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High-throughput determination of multi-mycotoxins in Chinese yam and related products by ultra fast liquid chromatography coupled with tandem mass spectrometry after one-step extraction

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Abstract

A simple, accurate and sensitive ultra fast liquid chromatography coupled with tandem mass spectrometry (UFLC-MS/MS) method was developed for high-throughput determination of aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂), ochratoxin A (OTA), fumonisins (FB₁ and FB₂) and zearalenone (ZEA) in Chinese yam, **yam flours and yam-derived products**. Mycotoxins were extracted from the samples with methanol-water-formic acid (79:20:1, v/v/v) and no further cleanup step before analysis. After optimization of some crucial parameters including sample preparation, chromatographic separation and MS/MS conditions, the method was successfully validated to exhibit excellent performance in terms of satisfactory linearity ($r \geq 0.9977$), limits of detection ($\leq 0.15 \text{ ng} \cdot \text{mL}^{-1}$) and quantification ($\leq 0.5 \text{ ng} \cdot \text{mL}^{-1}$) with good precision (RSD for intra- and inter-day variations of $\leq 4.65\%$ and 6.31% , respectively), **good accuracy (recoveries of 71.0-106.0%) and robustness, together with short run time (8 min/sample)**. The developed method was applied for simultaneous detection and quantification of the above 8 mycotoxins in 27 batches of Chinese yam and related products collected from different markets and pharmacies in China. The results revealed that 1 normal sample and 4 moldy samples were found to be contaminated with different mycotoxins. The detected concentrations of AFB₁ in 2 moldy samples exceeded the regulatory maximum residue levels. The proposed method was capable for simultaneous determination of mycotoxins in this and other types of complex matrices.

Keywords: Multi-mycotoxins; Chinese yam **and yam flours**; **Yam-derived products**; High-throughput determination; One-step extraction; UFLC-MS/MS

1. Introduction

Chinese yam (Shanyao in Chinese) is the tuberous rhizome of the *Dioscoreaceae* plants. In China, 93 species of the plants have been found with *Dioscorea opposita* Thunb. (Huai Shan Yao) as one of the most important varieties [1]. As reported in many literatures, Chinese yam contains a variety of functional compositions, such as polysaccharides, choline, saponins, cholesterol, phytic acid, crude fibre and others [2-4]. It has been used in traditional Chinese medicine for the treatment of diabetes, asthma, diarrhea and other ailments [5], as well as an important staple food in many counties because of its rich carbohydrate components [6].

Chinese yam and its related products have played important roles in domestic and abroad. However, because of the highly nutritional components, Chinese yam has been found to be readily contaminated by mycotoxins in the processes of drying, processing, storage and transportation [7-8]. Mycotoxins are toxic secondary metabolites produced by filamentous fungi [9] and many of them, such as aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), ochratoxin A (OTA), fumonisins (FB₁, FB₂) and zearalenone (ZEA), have been considered as cytotoxic, carcinogenic, mutagenic, or immunosuppressive compounds [10]. It has been reported that 54% of yam chips obtained from markets in western Nigeria were contaminated with AFB₁ and 32% with AFB₂ [11]. The mean contaminated levels of AFB₁ and AFG₁ in yam chips in Ibadan, Oyo state, Nigeria were 14 and 17 µg·kg⁻¹ respectively [12]. Also, yam flours obtained in south-western Nigeria were found to be contaminated with aflatoxins and fumonisins [13]. Contamination of agricultural and medicinal plants, foods and related products by mycotoxins can cause many diseases or deaths in humans and animals [14]. Due to their serious toxicities, maximum residue levels (MRLs) for some mycotoxins have been set by European Union and many other organizations [15-16]. In Chinese pharmacopeia (2010 edition), the MRL of AFB₁ in traditional Chinese medicines (TCMs) is set as 5 µg·kg⁻¹ [17]. To cater to these strict limits, it is therefore necessary to develop more rapid, accurate and highly sensitive methods for monitoring multiple mycotoxins in TCMs to well understand their global contamination impact.

