Multi-mycotoxins analysis in ginger and related products by UHPLC-FLR detection and LC-MS/MS confirmation

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A R T I C L E   I N F O

Article history:
Received 15 November 2013
Received in revised form 20 February 2014
Accepted 23 February 2014
Available online 5 March 2014

Keywords:
Ginger and related products
Aflatoxins
Ochratoxin A
Immunofluorescence (FLR)
UHPLC-FLR
LC-ESI-MS/MS

A B S T R A C T

The aim of this study was to optimize and validate a powerful method for the simultaneous analysis of aflatoxins $B_1$ (AFB1), $B_2$ (AFB2), $G_1$ (AFG1), $G_2$ (AFG2) and ochratoxin A (OTA) in ginger and related products collected from local markets in Beijing, China. The optimized analytical procedure was based on immunofluorescence column (IAC) clean-up, followed by ultra-high performance liquid chromatography with fluorescence (UHPLC-FLR) detection. Limits of detection (LOD) and quantification (LOQ) for the five mycotoxins were 0.005–0.2 and 0.0125–0.5 µg kg$^{-1}$, respectively. The average recoveries ranged from 84.2 to 97.3% with relative standard deviations (RSDs) from 0.63 to 7.86% at three spiking levels. Good linearity was observed for the analytes with correlation coefficients all higher than 0.9995. The established method was applied to 30 samples of 10 different species of ginger and related products, and all positive samples were confirmed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The results showed that 5 samples of ginger products were contaminated with AFB1 at 0.01–1.38 µg kg$^{-1}$, while 3 samples of ginger and 2 samples of ginger products were contaminated with OTA at 0.31–5.17 µg kg$^{-1}$. All the contamination levels were below the legally allowable limits.

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1. Introduction

Mycotoxins are secondary metabolites produced by toxigenic fungi which spread widely in the environment and are considered to be one of the most important contaminants in food and feed. According to estimations of the FAO (Food and Agriculture Organization of the United Nations), more than 25% of the world’s agricultural products are contaminated with mycotoxins (Food and Agriculture Organization, 2004). These mycotoxins pose serious threats to human and animal health because of their carcinogenicity, mutagenicity, teratogenicity and immunosuppression, and they can cause disease or death in humans and domestic animals when ingested, inhaled, or absorbed through the skin. The most commonly induced diseases include liver cancer, kidney failure, and effects on the brain or nervous system. Among these mycotoxins, aflatoxins (AFs) and ochratoxins are of great interest because they are more toxic and more common than others. AFs are mainly produced by strains of Aspergillus flavus and Aspergillus parasiticus, and are classified as having genotoxic and carcinogenic effects (Joint FAO/WHO Expert Committee on Food Additives, 2008, pp. 305–356). Evidence exists that aflatoxin exposure before birth and in early childhood is associated with stunted growth, which was defined by WHO as height for age being more than two standard deviations below average height for age in a given population (Pitt, 2013). AFs include aflatoxin $B_1$ (AFB1), $B_2$ (AFB2), $G_1$ (AFG1), $G_2$ (AFG2), $M_1$ (AFM1) and $M_2$ (AFM2), among which, AFB1, AFB2, AFG1, AFG2 were frequently found in agricultural products, and due to their hepatotoxic and hepatocarcinogenic properties, the content of AFB1, AFB2, AFG1, AFG2 in foods is restricted in many countries (Food and Agriculture Organisation, 2004). AFB1 is the most common one, usually comprising about 90% of the aflatoxins residue observed on contaminated foodstuffs. It is the most potent hepatocarcinogen known in mammals and classified by the International Agency for Research on Cancer (IARC) as Group 1A carcinogen (International Agency for Research on Cancer—IARC, 1993, pp. 359–362). Ochratoxins as ochratoxin A (OTA), ochratoxin B (OTB) and ochratoxin C (OTC) are mainly produced by Penicillium verrucosum and Aspergillus ochraceus in semitropical and temperate climate. OTA is distinctively more toxic and prevalent than OTB and is rapidly formed in the body from OTC, additionally it is a chronic
nephrotoxin and can affect kidney function, so it has attracted by far the most attention. It has carcinogenic properties as well, but the carcinogenic effects in animals are considered to be of less importance than the nephrotoxicity. Based on sufficient evidence of carcinogenicity in experimental animal studies and inadequate evidence in humans, the IARC has classified OTA as a possible human carcinogen (Group 2B) (International Agency for Research on Cancer—IARC, 1999). AFs and OTA have been considered as unavoidable contaminants of food and feed, even under good manufacturing practices conditions.

