Fig. 1. Optimization of the extraction conditions: (A) Extraction way, (B) Extraction solvent, (C) Added volume of extraction solvent, and (D) extraction time.

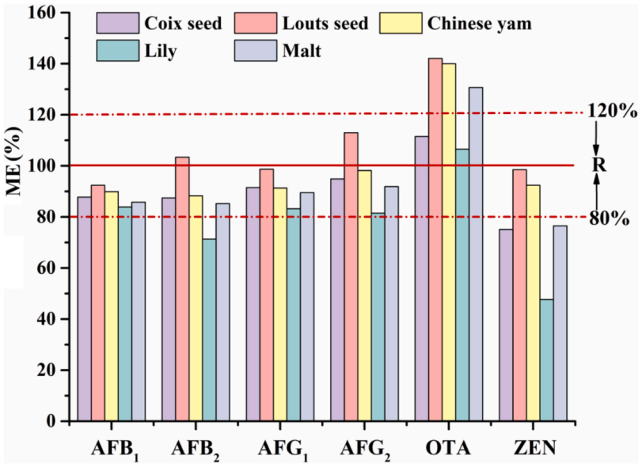

Fig. 2. Matrix effects of six mycotoxins in five kind of medicinal foods.

2020a) for trace analysis, a recovery rate of 70–125 % with relative standard deviation (RSD) ≤ 20 % was acceptable.

The reproducibility test through analyzing the spiked sample solution with 10.0 μg/kg of AFB_1, AFG_1 and OTA, 2.70 μg/kg of AFB_2 and AFG_2, and 20.0 μg/kg of ZEN by six times' consecutive injections to evaluate method precision.

2.7. Human health risk assessment of mycotoxins in medicinal foods

Given the severe toxicities of target mycotoxins, it is vital to assess the potential exposure risk of them in medicinal foods to human health through evaluating the contamination level of 6 target mycotoxins, together with the consumption data of the five kinds of medicinal foods. Since AFB_1 is classified as the Group 1A carcinogen, no relevant threshold dose is stipulated, and margin of exposure (MOE) is adopted. OTA and ZEN with reference dose (RfD) were evaluated by hazard quotient (HQ). Exposure risk assessment was conducted by calculating the estimated daily intake (EDI) according to the following equation:

$$EDI = (C \times F) / bw \quad (3)$$

where C is the mean contamination concentration (μg/kg) of mycotoxin in the medicinal food sample; F is the average consumption (g/day) of medicinal foods. According to the *Chinese Pharmacopoeia*, the F values for Coix seed, Lotus seed, Chinese yam, Lily and Malt are 19.5, 10.5, 22.5, 9, and 12.5 g/day, respectively (Chinese Pharmacopoeia Commission, 2020b). bw is the body weight of a human (kg), and the default international average weight of an adult is 60 kg (World Health Organization, 2011).

Here, MOE of aflatoxins was evaluated by the ratio of Benchmark dose lower limit for a 10 % response ($BMDL_{10}$) to EDI, and the $BMDL_{10}$ value of AFB_1 was 170 ng/kg·day·bw. Considering that AFB_1 was the main kind of aflatoxins in food samples, the toxicity of total aflatoxins was assumed to be equivalent to that of AFB_1 (European Food Safety Authority, 2007). Thus, MOE regarding aflatoxins was calculated according to the following formula:

$$MOE = BMDL_{10} / EDI \quad (4)$$

Generally, MOE > 10000 indicates little concern and small impact of aflatoxins on human health, while MOE < 10000 manifests a health threat through the intake of foods contaminated with aflatoxins. The smaller the MOE value is, the higher the risk of food to human health is