Analysis of mycotoxins in TCMs is difficult due to their complex matrix constituents. Current analytical techniques for mycotoxins mainly include thin-layer chromatography (TLC) [18-19], enzyme-linked immunosorbent assays (ELISA) [20-21], gas chromatography (GC) [22-23] or liquid chromatography (LC) [24-25]

coupled to various detectors. Among them, LC coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) method with high sensitivity, low detection limit and good confirmation reliability has become one of the most important analytical tools for multi-mycotoxins analysis. A large amount of studies on the analysis of several mycotoxins in foods and feeds, as well as some TCMs by using high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) are available [26-31]. Nevertheless, from then on, few studies were reported on the application of LC-MS/MS for high-throughput determination of multi-mycotoxins in Chinese yam, yam flour and yam-derived products. Owing to high edible and medicinal values, as well as high occurrence of mycotoxins in Chinese yam and related products, it is very important to develop a reliable and accurate LC-MS/MS method for objective evaluation the contamination levels of multiple mycotoxins in Chinese yam, yam flour and yam-derived products.

Normally, for mycotoxin analysis in complex matrices including TCMs, sample preparation and cleanup are necessary. Many strategies, such as matrix solid-phase dispersion (MSPD) [33], liquid-liquid extraction (LLE), “quick, easy, cheap, effective, rugged and safe” (QuEChERS) extraction [34] and solid phase extraction (SPE) [35] have been performed for these aims. Meanwhile, multifunctional columns or immunoaffinity columns have been widely used to purify the extracts. However, some of these techniques can be costly or time-consuming, and even the clean-up step will affect the whole analytical performance [36]. Therefore, injection of a crude sample extract with no further cleanup procedure has got wide attention, especially when LC-MS/MS method is used [37-39]. However, due to the complicated matrix components, injection of the crude extracts of TCMs will result in suppression or enhancement of the electrospray ionization (ESI) efficiency of the target analytes [40]. So, internal standard, matrix-matched standard calibration, and standard addition methods are used as possible approaches to control matrix effect for reliable quantification. Among them, the matrix-matched standard calibration method seems to be the most effective and accurate way to compensate the matrix effect, saving the laborious work of spiking each sample at different levels.

The objective of this work was to develop and validate a simple, accurate and sensitive UFLC-MS/MS method for high-throughput analysis of multi-class mycotoxins including AFB₁, AFB₂, AFG₁, AFG₂, OTA, FB₁, FB₂ and ZEA in Chinese yam, yam flours and yam-derived products collected from different markets

and pharmacies in China after one-step extraction. To the best of our knowledge, this is the first study on the application of a simple and high-throughput UFLC-MS/MS method for simultaneous determination of multiple mycotoxins in Chinese yam and its related products.

2. Material and methods

2.1. Material and reagents

The analytical standards of mycotoxins (AFB₁, AFB₂, AFG₁, AFG₂, OTA, FB₁, FB₂ and ZEA) were purchased from Pribolab (Singapore). Methanol and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid of analytical grade was purchased from Beijing Chemical Works (Beijing, China). Wahaha purified water (Wahaha, Hangzhou, China) was used in all experiments.

2.2. Standard solution preparation

The individual stock solution was prepared by dissolving each standard in methanol to get 2 µg·mL⁻¹ of AFB₁, AFG₁, OTA, 0.5 µg·mL⁻¹ of AFB₂, AFG₂, and 10 µg·mL⁻¹ of FB₁, FB₂, ZEA. One milliliter of the individual stock solution of OTA was mixed with 1 mL of AFs (AFB₁, AFB₂, AFG₁ and AFG₂), 1 mL of FBs (FB₁, FB₂) and 1mL of ZEA in a 10-mL volumetric flask to reach the final volume of 10 mL with methanol to prepare the mixed working solution containing 200 ng·mL⁻¹ of AFB₁, AFG₁ and OTA, 50 ng·mL⁻¹ of AFB₂ and AFG₂, and 1000 ng·mL⁻¹ of FB₁, FB₂ and ZEA. All the solutions were stored at -20 °C in amber glass vials and brought to room temperature before use.