Ginger is not only an important spice, but also a traditional Chinese medicine (TCM). Firstly, ginger has been used since antiquity as food additives for the purpose of flavoring. As a spice, ginger is widely used as a food condiment or a nutritional supplement all over the world and has been used in many industries with the food industry and catering being predominant users. It can be marketed in many forms such as fresh or dried products, liquid or solid extracts, tablets or capsules, powders and tea bags. What’s more, ginger is a frequently-used TCM and has been officially recorded in the Chinese Pharmacopoeia (Chinese Pharmacopoeia, 2010) for its therapeutic benefits to treat common cold especially nausea and vomiting (Kawai, Kinoshita, Koyama, & Takahashi, 1984). Up to now, ginger and its related products have played important roles in the economy of the countries producing, importing or exporting them. However, ginger is prone to be contaminated by mycotoxins during pre- and post-harvest, and a few publications have reported that AFs and OTA have been found in ginger and its related products with various contamination levels (Truckssess, Weaver, Oles, Fry Jr., & Noonan, 2008; Whitaker, Truckssess, Weaver, & Slate, 2009). Mohamed Hashem et al. have detected about 138 samples of 15 different spices collected from famous supermarkets from Aseer region, Saudi Arabia and found that ginger samples were contaminated the most heavily among all the spice samples (Hashem & Alamri, 2010). Based on the threats of AFs and OTA to human health and the fact that ginger can be easily contaminated by them, the European Union introduced measures to minimize the presence of AFs and OTA in different foodstuffs. Maximum residue levels of AFs and OTA in ginger (5 μg kg⁻¹ for AFB₁, 10 μg kg⁻¹ for total AFs and 15 μg kg⁻¹ for OTA) are laid down in Commission Regulation (EC) No. 1881/2006 (Commission Regulation, 2010a, 2010b).

Although few studies about determination of AFs and OTA in ginger have been done previously, a simple, rapid, sensitive, and specific method to measure AFs and OTA in a single test is required.

This study aimed to optimize and validate an ultra-high performance liquid chromatography with fluorescence (UHPLC-FLR) detection method for the simultaneous analysis of AFB₁, AFB₂, AFG₁, AFG₂ and OTA in ginger and its related products collected in Beijing, China. To the best of our knowledge, no single method that simultaneously analyzed these mycotoxins in ginger and related products using UHPLC-FLR has been published in the literature.

2. Experimental

2.1. Chemicals and reagents

Aflatoxins standard (2 μg of AFB₁, 2 μg of AFG₁, 0.5 μg of AFB₂, 0.5 μg of AFG₂ in 1 mL of acetonitrile) was purchased from PriboLab (Singapore), and OTA standard (1 mg, powder) was from ALEXIS (Lausen, Switzerland). The IAC-SEP® IAC-AFLA/OTA immunoaffinity columns (IACs) and GF/A glass microfiber filter (1.5 μm) were all purchased from VICAM (Waterworn, MA, USA). A KQ-500 ultrasonic cleaning bath (50 × 30 × 35 cm) was bought from Kunshan Ultrasonic Instrument Co. Ltd. (Jiangsu, China). HPLC-grade methanol was purchased from Honeywell (Burdick & Jackson, USA). Other reagents and chemicals were all analytical grades and purchased from Beijing Chemical Works (Beijing, China). Water used in this experiment was Wahaha purified water (Wahaha, Hangzhou, China).