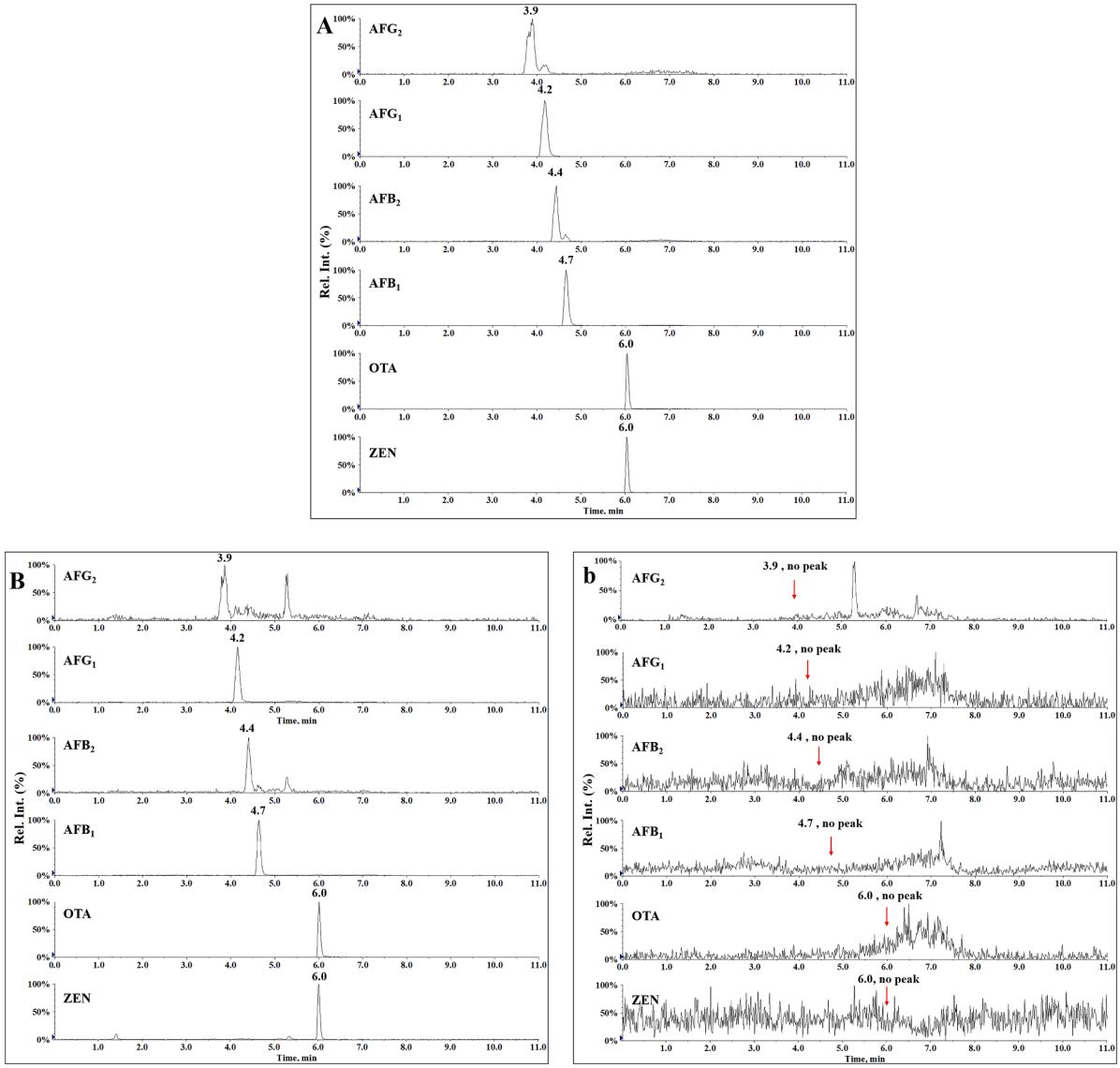

Fig. 3. UFLC-MS/MS MRM chromatograms of target mycotoxins in (A) Standard solution, and the spiked sample solutions of (B) Coix seed, (C) Lotus seed, (D) Chinese yam, (E) Lily, (F) Malt with 1.35, 5, 1.35, 5, 5 and 10 ng/mL of AFG_2, AFG_1, AFB_2, AFB_2, OTA and ZEN, and the blank matrix solutions of (b) Coix seed, (c) Lotus seed, (d) Chinese yam, (e) Lily, and (f) Malt, respectively.

(European Food Safety Authority, 2005; Cartus and Schrenk, 2017). Exposure to a dose that is 10,000 times lower than MOE will result in 1 in 100,000 people at risk (European Food Safety Authority Scientific Committee, 2012).

The European Food Safety Authority (EFSA) set the tolerable daily intake (TDI) and tolerable weekly intake (TWI) for assessing exposure risk to mycotoxins in foods under manageable conditions. The TWI for OTA is 120 ng/kg·week·bw, and TDI for ZEN is 250 ng/kg·day·bw (European Food Safety Authority, 2006, 2014). HQ is usually introduced to represent the risk level of OTA or ZEN dietary intake, which is calculated as the ratio of EDI to TDI according to the following formula:

$$HQ\ (\%) = (EDI / TDI) \times 100 \quad (5)$$

HQ < 100 % is considered as an acceptable dietary exposure level of OTA or ZEN without health threat on human, while, HQ > 100 % shows that the dietary exposure level exceeds the permissible limit with health threat, which, thus, will be considered as a severe safety incident (Wang et al., 2018).

In addition, as the interaction mechanism between OTA and ZEN is not clear, the total risk of them is estimated through calculating hazard index (HI) by directly combining the HQ values of OTA and ZEN according to following equation:

$$HI = HQ_{OTA} + HQ_{ZEN} \quad (6)$$

2.8. Data analysis

In some cases, the detected concentrations of some mycotoxins in a few samples are lower than LODs or LOQs. According to the official principle (World Health Organization, 2009), calculation analysis is carried out for the lower and upper bound, respectively. To calculate the dietary exposure level of these mycotoxins, medicinal food sample with monitored level of mycotoxin < LOD or LOQ was assigned a value of 0, which was the lower bound (LB); sample with detected level < LOD was given a value of LOD, which was the upper bound (UB); and sample with tested result between LOQ and LOD was allowed a value of LOQ. Meanwhile, the average content of mycotoxin in positive sample was

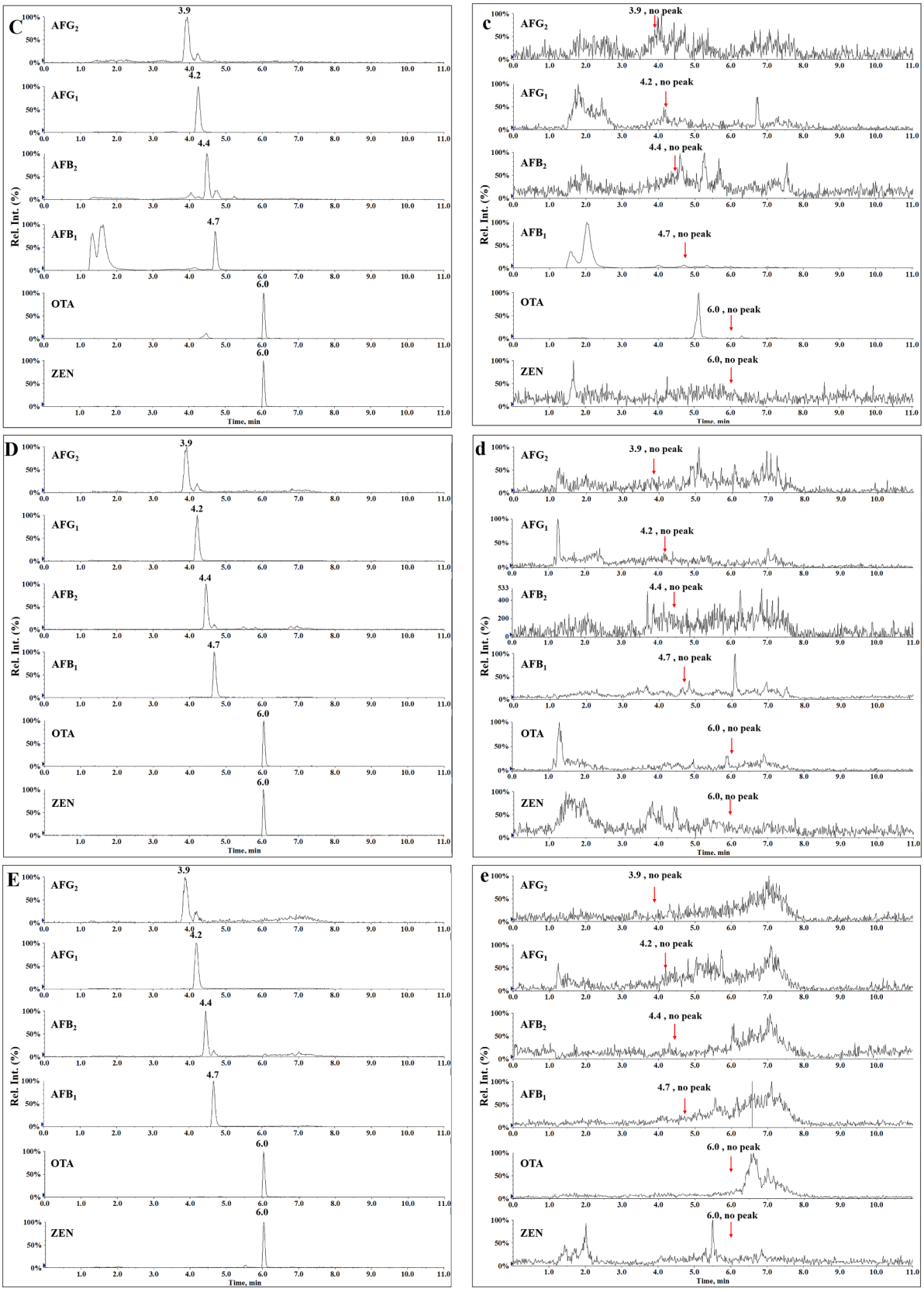

Fig. 3. (*continued*).