Mixed standard solutions were prepared by diluting the mixture working solution with methanol-water (80:20, v/v) to get 1, 2, 5, 10, 25, 50, 100 ng·mL⁻¹ of AFB₁, AFG₁, and OTA; 0.25, 0.5, 1.25, 2.5, 6.25, 12.5, 25 ng·mL⁻¹ of AFB₂ and AFG₂; 5, 10, 25, 50, 125, 250, 500 ng·mL⁻¹ of FB₁, FB₂ and ZEA, respectively.

2.3. Sample preparation

Twenty-seven Chinese yam and related product samples including 18 normal dried samples, 4 moldy samples, 2 commercial yam flours and 3 **yam-derived products** (such as Yam formula particles) were obtained from different markets and pharmacies in Henan, Hebei, Anhui, Jiangxi, Shandong, Shanxi, Fujian, Guizhou, Guangxi, Guangzhou and Beijing provinces of China. The 18 normal samples were stored in a refrigerator at -20 °C, 4 samples at high humidity and temperature to become moldy and the commercial yam flours and yam formula particles were stored at 4 °C in the darkness until further use.

1 Finely ground and homogenized sample ($1.0 \text{ g} \pm 0.1 \text{ mg}$) was weight into a 10-
2 mL centrifuge tube, and 4 mL of methanol-water-formic acid (79:20:1, v/v/v) was
3 added, which was shaken by vortex for 2 min. Afterward, the mixture was extracted
4 by ultrasonication in an ultrasonic bath at 500 W for 20 min. The extraction was
5 centrifuged at 10000 rpm for 10 min. Then, 1 mL of the supernatant was immediately
6 transferred to a 2-mL eppendorf tube and evaporated to dryness under a stream of
7 nitrogen gas at 40°C . The residue was re-dissolved with 0.5 mL methanol-water
8 (50:50, v/v). After being mixed by vortex for 15 s, the sample extraction was passed
9 through a $0.22 \mu\text{m}$ syringe filter. $2 \mu\text{L}$ of the filtrate was injected into the UFLC-
10 MS/MS system for analysis.

11 2.4. UFLC-MS/MS conditions

12 All experiments was carried out on an ultra-fast liquid chromatography (UFLC)
13 system (Shimadzu, Kyoto, Japan) coupled to an Applied Biosystem 5500 QTRAP[®]
14 mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with electrospray
15 ionization (ESI). Chromatographic separation of analytes was performed on a
16 SHISEIO Capcell core C18 column ($50 \times 2.1 \text{ mm}$, $2.7 \mu\text{m}$; Shiseio, Japan). 0.1%
17 formic acid in acetonitrile (solvent A) and 0.1% formic acid in aqueous (solvent B)
18 were selected as the mobile phase. The gradient elution program as follows: 0-0.01
19 min (85% B); 8 min (40% B); 9 min (0% B); 10 min (0% B) and 10.01-12 min (85%
20 B). The flow rate was set at $0.3 \text{ mL} \cdot \text{min}^{-1}$. Total run time was 12 min and the injection
21 volume was $2 \mu\text{L}$.

22 The MS/MS detection was operated at ESI in both positive and negative
23 ionization modes. Applied Biosystems Analyst software (version 1.6) was used for
24 instrument control and data analysis. The corresponding parameters of ionization source
25 were set as follows: curtain gas (CUR), 35 psi; nebulizer gas (GS1), 50 psi; auxiliary
26 gas (GS2), 50 psi; source temperature, 550°C ; ion spray voltage (IS), +5500 V for
27 ESI⁺ and -4500 V for ESI⁻. Multiple reaction monitoring (MRM) mode was employed
28 for quantitation and the MS/MS parameters of eight target mycotoxins have been
29 listed in [Table 1](#).