Phosphate-buffered saline (PBS) was prepared by dissolving 0.2 g of KCl, 0.2 g of KH₂PO₄, 8 g of NaCl and 1.2 g of Na₂HPO₄ in 1000 mL of water (pH was adjusted to 7.0 with 0.1 M HC₃). 2% tween-20PBS solution was prepared by dissolving 0.2 g of KCl, 0.2 g of KH₂PO₄, 8 g of NaCl and 1.2 g of Na₂HPO₄ and 20 mL of tween-20 in 1000 mL of water.

2.2. Standard solutions

A series of working solutions (0.05, 0.1, 0.3, 0.5, 1, 2, 3, 4, 5, 10 ng mL⁻¹ for AFG₁, AFB₁, OTA and 0.0125, 0.025, 0.075, 0.125, 0.25, 0.5, 0.75, 1, 1.25, 2.5 ng mL⁻¹ for AFG₂, AFB₂) were prepared freshly with the solution of methanol/water (50:50, v/v). The standard solutions were used to calculate the LC detector response and recovery studies.

2.3. Samples

Thirty samples of 10 different species of ginger and its related products including fresh ginger, moldy ginger, ginger powder, baked ginger, ginger peels, ginger tea bags, ginger black tea bags, ginger-red sugar tea bags, ginger-red jujube tea bags, ginger-black sugar tea bags were all purchased from different local markets and supermarkets (Beijing, China). They were packaged in plastic bags and stored at 4 °C in a fridge prior to analysis. All the samples were extracted and analyzed in triplicate.

2.4. Sample preparation

2.4.1. Extraction procedure

Ten grams of tested sample (fresh ginger should be chopped) and 2 g of NaCl were weighed and placed in a 100 mL Erlenmeyer flask, and then 50 mL of methanol/water (80:20, v/v) solution were added and the mixtures were extracted by ultrasonication in an ultrasonic cleaning bath at 500 W for 20 min. The extraction was filtered through a filter paper and 10 mL of the filtrate was diluted by 40 mL of 2% tween-20PBS solution in a 50 mL Erlenmeyer flask. Finally, the diluent was filtered through the GF/A glass microfiber filter and the final filtrate was obtained for analysis.

2.4.2. Immunoaffinity column cleanup

25 mL of the final filtrate was passed through an IAC-SEP® IAC-AFLA/OTA IAC. The column was washed with 20 mL of PBS solution (pH 7.0) until 2–3 mL of air passed through it and the investigated toxins were finally eluted with 3 mL of methanol. The elutes were evaporated to dryness under a stream of N₂ at 50 °C and the residue were re-dissolved in 1 mL of methanol/water (50:50, v/v). The solution was vortexed for 30 s and filtered through a 0.22 μm syringe filter. 3 μL of the filtrate was injected into the UHPLC system.

2.5. Apparatus and UHPLC conditions

UHPLC analysis was performed on a Waters ACQUITY UPLC® H-Class system (Waters, MA, USA) connecting with a fluorescence detector and the Waters Empower data software. UHPLC separations were performed on an ACQUITY UPLC® HSS T3 column (2.1 × 50 mm, 18 μm) and the column and sample temperatures were both kept at 30 °C. The mobile phase consisted of (A)
methanol and (B) water (0.5% aqueous acetic acid) at a flow rate of 0.2 mL min\(^{-1}\), freshly prepared every day. The optimized gradient elution procedure was as follows: 0–4 min (45%, A), 5–11 min (70%, A). The injection volume was 3 \(\mu\)L. The elution was monitored by a fluorescence detector. During the first 7 min of analysis, fluorescence conditions were optimized for AFs (excitation 360 nm and emission 440 nm wavelengths), and after that for OTA (excitation 333 nm and emission 460 nm wavelengths). The investigated mycotoxins could be well separated in 20 min under the above conditions.

