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Modified QuEChERS method combined with ultra-high performance liquid chromatography tandem mass spectrometry for the simultaneous determination of 26 mycotoxins in sesame butter

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ABSTRACT

A high-throughput method for the simultaneous determination of 26 mycotoxins in sesame butter was developed by coupling the modified Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method with ultra-high performance liquid chromatography triple quadrupole mass spectrometry (UHPLC–MS/MS). The samples were sequentially extracted using 20 mL (80:20, v/v) and 5 mL (20:80, v/v) acetonitrile aqueous solutions, followed by salting out by the addition of magnesium sulfate and sodium chloride. Finally, the samples were purified using hexane and dispersed C18 solid phase extraction (dSPE). The mycotoxins were further separated using a C18 column and detected by electrospray ionization (ESI) in the multiple reactions monitoring (MRM) mode. Using this detection technique, 16 mycotoxins were detected as positive ions using methanol and water containing 0.1% formic acid as the mobile phase, whereas the other 10 mycotoxins were detected as negative ions using methanol and water as the mobile phase. With the matrix-matched quantification calibration, the developed method showed a good linear dynamic range with regression coefficients of 0.995 or higher. This method allowed for the detection of the 26 mycotoxins at LOQs significantly lower than the available maximum residue levels currently regulated by EU regulations. Additionally, at the three spiking levels examined, the majority of recoveries were within 60–120%, with RSDs within 15%. The method developed herein has the advantages of high sensitivity, accuracy and throughput, and it can be applied to the target screening of mycotoxins in real samples.

1. Introduction

Agricultural products, including raw materials and processed food products, are susceptible to contamination by a variety of mycotoxins, which are mainly produced by *Aspergillus*, *Penicillium* and *Fusarium* species under certain climate conditions [1]. The frequently monitored mycotoxins include aflatoxins, ochratoxin A, patulin, zearalenone, groups of trichothecene and the fumonisins [2]. Patulin is normally found in fruit products, whereas the others are most often found in cereal products. Studies have shown that these mycotoxins have significant adverse effects on human health [3], most being highly toxic at very low concentrations. Among these mycotoxins, aflatoxins are carcinogenic to humans [4], ochratoxin A can induce renal pathologies [5], zearalenone has strong estrogenic effects, and trichothecene can inhibit protein synthesis and induce immune-modulatory effects [6]. Therefore, monitoring and inspecting the contamination levels of mycotoxins in foods and feeds have been a major focus of international and national actions over the years.

The European Union, United States and many other countries have established maximum residue levels (MRLs) and tolerable daily intake (TDI) for ochratoxin A, deoxynivalenol, T-2 and HT-2 toxins, fumonisins and zearalenone in different foodstuffs. The regulations of the EU are the most strict with MRLs ranging from 0.5 $\mu g\,kg^{-1}$ (aflaM1) to 1000 $\mu g\,kg^{-1}$ (deoxynivalenol) in food and

Abbreviations: NEO, neosolaniol; DAS, 4,5-diacetoxyscirpenol; DON, deoxynivalenol; FUS-X, fusarenon-X; DOM-1, de-epoxy-deoxynivalenol; 3-ADON, 3-acetyl deoxynivalenol; 15-ADON, 15-acetyl-deoxynivalenol; AFB1, aflatoxin B1; AFB2, aflatoxin B2; AFG1, aflatoxin G1; AFG2, aflatoxin G2; GLT, gliotoxin; FUM, fumagillin; FB1, fumonisin B1; FB2, fumonisin B2; FB3, fumonisin B3; ST, sterigmatocystin; VER, verruculogen; OTA, ochratoxinA; ZEN, zearalenone; MPA, mycophenolic acid; PAX, paxilline; IAC, immuno affinity column; dSPE, dispersed solid-phase extraction; QuEChERS, Quick Easy Cheap Effective Rugged and Safe.

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0.025 μg kg^{-1} (aflaM1) to 150 μg kg^{-1} (deoxynivalenol) in baby food [7]. Although the other toxins have not been regulated, their toxigenic potential has been evaluated in various studies [8–10]. Therefore, it is required to develop more rapid, accurate and highly sensitive methods for the routine analysis of mycotoxins to better understand their global contamination impact.

In earlier years, thin layer chromatography (TLC) and enzyme-linked immuno sorbent assay (ELISA) were used for the analysis of mycotoxins; however, these methods are semi-quantitative and cannot achieve high-throughput screening. In the past decade, high performance liquid chromatography coupled with mass spectrometry, especially tandem mass spectrometry (HPLC–MS/MS), represents the most efficient and reliable analytical technique for high efficiency isolation, unequivocal identification and accurate quantification [11]. This method has been widely applied in the determination of mycotoxins because of its rapidity, accuracy, high sensitivity and reproducibility [12].

In recent years, much effort has been put forth in the development of the immuno affinity column (IAC) [13–15] and the solid-phase extraction (SPE) [16–18] clean-up procedure in combination with HPLC–MS/MS methods for the determination of mycotoxins. The IAC clean-up is safe, rapid and highly selective; however, it is limited by the availability of immuno antibodies. Studies have also found that some mycotoxins are underestimated with IAC clean-up in some breakfast cereals and coffee [19]. SPE cartridges are less expensive and less selective than IAC and are by far the most popular technique currently used for analysis of FBs, AFB1 and OTA in food and feed [20]. However, because mycotoxins are a group of compounds with different chemical structures and polarities, SPE clean-up cartridges are difficult to use for the analysis of multiple mycotoxins with such diverse properties.