30 2.5. Method performance characterization

31 Performance characteristics of the established method were validated in terms of
32 linearity, specificity, limit of detection (LOD) and quantification (LOQ), precision
33 (intra-day and inter-day variation), recoveries, robustness and matrix effect (ME).

To verify linearity, matrix-matched calibration curves for AFB₁, AFB₂, AFG₁, AFG₂, OTA, FB₁, FB₂ and ZEA were constructed by spiking blank (mycotoxin-free) samples at seven concentration levels including 1-100 µg·kg⁻¹ of AFB₁, AFG₁ and OTA, 0.25-25 µg·kg⁻¹ of AFB₂ and AFG₂, and 5-500 µg·kg⁻¹ of FB₁, FB₂ and ZEA. It is considered satisfactory when correlation coefficient (*r*) is higher than 0.99. LOD and LOQ are determined at the lowest concentration of the mycotoxin that produce chromatographic peak at signal to noise ratio (S/N) of 3 and 10, respectively [41].

The specificity of the method was evaluated by analyzing a blank (mycotoxins-free) sample and mixed standard solution.

Intra- and inter-day variations were evaluated by analyzing the fortified samples spiked with target mycotoxins at medium concentration level (5 µg·kg⁻¹ of AFB₁, AFG₁ and OTA; 1.25 µg·kg⁻¹ of AFB₂ and AFG₂; 25 µg·kg⁻¹ of FB₁, FB₂ and ZEA) to estimate the method precision. Recovery study was performed on the fortified Chinese yam samples spiking with target mycotoxins at three concentrations (low, medium and high): 1, 5, 20 µg·kg⁻¹ for AFB₁, AFG₁ and OTA; 0.25, 1.25, 5 µg·kg⁻¹ for AFB₂, AFG₂; 5, 25, 100 µg·kg⁻¹ for FB₁, FB₂ and ZEA. Recoveries between 70-120% with relative standard deviations (RSDs) lower than 20% were considered satisfactory [42].

The robustness of the UFLC-MS/MS determination of eight mycotoxins was evaluated by changing the procedural conditions involving the use various flow rates of the mobile phase at gradient elution and different types of chromatographic columns. The aims were to investigate the reliability and practicability of the established method for accurate determination of multiple mycotoxins for different users.

The matrix effect (ME) was assessed by comparing the slope of matrix-matched calibration curve with the slope of pure standard solution calibration curve. No significant matrix interference is present when the ME values range from 70-120%.

3. Results and discussion

3.1. Optimization of extraction solvent

In this study, a simple and effective extract procedure without cleanup step for 8 mycotoxins analysis were established by simplifying the whole analysis process and reducing time and solvent consumption.

The optimization of extraction solvent is critical for the detection of multi-mycotoxins in complex matrices of Chinese yam and related products. Different

1 extraction solvent systems of various ratios of methanol with water containing formic
2 acid were investigated for achieving acceptable recoveries for each mycotoxin. As
3 shown in Fig. 1, except OTA, the recoveries of the other seven mycotoxins from the
4 extraction solvent of methanol-water (80:20, v/v) were lower than that from the
5 system of methanol-water-formic acid (79:20:1, v/v/v). Especially the recoveries of
6 fumonisins were below 60% when using methanol-water (80:20, v/v) due to the
7 complex matrix components of tested samples. The solvent system of methanol-
8 water-formic acid (79:20:1, v/v/v) gave satisfactory recoveries (>80%) for all
9 mycotoxins. Addition of different ratios of formic acid to the extraction solvent could
10 improve the recoveries of aflatoxins [43] and fumonisins [35], and it also could avoid
11 degradation of certain mycotoxins [39]. Consequently, the mixture of methanol-water-
12 formic acid (79:20:1, v/v/v) was selected as a compromise for the extraction of
13 analytes in Chinese yam and related products.