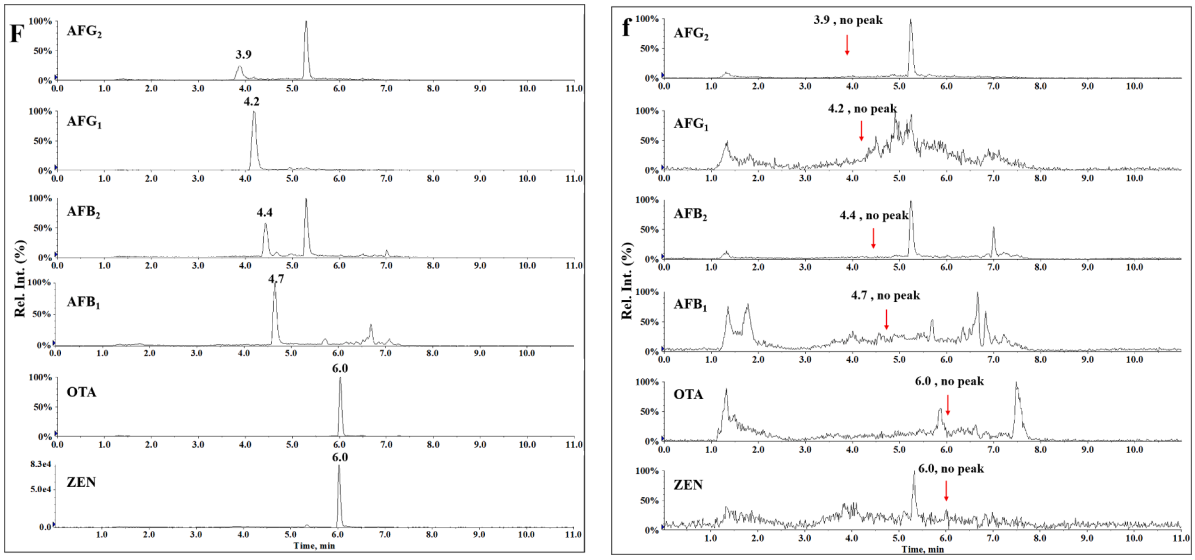

Fig. 3. (*continued*).

calculated for assessing the dietary exposure level. All charts were calculated and analyzed by Origin or Excel software.

3. Results and discussion

3.1. Optimization of extraction conditions

Aiming for highly-efficient extraction of six trace mycotoxins in the five kinds of medicinal food samples rich in starch, some important parameters and conditions, such as extraction way, extraction solvent and its volume, and extraction time, were systematically optimized. It was achieved with recovery of the six mycotoxins as the evaluation index and the spiked solution of Coix seed with 20.0 μg/kg of AFB_1, AFG_1 and OTA, 5.40 μg/kg of AFB_2 and AFG_2, and 40.0 μg/kg of ZEN as the tested matrix.

First, two simple and easy-to-operate extraction ways including shaking extraction and USAE were introduced for rapidly and efficiently extracting the six mycotoxins in the spiked Coix seed solution. As shown in Fig. 1A, the recoveries (56–108 %) of most of the mycotoxins were satisfactory within 70–125 % except OTA (<70 %) obtained from the shaking extraction method. While, the recovery values (89–119 %) of the six mycotoxins, especially OTA and ZEN, got from the USAE treatment was larger than that from the shaking extraction. Therefore, the USAE technique was chosen for effective extraction of target mycotoxins in the five kinds of complex medicinal food samples rich in starch.

Owing to their different physical and chemical properties of the six mycotoxins, the extraction solvent is essential for achieving high extraction efficiency, which was taken special consideration. Herein, different ratios of methanol/water (M/W) and acetonitrile/water (A/W) systems, as well as that with the addition of formic acid (FA) were investigated to assess their extraction efficiency for the six mycotoxins. Fig. 1B showed that the recovery values regarding the six mycotoxins were in an acceptable range of 70–125 %, and which got from acetonitrile/water system were in the whole slightly bigger than that from the methanol/water system. Thus, the acetonitrile/water system was given preference to be selected as the extraction solvent. Whereas, the addition of formic acid into the acetonitrile/water system has not remarkably improved the extraction efficiency, even lowered the recovery of some mycotoxins including AFB_1, AFB_2, AFG_1, AFG_2, and ZEN. In combination with the data in Table S2, the average recovery values of the six mycotoxins from acetonitrile/water (A/W, 80/20, *v/v*) and acetonitrile/water/formic acid (A/W/FA, 79/20/1, *v/v/v*) systems were 99.66 % and 99.96 %, respectively, which relatively got close to 100 %. Nevertheless, acetonitrile/water (A/W, 80/20, *v/v*) solvent gave smaller standard deviation (SD) value (7.04 %) than the acetonitrile/water/formic acid (79/20/1, *v/v/v*) system (15.33 %), which, thus, was selected as the predominant extraction solvent for the six mycotoxins.

Consequently, the added volume of the acetonitrile/water (80/20, *v/v*) system was optimized. Different ratios (1:2, 1:5 and 1:8, g/mL) of the spiked Coix seed sample to the added extraction solvent were compared. As seen in Fig. 1C, the six mycotoxins have not obtained sufficient extraction with low recovery when the added volume of acetonitrile/water (80/20, *v/v*) solvent was two times that of the spiked sample. The recovery of target mycotoxins was improved with increasing the added volume of extraction solvent. Taking environmental friendliness and saving cost into account, the ratio of the spiked Coix seed sample to the added acetonitrile/water (80/20, *v/v*) extraction solvent was controlled at 1:5.

Then, the extraction time from 10 min to 60 min was considered. As presented in Fig. 1D, except for OTA, the recoveries of other five mycotoxins were all satisfactory in an acceptable range of 70–125 %. With increasing the extraction time from 10 min to 60 min, the recovery of most mycotoxins was improved, while, the recovery of OTA became higher than 125 %, which might be due to that many matrix components with similar structure to OTA were extracted in the USAE process. Finally, 10 min was selected as the optimal ultrasonic time to completely extract these six mycotoxins from the spiked Coix seed sample and reduce the influence of matrix components.