### 2.6. UFLC-MS/MS conditions

A Shimadzu ultra-fast liquid chromatography (UFLC) system (Shimadzu, Kyoto, Japan) coupled to a 5500 QTRAP\textsuperscript{®} hybrid triple quadrupole/near ion trap (QqQLIT) mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source (AB SCIEX, Foster City, CA, USA) was used for the LC-MS/MS analysis. Applied Biosystems Analyst software (version 1.6) was used to control the LC-MS/MS system and for data acquisition and processing. AFs and OTA were separated on an Agilent Poroshell 120 EC-C18 column (4.6 mm \(\times\) 50 mm, 2.7 \(\mu\)m) operated at 30 \(^\circ\)C. The mobile phase consisted of (A) acetonitrile (0.1% formic acid) and (B) water (0.1% formic acid) at a flow rate of 0.3 mL min\(^{-1}\) and the injection volume was 2 \(\mu\)L. The optimized gradient elution procedure was as follows: 40% A for 4.00 min, linear gradient to 80% A between 4.00 and 5.00 min, held at 80% until 8.00 min, and then returned to 40% B in 0.01 min. The run time was 10.00 min. The mass spectrometer was operated in the positive ESI mode with MRM at unit resolution. The main MS parameters were optimized and finally set as follows: nebulizer gas (GS1), 50 psi; auxiliary gas (GS2), 50 psi; curtain gas (CUR), 35 psi; capillary temperature, 550 \(^\circ\)C; ion spray voltage (IS), 5500 V. Nitrogen was used as the nebulizer, heater, curtain and collision gas. The precursor-to-product ion transitions were \(m/z\) 313.1 → 285.1 and \(m/z\) 313.1 → 245.0 for AFB\(_1\), \(m/z\) 315.1 → 287.0 and \(m/z\) 315.1 → 259.0 for AFB\(_2\), \(m/z\) 329.1 → 243.0 and \(m/z\) 329.1 → 311.2 for AFG\(_1\), \(m/z\) 331.1 → 285.1 and \(m/z\) 331.1 → 241.0 for AFG\(_2\), \(m/z\) 404.2 → 358.0 and \(m/z\) 404.2 → 239.1 for OTA.

#### Table 1

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linear equation</th>
<th>(R^2)</th>
<th>Range (ng mL(^{-1}))</th>
<th>LOD (ng kg(^{-1}))</th>
<th>LOQ (ng kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFG(_2)</td>
<td>(y = 1,18,922x + 440.41)</td>
<td>0.9997</td>
<td>0.025–2.5</td>
<td>0.008</td>
<td>0.025</td>
</tr>
<tr>
<td>AFG(_1)</td>
<td>(y = 10,885x - 1609.8)</td>
<td>0.9995</td>
<td>0.5–10</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>AFB(_2)</td>
<td>(y = 3,11,786x - 1088.2)</td>
<td>0.9997</td>
<td>0.0125–2.5</td>
<td>0.005</td>
<td>0.0125</td>
</tr>
<tr>
<td>AFB(_1)</td>
<td>(y = 52,868x - 710.79)</td>
<td>0.9998</td>
<td>0.1–10</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>OTA</td>
<td>(y = 11,295x + 184.83)</td>
<td>0.9995</td>
<td>0.3–10</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Fig. 1. UHPLC-FLR chromatograms of (A) mixed standard solution of AFs and OTA; (B) blank ginger sample spiked with AFs and OTA at the level of 5 \(\mu\)g kg\(^{-1}\) for AFG\(_1\), AFB\(_1\), OTA and 1.25 \(\mu\)g kg\(^{-1}\) for AFG\(_2\), AFB\(_2\); and (C) blank ginger sample.
3. Results and discussion