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Ultrasound assisted extraction method is considered to be economic, rapid and
efficient for homogenization of complex matrices [44-45]. Herein, sonication was
chosen in the study and the optimized sonication time was 20 min. Further cleanup
step was avoided to reduce time and improve extraction efficiency.

3.2. Optimization of UFLC-MS/MS conditions

The optimal mobile phase composition was taken into consideration to achieve
excellent resolution and high sensitivity of eight target mycotoxins in UFLC-MS/MS
analysis. Methanol or acetonitrile and water containing formic acid, acetic acid or
ammonium are the commonly reported mobile phases for mycotoxin analysis. The
experiments showed that the mobile phase of acetonitrile could provide satisfactory
separation of the analytes, and the addition of formic acid would further improve the
elution efficiency, detection sensitivity, peak shape and ionization of the investigated
compounds. The solvent system of acetonitrile-water both containing 0.1% formic
acid was selected as the mobile phase. The UFLC-MS/MS MRM chromatograms of
fortified Chinese yam sample solution submitted to the proposed method were shown
in Fig. 2.

Fig. 2

For optimization of the MS/MS parameters, the individual standard solution of
each analyte was directly injected into the mass spectrometer in ESI(+/-) ionization
modes. The results revealed that AFB₁, AFB₂, AFG₁, AFG₂, OTA, FB₁ and FB₂ in the
form of [M+H]⁺ under positive ionization mode and ZEA in [M-H]⁻ under negative

ionization mode could get high MS/MS responses. The optimum parameters including mass number of precursor and production ion, declustering potential (DP), collision energy (CE), collision cell exit potential (CXP) of each analyte with the retention time have been listed in [Table 1](#).

[Table 1](#)

3.3. Method validation

3.3.1. Linearity, LOD and LOQ

The linearity was evaluated under the optimum chromatographic conditions by matrix-matched standard calibration curves obtained through analyzing fortified samples according to the previous validation protocol. Good linearity was obtained for all analytes within their concentration ranges with r in the range of 0.9977-0.9999. Low LODs and LOQs ([Table 2](#)) could satisfy the related regulatory levels of European Union and other organizations [46] for sensitive detection of mycotoxins at trace levels.

[Table 2](#)

3.3.2. Specificity, precision and recovery

The specificity of the method was tested by the analysis of blank (mycotoxin-free) samples. No interfering peaks were observed at the retention times of target analytes, suggesting high detection specificity of the developed method.

The precision of the method, expressed as RSDs, was estimated by repeated analysis ($n=6$) of the fortified sample spiked with the 8 analytes at medium concentration levels during the same day (intra-day) and on different six days (inter-day). The results in [Table 2](#) showed that the RSDs of intra- and inter-day variations were in the range of 1.63-4.65% and 2.14-6.31%, respectively, which were below the values recommended by European Union [42].

Recovery experiments were performed at three spiking levels (as indicated in [Table 2](#)) according to the MRLs set by the European Commission to check the trueness of the established method. The fortified samples spiked with the 8 analytes were incubated in the dark at room temperature for 1 h in order to ensure evaporation and let the compounds interact with the matrix. Then, the samples were treated and analyzed following the previously described procedure. Recovery is calculated as:

$$\text{Recovery (\%)} = 100 * (\text{mean measured concentration} / \text{spiked concentration})$$

The results in Table 2 showed satisfactory recoveries in the range of 71.0-106.0% with RSDs less than 16.5%, fulfilling with the current demands for mycotoxin analysis.

3.3.3. Robustness

As an intra-laboratory trial, robustness study is termed as the capacity of a UFL-MS/MS method to yield accurate quantitation results in the presence of small changes of chromatographic conditions [47]. Here, the changed procedural conditions involved various flow rates of the mobile phase and different types of chromatographic columns to evaluate the robustness of the established method. It could be seen in Fig. 3 that in comparison with the flow rate at 0.30 mL/min (Fig. 3A), the retention times of the eight analytes in Fig. 3B and C changed (increase or decrease) when using SHISEIO Capcell core C18 column (50 × 2.1 mm, 2.7 μm), and the retention times (Fig.3D) except ZEA when using Waters UPLC® BEH Phenyl column (50 × 2.1 mm, 1.7 μm). Although the retention times were changed, the peak intensities of the eight analytes expressed no obvious changes when different flow rates of the mobile phase and chromatographic columns were used, elucidating good robustness of the established UFL-MS/MS method for accurate quantitation of the eight mycotoxins in complex Chinese yam samples.