3.2. Matrix effect

In order to accurately quantify the six mycotoxins in complex medicinal food samples, possible matrix interferences were first evaluated. Fig. 2 showed that these medicinal food samples presented diverse MEs on the six mycotoxins. Significant signal suppression effects were observed for AFB_2 in Lily sample, and ZEN in Coix seed, Lily and Malt samples as the ME values were lower than 80 %. While, remarkable signal enhancement efforts were measured for OTA in Lotus seed, Chinese yam and Malt matrices since the ME values were bigger than 120 %. Diverse MEs would cause uncertain interferences on the ESI source for target mycotoxins in these complex matrices. Therefore, aiming at reducing ME for accurate quantitation of different mycotoxins in medicinal food samples, the matrix-matched calibration curves were established in the following evaluations.

Table 1
Matrix-matched calibration curve, linearity, LOD and LOQ of each mycotoxin in the five kinds of medicinal foods.

Matrix	Mycotoxin	Calibration curve	Linear range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Coix seed	AFB_1	$y = 145164x + 7475.9$	0.5–100	0.9998	0.06	0.16
	AFB_2	$y = 88112x - 13{,}792$	0.27–27	0.9993	0.05	0.15
	AFG_1	$y = 103181x + 8600$	0.5–100	0.9990	0.04	0.10
	AFG_2	$y = 48652x - 3979.2$	0.27–27	0.9999	0.05	0.17
	OTA	$y = 145302x - 28{,}898$	0.5–100	0.9995	0.06	0.18
	ZEN	$y = 45855x - 69{,}895$	1–200	0.9994	0.12	0.36
Lotus seed	AFB_1	$y = 152856x + 52795$	0.5–100	0.9997	0.06	0.16
	AFB_2	$y = 104249x - 21{,}787$	0.27–27	0.9997	0.05	0.13
	AFG_1	$y = 111247x - 69{,}031$	0.5–100	0.9991	0.06	0.16
	AFG_2	$y = 57948x - 3678.1$	0.27–27	0.9998	0.05	0.13
	OTA	$y = 185179x + 36568$	0.5–100	0.9999	0.06	0.18
	ZEN	$y = 60160x + 17346$	1–200	0.9998	0.12	0.36
Chinese yam	AFB_1	$y = 148789x + 58729$	0.5–100	0.9997	0.06	0.16
	AFB_2	$y = 88997x - 4554.2$	0.27–27	0.9999	0.05	0.13
	AFG_1	$y = 102983x + 4975$	0.5–100	0.9996	0.06	0.16
	AFG_2	$y = 50363x + 4230$	0.27–27	0.9997	0.06	0.13
	OTA	$y = 182366x - 55{,}636$	0.5–100	0.9999	0.06	0.18
	ZEN	$y = 56438x - 59{,}007$	1–200	0.9997	0.12	0.36
Lily	AFB_1	$y = 138750x - 45{,}900$	0.5–100	0.9996	0.06	0.16
	AFB_2	$y = 71942x - 16{,}485$	0.27–27	0.9996	0.04	0.13
	AFG_1	$y = 93820x - 61{,}207$	0.5–100	0.9995	0.06	0.16
	AFG_2	$y = 41800x - 12{,}779$	0.27–27	0.9993	0.08	0.13
	OTA	$y = 138742x - 72{,}822$	0.5–100	0.9994	0.06	0.18
	ZEN	$y = 29151x - 69{,}036$	1–200	0.9994	0.25	0.67
Malt	AFB_1	$y = 141852x - 106{,}170$	0.5–100	0.9993	0.06	0.19
	AFB_2	$y = 85906x - 18{,}642$	0.27–27	0.9992	0.06	0.16
	AFG_1	$y = 100871x - 84{,}092$	0.5–100	0.9993	0.06	0.19
	AFG_2	$y = 47098x + 1006.6$	0.27–27	0.9998	0.06	0.16
	OTA	$y = 170266x - 159{,}992$	0.5–100	0.9990	0.06	0.19
	ZEN	$y = 46699x - 50{,}412$	1–200	0.9998	0.12	0.36

3.3. UFLC-MS/MS method validation

The analytical performance of the established UFLC-MS/MS method was validated for its real application.

3.3.1. Selectivity

The UFLC-MS/MS MRM chromatograms in Fig. 3A-F implied that the six target mycotoxins in the standard solution and the spiked medicinal food extraction have obtained satisfactory separation without interferences each other at the corresponding retention time of 3.9, 4.2, 4.4, 4.7, 6.0 and 6.0 min for AFG_2, AFG_1, AFB_2, AFB_1, OTA and ZEN, respectively. No interference peaks were observed at the corresponding retention time of each mycotoxin in these blank (mycotoxin-free) sample solutions in Fig. 3b-f. Furthermore, Fig. 3B-F showed no extra peaks at the retention time of each mycotoxin, indicating that the complex matrix constituents in those samples did not interfere the determination of the six mycotoxins. These observations elucidated the excellent selectivity of the established UFLC-MS/MS method.

3.3.2. Sensitivity and linearity

The matrix-matched calibration curves between peak area and concentration of the six mycotoxins in the five kinds of spiked medicinal food matrices were constructed in Table 1, which presented wide concentration ranges with R^2>0.9990. The obtained LODs and LOQs were in the range of 0.04–0.25 ng/mL in solvent (equal to 0.2–1.25 μg/kg in sample) and 0.10–0.67 ng/mL in solvent (equal to 0.5–3.35 μg/kg in sample), respectively. These results well met the relevant regulations (Chinese Pharmacopoeia Commission, 2020a). High sensitivity and broad linear range of the established UFLC-MS/MS method could ensure reliable detection of target mycotoxins at trace level in complex medicinal food samples.

3.3.3. Accuracy and precision

The trueness or accuracy of the established method for these mycotoxins was evaluated by measuring the recoveries from the five kinds of spiked samples with three concentration levels of mycotoxins. Table 2 indicated that the average recoveries ranged from 75.33 % to 118 % with RSDs of 0.32–7.77 %, which met the relevant regulations (Chinese Pharmacopoeia Commission, 2020a), verifying high accuracy of the UFLC-MS/MS method for accurately detecting the six mycotoxins in the tested samples.

The RSD values regarding consecutive analysis of the fortified sample solution were 0.83 %, 2.32 %, 0.84 %, 0.74 %, 8.83 %, and 2.31 % for AFB_1, AFG_1, OTA, AFB_2, AFG_2, and ZEN, respectively, which were<10 %, confirming high repeatability and precision of the UFLC-MS/MS method.

The comparison of different LC-MS/MS developed for multi-mycotoxin detection in various food and medicinal plants matrices was listed in Table 3, confirming the remarkable simplity without clean-up step, and significant rapidity with small pretreatment and analysis time of the current validated UFLC-MS/MS method. Then, following the USAE pretreatment optimization and method validation, the highly sensitive and rapid UFLC-MS/MS method was utilized for simultaneous determination of 6 mycotoxins belonging to different classes in 103 batches of commercial medicinal food samples collected from various origins in China, the Netherlands, and Vietnam.