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method was initially developed for the analysis of pesticide residues in fresh fruits and vegetables and was soon expanded to the analysis of food additives, veterinary drug residues and mycotoxins in foods. For the determination of mycotoxins, Sospedra et al. [21] reported an analytical method of three type A and two type B-trichothecenes in wheat flour using QuEChERS; Zachariasova et al. [22] developed a method for the determination of 11 Fusarium mycotoxins in cereals; Basmussen et al. [23] presented a method for the simultaneous determination of 27 mycotoxins in feeds made of corn; and Zhang et al. [24] reported a method for the simultaneous determination of 22 carbamate insecticides and 17 mycotoxins in cereals. However, these applications focused only on common matrices such as wheat, corn and cereals. Sesame sauce, a popular sauce in Asia, is produced mainly from sesame seeds and peanuts and thus can be easily contaminated by mycotoxins. This sauce is rich in fat, carbohydrates, protein, and natural pigments; therefore, it is difficult to clean up. The aim of this study was to develop a reliable method by coupling the universal QuEChERS extraction and clean-up method with ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) for the simultaneous determination of 26 predominant mycotoxins in the complex matrix of sesame butter.

2. Experimental methods

2.1. Materials and reagents

The standard compounds of neosolaniol (NEO), 4,5-diacetoxyscirpenol (DAS), deoxynivalenol (DON), fusarenon-X (FUS-X), de-epoxy-deoxynivalenol (DOM-1), 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), aflatoxin M2 (AFM2), T2 toxin, HT-2 toxin, gliotoxin (GLT), fumagillin (FUM), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), sterigmatocystin (ST), verruculogen (VER), ochratoxin A (OTA), zearalenone (ZEN), Mycophenolic acid (MPA) and paxilline (PAX) were purchased from Alexis Corporation (Lausen, Switzerland).

Methanol, acetonitrile and formic acid of HPLC grade were purchased from Thermo Fisher Scientific (New Jersey, US). The water used was purified by a Mill-Q apparatus (Millipore, US). Hexane, magnesium sulfate, sodium chloride, sodium citrate, sodium hydrogen citrate sesquihydrate, dSPE sorbent C18 and other reagents used were of analytical grade. The SPE dispersive tubes were purchased from Agilent Technologies.

Sesame butter and peanut butter samples were purchased randomly from local grocery stores.

2.2. Preparation of standard solutions

Solid portions of each standard were weighed and dissolved directly in acetonitrile to prepare 0.1 mg mL^{-1} stock solutions, which were stored in the dark at −18 °C. An accurate volume of each standard solution was transferred into the combined solution and diluted step-by-step to prepare a sequence of working mix solutions with concentrations of 1, 5, 10, 25, 50, 500 and 1000 ng mL^{-1}. All of the working solutions were freshly prepared just before use with the blank matrix.

2.3. UHPLC conditions

An Agilent infinity 1290 ultra-high performance liquid chromatography system (Agilent Technologies, Woldbroon, Germany) was utilized throughout the study. This instrument consisted of a UHPLC pump with a built-in micro-degasser, an infinity autosampler with back flush function and a temperature control compartment. Chromatographic separation was achieved using an Agilent Zorbax Eclipse Plus column (1.8 μm, 100 mm × 2.1 mm, Agilent Technologies, Wilmington, DE, US) with the mobile phase at a flow rate of 0.4 mL min^{-1}. The mobile phase was an in-line mixture of solvent A and solvent B; where solvent A was either water containing 0.1% formic acid for the positive ESI mode (ESI+) or pure water for the negative ESI mode (ESI−), and solvent B was either methanol containing 0.1% formic acid for the ESI+ mode or pure methanol for the ESI− mode. Two gradient elution programs for the ESI+ and ESI− modes were established. For the ESI+ mode, %B started from 30% and linearly increased to 35% within 8 min, then rapidly increased to 50% B within 8–8.5 min, followed by a slow increase to 80% within 8.5–15 min. For the ESI− mode, %B was initially maintained at 10% for 3 min, rapidly increased to 15% within 3–3.1 min and then subsequently increased to 20% within 3.1–4.5 min, 30% within 4.5–8 min, 45% within 8–8.5 min and 90% within 8.5–14 min. The post-time for column re-equilibration was set at 2 min between two consecutive injections. The column temperature was maintained at 40 °C and the injection volume was set at 3 μL.

2.4. MS/MS conditions

MS/MS was performed on an Agilent 6460 Triple Quadrupole mass spectrometer equipped with a JetStream electrospray ionization source (Agilent Technologies, Wilmington, DE, US). Based on the structural properties of the analytes, both the positive and negative ionization modes were applied. The parameters used for the mass spectrometric detection were set as follows: capillary voltage, 3.5 kV (+/−); nozzle voltage, 0 V (+)/2000 V (−); nebulizer gas (N_2), 45 psi (+/−); drying gas (N_2) temperature, 300 °C (+)/350 °C (−); drying gas flow rate, 6 L min^{-1} (+)/10 L min^{-1} (−); sheath gas (N_2) temperature, 300 °C (+)/350 °C (−); sheath gas flow rate, 12 L min^{-1}