Fig. 3

3.3.4. Matrix effect

Matrix effect (ME), due to the presence of impurities, will affect the accuracy of a method leading to incorrect results. Herein, injection of crude extracts of Chinese yam without any purification should take careful consideration of ME. The response of target mycotoxin could be suppressed or enhanced by the matrix components, which was commonly known as signal suppression/enhancement effect (SSE). And, the SSE is calculated to correct the response according to the following equation:

$$\text{SSE\%} = 100 * (\text{slope matrix} - \text{matched standard} / \text{slope solvent} - \text{based standard})$$

If the SSE <100%, ion suppression occurs; if the SSE >100%, ion enhancement is taking place and the SSE of 70-120% is acceptable. As can be seen in Table 2, ion suppression effect was observed for AFB₁, AFB₂, AFG₁, AFG₂ and ZEA, while ion enhancement effect for OTA, FB₁ and FB₂, which will interfere the accurate quantitation of target analytes. Therefore, the previously proposed matrix-matched standard calibration curves were could be used for quantification purposes.

3.4. Application to real samples

The developed method was verified by its application in 27 Chinese yam samples, including 8 normal dried samples, 4 moldy samples, 2 commercial yam flours, as well as 3 yam formula particles in China. As shown in Table 3, only 1 out of the 18 normal dried samples were detected with FB₁. But, the contamination level was below the regulatory MRLs suggested by EU. AFB₁, FB₁ and FB₂ were all detected in the moldy samples, and the contamination concentrations of AFB₁ in samples S21 and S22 exceeded the suggested MRLs. The typical MRM chromatograms of AFB₁, FB₁ and FB₂ in positive Chinese yam sample were shown in Fig. 4. The mycotoxins are in agreement with previous reports, OTA and ZEA did not contaminate yams whereas contamination by AFs and FBs was observed [13]. In addition, the commercial yam flours and formula particles were not contaminated by any mycotoxin (as indicated in Fig. 4).

Fig. 4

The proposed method was proved to be simple, quick and effective for high-throughput detection of multi-mycotoxins in Chinese yam, yam flours and yam-derived products, which is acceptable for routine monitoring of trace components in other complex matrices.

4. Conclusions

A simple, highly sensitive and specific UFLC-MS/MS method in ESI⁺ and ESI⁻ ionization modes was developed and validated for simultaneous determination of multi-class mycotoxins (AFB₁, AFB₂, AFG₁, AFG₂, FB₁, FB₂ and ZEA) in Chinese yam, yam flours and yam-derived products. All the 8 analytes were well separated in 8 min on a C18 column by using with mobile phase of acetonitrile-water both containing 0.1% formic acid at gradient elution. The samples were treated by one-step extraction without any clean-up step and the matrix effect was calculated to correct the determined concentrations. The developed method exhibited excellent performance in terms of selectivity, specificity, robustness, short run time of analysis and simplicity of sample preparation for multi-mycotoxin analysis at trace level in Chinese yam and related products. Real application of the developed method showed that 1 normal sample and 4 moldy samples were contaminated with different mycotoxins and the concentrations of AFB₁ in 2 moldy samples were found to exceed the suggested MRLs by EU. Therefore, it is necessary to be alert for Chinese yam contamination, as well as is in urgent need for regular monitoring of multi-class mycotoxins to warrant the safety and quality of Chinese yam and related products.

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Figure legends

Fig. 1. Effects of different solvents on the extraction efficiency of mycotoxins from Chinese yam.