3.4. Real samples analysis

It could be found in Table 4 that 58 samples out of 103 batches of medicinal foods were detected with one or more mycotoxins with a positive rate of 56.31 % (58/103). Regarding different types of medicinal foods, it could be found that: (1) Coix seed samples gave the highest positive rate of 96.15 % (25/26), followed by Lily of 90 % (9/10), Chinese yam of 50 % (5/10), Lotus seed of 34.04 % (16/47), and Malt of 30 % (3/10). (2) Coix seed samples were predominantly contaminated

Table 2
Recovery of six mycotoxins in five kinds of medicinal food samples ($n = 3$).

Analytes	Recovery (RSD, %)														
	Coix seed			Lotus seed			Chinese yam			Lily			Malt		
	Low [a]	Medium [b]	High [c]	Low	Medium	High	Low	Medium	High	Low	Medium	High	Low	Medium	High
AFB_1	98.07 (2.70)	94.50 (3.31)	94.18 (0.85)	80.36 (3.84)	83.03 (2.73)	97.29 (0.92)	91.79 (3.25)	93.23 (3.09)	88.35 (3.74)	83.02 (1.24)	81.51 (4.03)	91.36 (2.41)	80.40 (5.40)	87.77 (3.55)	97.68 (4.13)
AFB_2	88.78 (1.03)	78.75 (7.40)	75.33 (1.27)	97.19 (4.11)	89.83 (5.69)	97.09 (3.25)	99.81 (3.97)	85.58 (3.40)	107.19 (7.77)	82.50 (1.93)	84.59 (2.70)	90.22 (3.10)	93.29 (4.03)	92.99 (1.54)	102.69 (0.54)
AFG_1	90.04 (3.38)	86.66 (2.79)	83.31 (2.44)	118.00 (1.27)	105.34 (3.85)	98.13 (2.77)	84.74 (3.99)	102.31 (5.77)	87.42 (4.99)	82.39 (1.18)	83.40 (4.51)	93.47 (2.17)	86.75 (3.17)	90.44 (1.07)	102.36 (3.63)
AFG_2	84.40 (6.48)	78.20 (1.52)	77.03 (3.63)	87.66 (4.83)	80.94 (5.37)	84.19 (3.82)	90.47 (4.30)	95.71 (5.87)	83.24 (1.48)	100.51 (1.20)	90.21 (5.07)	93.90 (3.12)	83.73 (2.22)	77.06 (4.85)	98.60 (1.55)
OTA	79.62 (2.06)	76.14 (1.77)	75.88 (1.26)	97.60 (0.88)	97.22 (1.69)	97.10 (2.93)	90.40 (0.37)	100.16 (3.74)	110.30 (1.74)	96.32 (3.78)	92.40 (1.24)	105.22 (2.40)	76.63 (0.85)	75.94 (5.22)	87.27 (0.50)
ZEN	92.32 (3.58)	90.05 (2.04)	94.12 (1.01)	117.24 (3.39)	112.84 (2.51)	107.53 (1.62)	81.47 (3.51)	97.48 (5.00)	103.70 (0.32)	103.00 (4.21)	102.24 (3.25)	114.09 (3.86)	77.28 (3.11)	81.71 (2.36)	90.82 (4.62)

[a] 5.0 μg/kg of AFB_1, AFG_1 and OTA, 1.35 μg/kg of AFB_2 and AFG_2, and 10.0 μg/kg of ZEN;
[b] 10.0 μg/kg of AFB_1, AFG_1 and OTA, 2.70 μg/kg of AFB_2 and AFG_2, and 20.0 μg/kg of ZEN;
[c] 15.0 μg/kg of AFB_1, AFG_1 and OTA, 4.05 μg/kg of AFB_2 and AFG_2, and 30.0 μg/kg of ZEN.

Table 3
Comparison of diverse LC-MS/MS methods for multi-mycotoxin detection.

Matrix	No. of mycotoxin	Extraction solvent	Clean-up	Pretreatment time (min)	Analysis time (min)	LOQ (μg/kg)	Reference
Atractylodis rhizoma	7	A/W (90/10, *v/v*)	QuEChERS	27	12	0.1–0.5	Liu et al., 2019
Origanum vulgare, Rosmarinus officinalis, Matricaria chamomilla, Myrtus communis, and Verveine officinale	15	(1) boiling water; (2) acetonitrile, ethyl acetate	–	17	21	0.09–12.9	El Jai et al., 2021b
Green tea	15	(1) boiling water; (2) acetonitrile, ethyl acetate	–	18	25	0.1–14.32	El Jai et al., 2021a
Coix seed	24	A/W/FA (70/29/1, *v/v/v*)	Dilution	20.5	21	0.5–100	Wu et al., 2021
Coix seed, Lotus seed, Chinese yam, Lily, and Malt	6	A/W (80/20, *v/v*)	–	16	11	0.5–3.35	**This work**

A: acetonitrile; W: water; FA: formic acid.

with ZEN at an occurrence rate of 96.15 % (25/26), followed by AFB_1 at 26.92 % (7/26), and AFG_1 at 15.38 % (4/26). Lotus seed matrices were mainly polluted by AFB_1 at an occurrence rate of 34.04 % (16/47), followed by AFB_2 at 19.15 % (9/47), AFG_1 at 8.51 % (4/47), and AFG_2 and OTA at 4.26 % (2/47). Chinese yam samples were majorly contaminated with AFB_1 at 50 % (5/10), followed by OTA at 10 % (1/10). Lily and Malt matrices were only polluted by OTA at an occurrence rate of 90 % (9/10), and ZEN at an occurrence rate of 30 % (3/10), respectively. (3) One Lotus seed sample (LS20) collected from Hubei province, China, was simultaneously contaminated with 5 mycotoxins including AFB_1, AFB_2, AFG_1, OTA, and ZEN, and two Lotus seed samples from Anhui (LS6) and Jiangxi (LS42) provinces, China, were simultaneously monitored with 4 aflatoxins. One Lotus seed (LS12) and four Coix seed (CS5, CS20, CS21, and CS26) samples were detected with 3 mycotoxins.