3.1. Sample extraction and clean-up

The sample extraction should allow good recoveries for all analytes of interest in the specific matrix and the selection of extraction solvent mixture is very important for achieving the true value. As reported previously, a high percentage of organic solvent is widely used for the extraction of AFs and OTA from real samples. Acetonitrile based extractant could lead to absorption of water by the dry matrix, and methanol/water mixtures could prevent this (Stroka, Petz, Joerissen, & Anklam, 1999), so methanol/water system is commonly used for the extraction of AFs and OTA from samples. Methanol/water (80:20, v/v) was chosen as the extraction solvent in this study since it provided the highest recoveries for AFs and OTA as reported (Rahmani, Jinaip, Soleimany, Khatib, & Tan, 2011). Sonication is a convenient, economic and widely used method for extraction of mycotoxins with no need of special instrument compared with other extraction procedures and it can provide high recoveries as reported (Romero-González, Vidal, Aguilera-Luiz, & Frenich, 2009; Yang et al., 2010). Herein, sonication was chosen in this study and the optimized ultrasonication time was 20 min, after repeated experiments for high extraction efficiencies of AFs and OTA. Sodium chloride was added in the step of extraction for the purpose of salting out.

In order to reduce the matrix effects caused by the complex matrices such as ginger powder and baked ginger, purification was performed with IAC-SEP® AFLA/OTA IAC due to its efficiency and specificity for AFB1, AFB2, AFG1, AFG2 and OTA (Kong, Liu, Qiu, Xiao, & Yang, 2013; Wei et al., 2013).

3.2. Optimization of UHPLC-FLR conditions

In order to get a high separation efficiency of the five investigated analytes, some important parameters of chromatographic conditions including the proportion and flow rate of mobile phase, column temperature and elution procedure were optimized.

Methanol/water system was widely used as mobile phase to separate mycotoxins and it can provide high separation efficiency, some important parameters of chromatographic conditions including the proportion and flow rate of mobile phase, column temperature and elution procedure were optimized.

Acetonitrile based extractant could lead to absorption of water by the dry matrix, and methanol/water mixtures could prevent this (Stroka, Petz, Joerissen, & Anklam, 1999), so methanol/water system is commonly used for the extraction of AFs and OTA from samples. Methanol/water (80:20, v/v) was chosen as the extraction solvent in this study since it provided the highest recoveries for AFs and OTA as reported (Rahmani, Jinaip, Soleimany, Khatib, & Tan, 2011). Sonication is a convenient, economic and widely used method for extraction of mycotoxins with no need of special instrument compared with other extraction procedures and it can provide high recoveries as reported (Romero-González, Vidal, Aguilera-Luiz, & Frenich, 2009; Yang et al., 2010). Herein, sonication was chosen in this study and the optimized ultrasonication time was 20 min, after repeated experiments for high extraction efficiencies of AFs and OTA. Sodium chloride was added in the step of extraction for the purpose of salting out.

In order to reduce the matrix effects caused by the complex matrices such as ginger powder and baked ginger, purification was performed with IAC-SEP® AFLA/OTA IAC due to its efficiency and specificity for AFB1, AFB2, AFG1, AFG2 and OTA (Kong, Liu, Qiu, Xiao, & Yang, 2013; Wei et al., 2013).

3.3. Method validation

The above-optimized chromatographic conditions were assessed for stability, linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, reproducibility and precision. Reproducibility and recovery were in agreement with requirements established by EU Regulation No. 401/2006. The results below proved that the established UHPLC-FLR method was satisfactory for the simultaneous multi-mycotoxins determination of AFs and OTA in ginger samples after ultrasonic extraction and IAC clean-up.

3.3.1. Stability, precision and reproducibility

The stability of spiked ginger samples (5 μg kg⁻¹ for AFB1, AFG1 and OTA, 1.25 μg kg⁻¹ for AFB2 and AFG2) was determined by monitoring the peak area of each analyte over a period of 24 h on the same day. The relative standard deviation (RSD) values for the peak areas of AFG2, AFG1, AFB2, AFB1 and OTA were 0.31%, 2.62%, 1.87%, 2.30%, 2.85%, respectively, demonstrating good stability.