Fig. 2. UFLC-MS/MS chromatograms of (A) blank Chinese yam sample; and (B) fortified Chinese yam sample spiked with $5 \mu\text{g}\cdot\text{kg}^{-1}$ of AFB₁, AFG₁ and OTA; $1.25 \mu\text{g}\cdot\text{kg}^{-1}$ of AFB₂ and AFG₂; $25 \mu\text{g}\cdot\text{kg}^{-1}$ of FB₁, FB₂ and ZEA.

Fig. 3. Robustness study for the UFLC-MS/MS for eight mycotoxins in Chinese yam samples.

Flow rates of (A) $0.3 \text{ mL}\cdot\text{min}^{-1}$, (B) $0.25 \text{ mL}\cdot\text{min}^{-1}$ and (C) $0.35 \text{ mL}\cdot\text{min}^{-1}$ by using SHISEIO Capcell core C18 column ($50 \times 2.1 \text{ mm}$, $2.7 \mu\text{m}$); and (D) flow rate of $0.3 \text{ mL}\cdot\text{min}^{-1}$ by using Waters UPLC[®] BEH Phenyl column ($50 \times 2.1 \text{ mm}$, $1.7 \mu\text{m}$).

Fig. 4. UFLC-MS/MS MRM chromatograms of (A) fortified sample with $5 \mu\text{g}\cdot\text{kg}^{-1}$ of AFB₁, $25 \mu\text{g}\cdot\text{kg}^{-1}$ of FB₁ and $25 \mu\text{g}\cdot\text{kg}^{-1}$ of FB₂; (B) positive sample contaminated with AFB₁; (C) positive sample contaminated with FB₁ and FB₂, and (D) related product sample without contamination.

Figure

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Table 1. MS/MS parameters of the eight target mycotoxins.

Mycotoxins	Retention time (min)	Precursor ion	Polarity	Dwell time (ms)	Production ions ^a	Dp (V)	CE (eV)	CXP (V)
AFG ₂	3.51	331.1	ESI ⁺	80	245.0(Q)	120	40	13
					285.1(q)	120	37	13
AFB ₂	3.91	315.1	ESI ⁺	80	287.0(Q)	90	34	13
					259.0(q)	90	38	13
AFG ₁	3.97	329.1	ESI ⁺	80	243.0(Q)	70	35	13
					311.2(q)	70	28	13
FB ₁	4.13	722.5	ESI ⁺	80	352.4(Q)	120	48	13
					334.5(q)	120	52	14
AFB ₁	4.36	313.1	ESI ⁺	80	285.1(Q)	100	30	13
					241.0(q)	100	37	13
FB ₂	5.07	706.5	ESI ⁺	80	336.2(Q)	110	47	15
					318.5(q)	110	50	14
ZEA	7.03	317.1	ESI ⁻	80	131.0(Q)	-70	-41	-11
					175.0(q)	-70	-25	-7
OTA	7.06	404.2	ESI ⁺	80	358.0(q)	100	36	13
					239.1(Q)	100	30	13

^a Product ions: (Q) quantification, (q) qualitation;

DP: Declustering potential, CE: Collision energy, CXP: Collision cell exit potential.

Table 2. Linearity, limits of detection, precision and recoveries for 8 mycotoxins using the developed UFLC-MS/MS method.