As regard to the six mycotoxins listed in Tables 4 and 5, it could be concluded that: (1) ZEN was monitored in 29 samples with the highest positive rate of 28.16 % (29/103) at the detected contents of 5.58–2721.87 μg/kg. The contents of ZEN in 5 Coix seed samples (4.85 %) exceeded the maximum residue limit (MRL) of 500 μg/kg set by *Chinese Pharmacopoeia* (Chinese Pharmacopoeia Commission, 2020b). (2) AFB_1 was found in 28 samples with high residual rate of 27.18 % (28/103) at contents of 1.39–369.51 μg/kg. The contents of AFB_1 in 17 samples (16.5 %) including 7 Coix seed and 10 Lotus seed samples were over its MRL of 5 μg/kg (Chinese Pharmacopoeia Commission, 2020b). Five Chinese yam samples were contaminated with AFB_1 < LOQ (0.16 ng/mL). (3) The total contents of aflatoxins (AFs) regarding the sum of $AFB_1 + AFB_2 + AFG_1 + AFG_2$ ranged from 1.39 μg/kg to 388.59 μg/kg, and that in 17 samples (16.5 %) including 7 Coix seed and 10 Lotus seed samples were bigger than the officially-set MRL of 10 μg/kg (Chinese Pharmacopoeia Commission, 2020b). (4) OTA was observed in 12 samples with positive rate of 11.65 % (12/103) at contents of 1.78–701.76 μg/kg. The contents of OTA in 6 samples (5.83 %) including 1 Lotus seed and 5 Lily samples were greater than the MRL of 5 μg/kg (European Commission, 2006). (5) In all, ZEN displayed the highest positive rate in these medicinal food samples, followed by AFB_1, OTA, AFB_2, AFG_1 and AFG_2, while, AFB_1 presented the highest occurrence rate exceeding its corresponding MRL, followed by OTA and ZEN.

In order to clearly analyze the contamination status, the mycotoxin-positive rate in five kinds of medicinal food samples was compared in Fig. 4. Obvious differences regarding the types of mycotoxins detected in these samples could be observed. Coix seed samples were the most susceptible to ZEN with a positive rate of 96.15 %, followed by AFB_1 at 26.92 %. Lotus seed samples were heavily contaminated by AFs with an AFB_1-positive rate as high as 34.04 %. The AFs-positive rate in Chinese yam samples was high, but the absolute contents of AFs were all lower than LOQ. The 5 batches of AFB_1-positive Chinese yam samples were all processed by ourselves in our laboratory. The delay in dehydration might aggravate the AFB_1 contamination, highlighting the importance of cautious processing to avoid mycotoxins contamination. Lily samples were extremely susceptible to OTA contamination with a positive rate as high as 90 %. The ZEN-positive rate in malt was relatively high reaching

Table 4
Detected mycotoxins in 103 batches of medicinal food samples.

Sample	Origin	Mycotoxin (μg/kg)					
		AFB_1	AFB_2	AFG_1	AFG_2	OTA	ZEN
CS1	Anhui province	- [a]	–	–	–	–	204.82
CS2	Fujian province	–	–	–	–	–	74.42
CS3	Fujian province	–	–	–	–	–	77.83
CS4	Fujian province	–	–	–	–	–	668.89
CS5	Fujian province	16.32	–	28.87	–	–	110.96
CS6	Guangdong province	–	–	–	–	–	444.52
CS7	Guizhou province	–	–	–	–	–	1671.07
CS8	Guizhou province	–	–	–	–	–	478.18
CS9	Guizhou province	–	–	–	–	–	155.46
CS10	Guizhou province	–	–	–	–	–	94.80
CS11	Guizhou province	–	–	–	–	–	129.28
CS12	Guizhou province	–	–	–	–	–	110.96
CS13	Guizhou province	16.85	–	–	–	–	350.66
CS14	Guizhou province	20.35	–	–	–	–	142.00
CS15	Guizhou province	–	–	–	–	–	199.21
CS16	Guizhou province	–	–	–	–	–	156.58
CS17	Guizhou province	–	–	–	–	–	190.24
CS18	Guizhou province	16.87	–	–	–	–	474.44
CS19	Guizhou province	–	–	–	–	–	156.58
CS20	Heilongjiang province	20.43	–	27.76	–	–	1476.62
CS21	Heilongjiang province	18.81	–	27.98	–	–	253.43
CS22	Netherlands	–	–	–	–	–	–
CS23	Henan province	–	–	–	–	–	444.52
CS24	Liaoning province	–	–	–	–	–	229.50
CS25	Shandong province	–	–	–	–	–	796.03
CS26	Vietnam	33.12	–	28.81	–	–	2721.87
LS1	Anhui province	–	–	–	–	–	–
LS2	Anhui province	–	–	–	–	–	–
LS3	Anhui province	–	–	–	–	–	–
LS4	Anhui province	1.39	–	–	–	–	–
LS5	Anhui province	–	–	–	–	–	–
LS6	Anhui province	1.89	2.86	4.30	3.70	–	–
LS7	Fujian province	–	–	–	–	–	–
LS8	Fujian province	2.51	–	–	–	–	–
LS9	Fujian province	–	–	–	–	–	–
LS10	Fujian province	–	–	–	–	–	–
LS11	Fujian province	–	–	–	–	–	–
LS12	Hebei province	200.84	20.71	6.07	–	–	–
LS13	Hebei province	–	–	–	–	–	–
LS14	Hubei province	–	–	–	–	–	–
LS15	Hubei province	–	–	–	–	–	–
LS16	Hubei province	21.61	–	–	–	–	–
LS17	Hubei province	–	–	–	–	–	–
LS18	Hubei province	–	–	–	–	–	–
LS19	Hubei province	–	–	–	–	–	–
LS20	Hubei province	63.16	6.72	6.99	–	1.78	49.87
LS21	Hunan province	369.51	19.08	–	–	–	–
LS22	Hunan province	39.81	5.17	–	–	–	–
LS23	Hunan province	–	–	–	–	–	–
LS24	Hunan province	176.74	13.17	–	–	–	–
LS25	Hunan province	83.10	17.20	–	–	–	–
LS26	Hunan province	7.53	–	–	–	–	–
LS27	Hunan province	–	–	–	–	–	–
LS28	Hunan province	–	–	–	–	–	–
LS29	Hunan province	–	–	–	–	–	–
LS30	Hunan province	–	–	–	–	–	–
LS31	Hunan province	4.70	–	–	–	5.79	–
LS32	Hunan province	3.48	–	–	–	–	–
LS33	Hunan province	–	–	–	–	–	–
LS34	Hunan province	–	–	–	–	–	–
LS35	Jiangxi province	–	–	–	–	–	–
LS36	Jiangxi province	–	–	–	–	–	–
LS37	Jiangxi province	–	–	–	–	–	–
LS38	Jiangxi province	8.26	–	–	–	–	–
LS39	Jiangxi province	–	–	–	–	–	–
LS40	Jiangxi province	–	–	–	–	–	–
LS41	Jiangxi province	–	–	–	–	–	–
LS42	Jiangxi province	3.86	2.61	4.30	4.92	–	–
LS43	Jiangxi province	–	–	–	–	–	–
LS44	Shandong province	–	–	–	–	–	–
LS45	Shandong province	161.79	19.96	–	–	–	–
LS46	Shandong province	–	–	–	–	–	–
LS47	Shandong province	–	–	–	–	–	–