The precision of the established method was determined by measuring intra- and inter-day precision from analysis of mixed standard solutions containing 5 ng mL⁻¹ of AFB1, AFG1 and OTA, 1.25 ng mL⁻¹ of AFB2 and AFG2. The intra-day precision was determined by six consecutive injections of the mixed standard solution within one day. The inter-day precision was determined by five consecutive injections of the mixed standard solution during five consequent days. Intra- and inter-day precisions were expressed by the RSD of replicate results and the RSD values were 2.2–3.5% and 2.8–5.2%, respectively, which were well below the reference values calculated for the five analytes as recorded by

| Table 3 |

<table>
<thead>
<tr>
<th>Contents (μg kg⁻¹)</th>
<th>AFG2</th>
<th>AFG1</th>
<th>AFB2</th>
<th>AFB1</th>
<th>OTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fresh ginger</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Moldy fresh ginger</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.32</td>
</tr>
<tr>
<td>Ginger powder</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Baked ginger</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ginger tea bag</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.13</td>
<td>-</td>
</tr>
<tr>
<td>Ginger black sugar tea bag</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.38</td>
<td>-</td>
</tr>
<tr>
<td>Ginger red sugar tea bag</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.23</td>
<td>-</td>
</tr>
<tr>
<td>Ginger red jujube tea bag</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.15</td>
</tr>
<tr>
<td>Ginger-black sugar tea bag</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Not detected.
European Commission Regulation. The above results indicated good precision.

The reproducibility of the method was determined by analyzing the blank ginger samples (which didn’t contain any of the five mycotoxins) spiked with 5 µg kg⁻¹ of AFB₁, AFG₁ and OTA, 1.25 µg kg⁻¹ of AFB₂ and AFG₂ and separately treated by the above-described procedure. Each analysis was repeated six times and the peak area of each analyte was recorded. The RSD values of the peak areas of five analytes were 6.5–10.2%, among which, the values of AFG₂ and AFG₁ were a bit worse than that of AFB₁, AFG₁ and OTA.

3.3.2. Linearity, LOD and LOQ

Calibration curves were constructed with nine levels of investigated standard analytes in the range of 0.1–10 ng mL⁻¹ for AFB₁, 0.0125–2.5 ng mL⁻¹ for AFB₂, 0.5–10 ng mL⁻¹ for AFG₂, 0.025–2.5 ng mL⁻¹ for AFB₂ and 0.3–10 ng mL⁻¹ for OTA, each standard solution was injected triplicate. The calibration curves were constructed by plotting the peak area (y) versus concentration (x) of each analyte obtained from UHPLC analysis. The linearity was determined by linear regression analysis and expressed as correlation coefficient (R).

The LOD and LOQ were used to assess the sensitivity of the UHPLC-FLR method, which were determined by injecting serial diluted the blank ginger samples spiked with a certain concentration of mixed standard solutions and were calculated as signal-to-noise (S/N) ratio of 3 and 10.

The detailed results of linearity, LOD and LOQ were concluded in Table 1 and the results indicated the good sensitivity of the established method. It can be seen from the table that the linearities of the five analytes were very good with R higher than 0.9995, and the LODs and LOQs were 0.008–0.2 µg kg⁻¹, 0.0125–0.5 µg kg⁻¹, respectively.