Mycotoxins	Linear data			LOD (ng·mL ⁻¹)	LOQ (ng·mL ⁻¹)	SSE (%)	Precision		Recoveries (RSD, %)		
	Linear equation	<i>r</i>	Ranges (ng·mL ⁻¹)				Intra-day	Inter-day	High level ^a	Medium level ^b	Low level ^c
AFB ₁	y=26900x-14700	0.9996	1-100	0.02	0.06	20.15	2.31	2.14	94.20(2.78)	97.27 (5.11)	105.60 (2.14)
AFB ₂	y=19700x-2620	0.9991	0.25-25	0.06	0.20	78.80	4.65	4.86	93.20(5.20)	94.13 (1.96)	95.04 (7.14)
AFG ₁	y=19600x+1770	0.9999	1-100	0.05	0.15	89.91	2.32	4.56	95.10(7.21)	96.53 (4.27)	92.20 (2.76)
AFG ₂	y=6690x+2730	0.9977	0.25-25	0.06	0.20	72.71	1.63	5.10	91.73(16.5)	92.00 (8.29)	74.96 (4.07)
OTA	y=26900x-457	0.9999	1-100	0.05	0.15	103.07	2.08	6.31	94.43(2.90)	96.33 (3.18)	98.20 (7.20)
FB ₁	y=1770x+1120	0.9999	5-500	0.05	0.15	199.78	3.13	3.97	105.73(0.89)	99.20 (8.47)	106.00 (5.87)
FB ₂	y=3740x-6100	0.9995	5-500	0.15	0.50	212.50	1.66	2.87	91.47(3.11)	97.20 (1.48)	102.40 (11.04)
ZEA	y=20700x+108000	0.9998	5-500	0.15	0.50	99.52	2.53	3.31	95.80(2.57)	90.40 (4.79)	71.00 (8.76)

^a AFB₁, AFG₁, OTA: 20 µg·kg⁻¹; AFB₂, AFG₂: 5 µg·kg⁻¹; FB₁, FB₂, ZEA: 100 µg·kg⁻¹

^b AFB₁, AFG₁, OTA: 5 µg·kg⁻¹; AFB₂, AFG₂: 1.25 µg·kg⁻¹; FB₁, FB₂, ZEA: 25 µg·kg⁻¹

^c AFB₁, AFG₁, OTA: 1 µg·kg⁻¹; AFB₂, AFG₂: 0.25 µg·kg⁻¹; FB₁, FB₂, ZEA: 5 µg·kg⁻¹

SSE: signal suppression/enhancement effect

Table 3. Contamination levels of 8 mycotoxins in Chinese yam and related products.

Samples	Sample no.	Source	Mycotoxins detected	Mycotoxin residue level ($\mu\text{g}\cdot\text{kg}^{-1}$)	MRLs suggested ($\mu\text{g}\cdot\text{kg}^{-1}$)
Normal samples	S1	Henan province	ND	-	
	S2	Henan province	ND	-	
	S3	Henan province	ND	-	
	S4	Henan province	ND	-	
	S5	Henan province	ND	-	
	S6	Henan province	FB ₁	0.878	1000 $\mu\text{g}\cdot\text{kg}^{-1}$ for FBs
	S7	Henan province	ND	-	
	S8	Hebei province	ND	-	
	S9	Hebei province	ND	-	
	S10	Fujian province	ND	-	
	S11	Fujian province	ND	-	
	S12	Guangzhou province	ND	-	
	S13	Guizhou province	ND	-	
	S14	Anhui province	ND	-	
	S15	Shanxi province	ND	-	
	S16	Shandong province	ND	-	
	S17	Jiangxi province	ND	-	
	S18	Guangxi province	ND	-	
Moldy samples	S19	Fujian province	FB ₁	1.905	1000 $\mu\text{g}\cdot\text{kg}^{-1}$ for FBs
	S20	Henan province	AFB ₁	1.305	5 $\mu\text{g}\cdot\text{kg}^{-1}$ for AFB ₁
			FB ₁	3.727	1000 $\mu\text{g}\cdot\text{kg}^{-1}$ for FBs
			FB ₂	< LOQ	
	S21	Shanxi province	AFB ₁	7.737	5 $\mu\text{g}\cdot\text{kg}^{-1}$ for AFB ₁
			FB ₁	< LOQ	
Yam flours	S22	Hebei province	AFB ₁	9.688	5 $\mu\text{g}\cdot\text{kg}^{-1}$ for AFB ₁
	S23	Shandong province	ND	-	
	S24	Shandong province	ND	-	
Formula particles	S25	Beijing province	ND	-	
	S26	Beijing province	ND	-	
	S27	Beijing province	ND	-	

ND: Not detected.