(*continued on next page*)

Table 4 (*continued*)

Sample	Origin	Mycotoxin (μg/kg)					
		AFB_1	AFB_2	AFG_1	AFG_2	OTA	ZEN
CY1	Beijing city	<LOQ	–	–	–	–	–
CY2	Beijing city	<LOQ	–	–	–	–	–
CY3	Beijing city	<LOQ	–	–	–	–	–
CY4	Beijing city	–	–	–	–	–	–
CY5	Beijing city	–	–	–	–	–	–
CY6	Beijing city	<LOQ	–	–	–	2.59	–
CY7	Henan province	<LOQ	–	–	–	–	–
CY8	Henan province	–	–	–	–	–	–
CY9	Henan province	–	–	–	–	–	–
CY10	Henan province	–	–	–	–	–	–
L1	Gansu province	–	–	–	–	8.32	–
L2	Guizhou province	–	–	–	–	9.90	–
L3	Guizhou province	–	–	–	–	3.21	–
L4	Hunan province	–	–	–	–	701.76	–
L5	Hunan province	–	–	–	–	3.32	–
L6	Hunan province	–	–	–	–	366.61	–
L7	Hunan province	–	–	–	–	7.89	–
L8	Jiangsu province	–	–	–	–	–	–
L9	Sichuan province	–	–	–	–	3.29	–
*L*10	Sichuan province	–	–	–	–	2.86	–
M1	Anhui province	–	–	–	–	–	–
M2	Anhui province	–	–	–	–	–	–
M3	Hebei province	–	–	–	–	–	–
M4	Hebei province	–	–	–	–	–	5.58
M5	Hebei province	–	–	–	–	–	–
M6	Hebei province	–	–	–	–	–	–
M7	Hebei province	–	–	–	–	–	–
M8	Hebei province	–	–	–	–	–	5.64
M9	Sichuan province	–	–	–	–	–	–
M10	Sichuan province	–	–	–	–	–	7.53

[a] Not detected. CS: Coix seed; LS: Lotus seed; CY: Chinese yam; L: Lily; M: Malt.

Table 5
Occurrence rate and content of mycotoxins in tested medicinal food samples.

Mycotoxin	Positive samples		Content over MRL [b]			Mean [f] (μg/kg)	Range (μg/kg)
	n	Occurrence rate (%)	MRL	n	Occurrence rate (%)		
AFB_1	28	27.18	5 [c]	17	16.5	12.55	1.39–369.51
AFB_2	9	8.74	- [d]	–	–	1.04	2.61–20.71
AFG_1	8	7.77	–	–	–	1.31	4.30–28.87
AFG_2	2	1.94	–	–	–	0.08	3.70–4.92
AFs [a]	28	27.18	10[c]	17	16.5	14.99	1.39–388.59
OTA	12	11.65	5 [e]	6	5.83	10.85	1.78–701.76
ZEN	29	28.16	500[c]	5	4.85	115.35	5.58–2721.87

[a] $AFB_1 + AFB_2 + AFG_1 + AFG_2$.
[b] Maximum residue limit.
[c] The contamination level exceeded MRLs set by the Chinese Pharmacopoeia (2020 edition);
[d] No relevant MRL.
[e] The contamination level exceeded the requirements of EC regulations.
[f] Mean value of all the analyzed samples.

30 %. High occurrence rate and contaminated contents of these mycotoxins should be taken special attention on their potential exposure risks to human health in consuming these medicinal foods in practice.

3.5. Risk assessment of mycotoxins in medicinal foods

The above findings have shown that AFs, especially AFB_1, were mainly detected in Coix seed and Lotus seed samples with high positive rate. Regarding the exposure risk assessment of AFs in Coix seed and Lotus seed, the MOE was taken into consideration. Table 6 showed that the calculated MOE values regarding AFB_1 (25.65 and 13.51) and AFs (14.29 and 12.07) in Coix seed and Lotus seed positive samples (PSs) were all far<10000. These findings indicated that the intake of Afs-contaminated Coix seed and Louts seed might pose threats and risks to human health (European Food Safety Authority, 2005). In addition, both the LB and UB values for MOE, such as 95.28 and 91.62, as well as 39.7 and 39.38 regarding AFB_1, together with 53.09 and 48.7, as well as 35.45 and 34.31 regarding AFs for Coix seed and Lotus seed, respectively, were close. Furthermore, the values for MOE of AFB_1- and AFs-positive Coix seed and Lotus seed were lower than their respective LB and UB values. All these indicated great exposure risks of AFs and AFB_1 in these two kinds of medicinal foods to human health. In regard to the other three kinds of medicinal foods, the PS and LB for MOE values were all not obtained. Thus, when the sample was AFs-negative, the exposure risk was low. However, the UB values (3777.78 for Lily and 2720.0 for Malt) were smaller than 10000, illustrating high exposure risks and potential threats of Lily and Malt when they were polluted by AFs. While, the UB values of 824.24 regarding AFB_1 and 323.81 regarding AFs for Chinese yam were smaller than 10000, manifesting possible exposure risk of AFB_1 and AFs in this medicinal food to human health. It should be noticed that the EDI data were obtained according to the regulation (Chinese Pharmacopoeia Commission, 2020b), which should

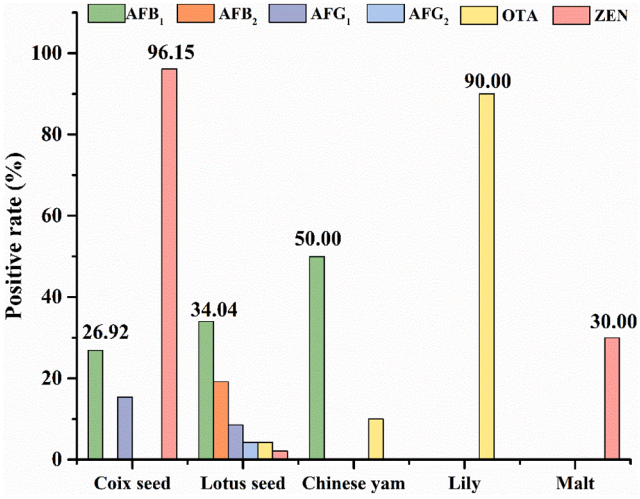

Fig. 4. Contamination rate of mycotoxins in five kinds of medicinal food samples.

be confirmed based on practical consumption amount of these medicinal foods for actual evaluation in the future. The five kinds of tested samples with diverse edible values and medicinal functions have a relatively large consumption amount in the global market, therefore, more attention should be focused on their exposure risks of AFB_1 and AFs to human health.