3.3.3. Recovery

The recovery was used to evaluate the accuracy of the established method. The recovery experiments were performed at three spiking levels (1.0, 5.0, 25.0 µg kg⁻¹ for AFB₁, AFG₁, OTA and 0.25, 1.25, 6.25 µg kg⁻¹ for AFB₂, AFG₂) by adding an appropriate amount of AFs and OTA standard solutions to the blank ginger samples. The spiked samples were extracted, cleaned-up, derivatized and analyzed by UHPLC-FLR as previously described. The recovery was calculated by the following equation:

\[
\text{Recovery} = \left( \frac{\text{measured concentration for spiked sample}}{\text{spiked concentration}} \right) \times 100\%
\]

The typical UHPLC-FLR chromatograms of blank ginger samples and spiked ginger samples were shown in Fig. 1A and B and the recovery values of the method for the five analytes were displayed in Table 2. The average recoveries ranged from 84.2 to 97.3% for AFs with RSDs of 0.6–7.9% and from 89.3 to 97.4% for OTA with RSDs of 2.1–4.5%, which were in agreement with Commission Regulation No. 401/2006.

3.4. Real sample analysis

The established method was applied for the determination of AFB₁, AFB₂, AFG₁, AFG₂ and OTA in 30 samples of 10 different species of ginger and related products samples collected from local markets and supermarkets in Beijing, China. The occurrence and levels of AFs and OTA in all tested samples for this survey were summarized in Table 3.

It can be seen from Table 3 that total 13 samples of 4 species of ginger and related products including moldy ginger, ginger powder, ginger tea bags and ginger black tea bags were contaminated with AFB₁ or OTA at different levels, while none of the five mycotoxins was detected in fresh ginger, baked ginger, ginger peels, ginger-red sugar tea bags, ginger-red jujube tea bags and ginger-black sugar tea bags. OTA was detected in moldy ginger and ginger powder samples ranging from 0.32 to 5.17 µg kg⁻¹, while AFB₁ was detected in ginger tea bags and ginger black tea bags ranging from 0.13 to 1.38 µg kg⁻¹. All the AFB₁ and OTA levels in the contaminated ginger samples did not exceed the maximum limit set for spices by EU regulation.

3.5. Confirmatory results by LC-ESI-MS/MS

All positive samples were further confirmed by liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS). Retention time and fragment ions of AFB₁ (m/z 313.1 → 285.1, m/z 313.1 → 245.0), AFB₂ (m/z 315.1 → 287.0, m/z 315.1 → 259.0), AFG₁ (m/z 329.1 → 243.0, m/z 329.1 → 311.2), AFG₂ (m/z 331.1 → 285.1, m/z 331.1 → 241.0) and OTA (m/z 404.2 → 358.0, m/z 404.2 → 239.1) from LC-ESI-MS/MS analysis were used to confirm the positive samples. As a result, there were no false-positive samples, which indicated that the proposed method was sensitive and reliable. Typical MRM chromatograms of AFB₁ and OTA in positive ginger samples were shown in Fig. 2a and b.

4. Conclusions

A simple, sensitive and rapid method based on IAC clean-up coupled with UHPLC and FLR detection was developed for simultaneous determination of AFB₁, AFB₂, AFG₁, AFG₂ and OTA in ginger and related products, and the validated method shows satisfactory linearity, precision and accuracy. The four AFs and OTA were separated in less than 10.0 min and no interference peaks were observed.

The developed method was successfully applied for the analysis of 30 ginger and related products samples. All positive samples were confirmed by LC-ESI-MS/MS and there were no false-positive
samples, indicating that the proposed method was sensitive and reliable. The results showed that ginger and related products were more easily contaminated by 

AFB1 and OTA than AFB2, AFG1 and AFG2. Although all the contamination levels of the samples analyzed by the developed method were below the maximum limit set for AFs and OTA in spices by EU regulation, considering both the toxicity of AFs and OTA and the large consumption of ginger and related products all over the world, more attention should be paid to not only the development of more sensitive detection methods but also the prevention strategies to manage the AFs and OTA contamination of ginger and related products during storage and processing.

Acknowledgment

The work was supported by the National Science Foundation of China (No. 81274072) and PUMC Youth Fund and the Fundamental Research Funds for the Central Universities (3332013078).

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