The exposure risks of OTA and ZEN, and the total risk of them in the tested medicinal food samples were evaluated by HQ and HI, respectively. As shown in Table 6, the HQ_{OTA}, HQ_{ZEN} and $HI_{OTA+ZEN}$ values for Coix seed, Lotus seed, Chinese yam and Malt samples were all<100 %. It indicates that the contamination levels of OTA and ZEN, as well as OTA + ZEN were within the tolerable range of human body, and the exposure risks through intake of these four kinds of medicinal food would not cause threats to human health. Whereas, the HQ_{OTA} and $HI_{OTA+ZEN}$ values for OTA- and ZEN-positive Lily samples were both 107.6405 % >100 %, illustrating that the dietary exposure levels of OTA or OTA + ZEN exceeded the permissible limit, which might pose health threats to human through intake of Lily contaminated with OTA or OTA + ZEN. In addition, the LB (96.626 %) and UB (96.9025 %) values for OTA in Lily sample were extremely close to 100 %, declaring that the long-term consumption of Lily contaminated with OTA would harm human health. Thereby, it was urgent and necessary to strengthen the monitoring of OTA in Lily samples.

In our daily life, much vigilance should be raised on the contaminated medicinal foods with mycotoxins to avoid the potential risks and damages to human health.

4. Conclusions

Medicinal foods with fascinating edible values and pharmaceutical functions are widely used in the world, but are susceptible to mycotoxins at trace levels. Regular detection of multiple mycotoxins in these samples is of great significance and urgency to ensure their quality and safety. This study has witnessed the comprehensive validation and successful application of a simple and sensitive USAE-based UFLC-MS/MS method for simultaneous determination of multi-mycotoxins in complex medicinal foods rich in starch. Pivotal experimental conditions affecting the effective extraction of mycotoxins were systematically optimized to eliminate some component interferences in five kinds of medicinal food matrices for improving the extraction efficiency and detection accuracy. It was highlighted that the developed UFLC-MS/MS strategy displayed excellent analytical performances regarding selectivity, sensitivity, linearity, precision, and accuracy for all target mycotoxins. Its practicability was verified through the successful

Table 6
Exposure risk assessment of mycotoxins in the five kinds of medicinal foods.

Mycotoxin	Parameter	Coix seed			Lotus seed			Chinese yam			Lily			Malt		
		PS	LB	UB	PS	LB	UB	PS	LB	UB	PS	LB	UB	PS	LB	UB
AFB_1	c (μg/kg)	20.39	5.49	5.71	71.89	24.47	24.67	<LOQ	ND	0.55	ND	ND	0.3	ND	ND	0.3
	EDI (ng/kg·day·bw)	6.6274	1.7843	1.8556	12.5801	4.2826	4.3172	-[a]	-	0.2063	-	-	0.045	-	-	0.0625
	MOE	25.65	95.28	91.62	13.51	39.7	39.38	-	-	824.24	-	-	3777.78	-	-	2720.0
AFs	c (μg/kg)	36.6	9.85	10.74	80.5	27.4	28.32	<LOQ	ND	1.40	ND	ND	1.20	ND	ND	1.20
	EDI (ng/kg·day·bw)	11.8936	3.2021	3.4909	14.0867	4.7955	4.9554	-	-	0.525	-	-	0.18	-	-	0.25
	MOE	14.29	53.09	48.7	12.07	35.45	34.31	-	-	323.81	-	-	944.44	-	-	680.0
OTA	c (μg/kg)	ND	ND	0.3	3.78	0.16	0.45	2.59	0.26	0.53	123.02	110.43	110.75	ND	ND	0.3
	EDI (ng/kg·day·bw)	-	-	0.0975	0.6621	0.0282	0.0784	0.9713	0.0971	0.1982	18.4527	16.5645	16.6119	-	-	0.0625
	HQ (%)	-	-	0.5688	3.862	0.1643	0.4576	5.6656	0.5666	1.1564	107.6405	96.626	96.9025	-	-	0.3646
ZEN	c (μg/kg)	472.51	454.34	454.36	49.87	1.06	1.65	ND	ND	0.6	ND	ND	1.25	6.25	1.88	2.3
	EDI (ng/kg·day·bw)	153.5673	147.6609	147.6684	8.7267	0.1857	0.2884	-	-	0.225	-	-	0.1875	1.3023	0.3907	0.4782
	HQ (%)	61.4269	59.0643	59.0673	3.4907	0.0743	0.1154	-	-	0.09	-	-	0.0750	0.5209	0.1563	0.1913
OTA+ZEN	HI (%)	61.4269	59.0643	59.6361	7.3527	0.2386	0.5729	5.6656	0.5666	1.2464	107.6405	96.626	96.9775	0.5209	0.1563	0.5559

C: mean contamination concentration of mycotoxin in the medicinal foods PS: positive sample; LB: lower bound; UB: upper bound; ND: not detected.
[a] No regulation.

identification and quantification of six mycotoxins belonging to different classes at trace levels in 103 batches of medicinal food samples that were easily polluted by mycotoxins.

Fifty-eight samples were positive with one or more mycotoxins. Coix seed had the highest positive rate, followed by Lily, Chinese yam, Lotus seed and Malt. One Lotus seed sample was simultaneously contaminated with 5 mycotoxins, and one Lotus seed and four Coix seed samples were detected with 3 mycotoxins. ZEN gave the highest positive rate with the contents in 5 Coix seeds exceeding its MRL, followed by AFB_1 and that in 7 Coix seed and 10 Lotus seed samples over its MRL, and OTA in 1 Lotus seed and 5 Lily samples greater than the corresponding MRL. Exposure risk assessment of the detected mycotoxins indicated that Coix seed and Lotus seed samples, which were susceptible to aflatoxins, presented great threats to human health. Long-term consumption of Lily samples, which were easily contaminated with OTA, would also harm human health. In addition, when multiple mycotoxins were present simultaneously in one medicinal food, synergistic toxic effects would be caused, resulting in more substantial harms to human (Alassane-Kpembi et al., 2017). In the future, we should raise much vigilance on these medicinal foods rich in starch that are easily contaminated by mycotoxins to avoid potential risks and severe damages to human health.

Based on the findings of the current study, it is of great necessity and urgency to constitute detailed MRLs for more mycotoxins in sufficient foods to guarantee the quality and safety of medicinal foods and their ending products, as well as the health of consumers.

CRediT authorship contribution statement

Xiaofang Liao: Methodology, Investigation, Validation, Writing – original draft. **Ying Li:** Software, Validation. **Nan Long:** Writing – original draft. **Qingbin Xu:** Validation. **Peng Li:** Formal analysis, Validation. **Jiabo Wang:** Validation. **Lidong Zhou:** Conceptualization, Supervision, Data curation. **Weijun Kong:** Supervision, Conceptualization, Writing – review & editing, Resources, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112456.

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