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Rapid microwave-assisted dispersive micro-solid phase extraction of mycotoxins in food using zirconia nanoparticles

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ABSTRACT

Mycotoxins are a group of secondary fungi metabolites present in foods that cause adverse effects in humans and animals. The objective of this study was to develop and validate a reliable and sensitive method to determine the presence of fumonisin B1, aflatoxin B1, ochratoxin B, T-2 toxin, ochratoxin A and zearalenone. A rapid, effective process, which involves microwave-assisted dispersive micro-solid phase extraction (MA-D-μ-SPE), has been proposed for the extraction and detection of 6 mycotoxins in peach seed, milk powder, corn flour and beer sample matrixes, for subsequent analysis by ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF/MS). Several experimental parameters (type of dispersant, concentration of dispersant, vortex time, type of desorption solvent and pH) affecting the extraction efficiency were systematically studied and optimized. The optimum extraction conditions involved immersing 2.5 μg/mL of nano zirconia (as dispersant) in a 5 mL sample solution. After 2 min of extraction by vigorous shaking, the target analytes were desorbed by 100 μL of chloroform at pH 4.5. The results indicated good linearity in the range of 0.0074-3.6 μg/mL ($r \geq 0.9982$), low limits of detection (0.0036-0.033 μg/kg for solid samples and 0.0022–0.017 ng/mL for beer), acceptable reproducibility (relative standard deviation (RSD%) 2.08–2.76% for retention time and 3.51–4.59% for peak area, n = 3), and satisfactory spiked recoveries (84.27–104.96%) for studied mycotoxins in sample matrixes, which demonstrated that MA-D-μ-SPE coupled with UHPLC-Q-TOF/MS is a useful tool for analysis of multi-mycotoxin.

1. Introduction

Mycotoxins are a group of secondary fungi metabolites, produced by different fungal genera, which cause potential illness or even death in humans and animals due to a variety of teratogenic, hepatotoxic and carcinogenic effects [1–3]. Among these mycotoxins, aflatoxin B1 (AFB1) is a group 1 classified as human carcinogen by the International Agency of Research on Cancer due to the most toxic and the strongest natural carcinogens, whereas fumonisin B1 (FB1) and ochratoxin A (OTA) are classified as possible group 2 B human carcinogens [4]. Such determination and classification of multiple mycotoxins is necessary to protect humans from health risks. So far, different methods have been used for the determination of multiple mycotoxins, including high-performance liquid chromatography with fluorescence detection (HPLC-FLD) (40 min) [5], gas chromatography–mass spectrometry (GC–MS) (17 min) [6] and liquid chromatography tandem mass spectrometry (LC–MS/MS) (16.5 min) [7]. However, most of these analytical methods required relatively longer instrumental analysis times. Therefore, development of a strategy to utilize ultra-performance liquid chromatography coupled to tandem quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS) [8] for identification of multiple mycotoxins is necessary. Effective extraction of multiple mycotoxins from solid samples is the first step prior to qualitative and quantitative analysis. In previous studies, various extraction techniques have been reported, such as soxhlet extraction [9], magnetic stirring [10] and ultrasound-assisted extraction [11]. However, most of these methods require a long extraction time, a large amount of organic solvent, or exhibit poor performance. Recently, microwave-assisted extraction (MAE) has been considered an advanced and effective “green technique”. MAE is a procedure that uses microwave energy to heat rapidly organic solvents in contact with a sample in order to partition interest analytes from the sample matrix into the solvent. Compared with

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conventional extraction methods, it has the advantage of shorter extraction time, reduced organic solvent as well as increased extraction yield. With the aim to improve the extraction efficiency as much as possible, a large number of sample preparations, such as immunoaffinity columns (IACs) [12,13], liquid–liquid extraction (LLE) [14], dispersive liquid–liquid microextraction (DLLME) [15], QuEChERS (quick, easy, cheap, effective, rugged and safe) method [16], and solid phase extraction (SPE) [17] were reported. However, these proposed methods are relatively expensive, time consuming and complicated. Therefore, it is necessary to develop an economic, quick and simple method for simultaneous analysis of multiple mycotoxins.

Solid phase extraction (SPE) is a widely used sample preparation method for the enrichment of target analytes from samples, and plays a significant part in modern analytical chemistry [18,19]. However, the SPE method required multiple steps and is still time-consuming, complicated and relatively expensive [20,21]. Recently, sample extraction trends for miniaturization and simplicity have become increasingly popular. To overcome the above limitations, tremendous efforts have been made to develop some novel fast, efficient, economical, miniaturized and simplistic sample preparation methods. The dispersive micro-solid phase extraction (D-u-SPE) process using zirconia nanoparticles as a dispersant per se is novel in terms of application for mycotoxin extraction[22,23]. It has been introduced as a new version of SPE which holds the advantages of dispersive liquid–liquid extraction (DLLE) and SPE simultaneously [24,25]. Compared with QuEChERS method [26], D-u-SPE was found to be advantageous in terms of short time requirement, speed and reduced solvent and sorbent consumption. Moreover, as far as we know, most reported D-u-SPE methods for the determination of mycotoxin are combined with multi-step preparation (dispersive liquid–liquid microextracton) and used for single-target analyses [27]. Thus, the D-u-SPE procedure, which performed on a single separation and enrichment step and was used for the extraction and detection of 6 mycotoxins is meaningful. The selection of the dispersant is a key point to achieve the appropriate selectivity, the satisfactory extraction efficiency and the high enrichment factor in the D-μ-SPE process [28]. Over the past few years, the conventional D-μ-SPE materials have been substituted by nano materials due to their high surface-to-volume ratio and high sorption properties [29]. The application of new dispersant promoted the development of this method.

Nano materials, which have nano-scaled size leading to an increased of surface active sites, have attracted great attention from investigators in the past few years. Their high reactivity and efficient chemical stability has led them to be widely used in various research fields of medicine, technology, personal care applications and others [30,31]. Nano materials are generally classified as nano particles, carbonaceous nanomaterials, silicon nanomaterials. Nano zirconia (MHZR) is an ideal building block for nano particles because they offered multiple potential properties such as chemical inertness, high mechanical strength, excellent thermal stability and good tenacity [32]. On account of these properties, MHZR was commonly used in various practical fields, including ceramic glazes [33], sorbents [34], catalyst [32], refractory materials [35] and dental restorations [36]. Based on their application in adsorption field, MHZR offers an obvious higher surface area-to-volume ratio and a shorter diffusion route in comparison with conventional sorbents, which results in high extraction efficiency and rapid extraction dynamics. With the increasing advances in nano material science and technology, MHZR presents broader and promising prospects for extraction. As far as we know, MHZR used as a dispersant in the D-μ-SPE process has not been previously reported.

The aim of this study was to combine a MA-D-μ-SPE with UHPLC-Q-TOF/MS to develop a rapid and efficient method for determination of multiple mycotoxins in peach seed and milk powder samples. This work presents a novel application of MHZR as a dispersant phase in D-μ-SPE procedure. Main experimental parameters affecting the quantitative extraction of target analytes were investigated and optimized, including type of dispersant, dispersant amount, vortex time and type of desorption solvent. The linear, precision and limits of detection of this method were assessed, and its applicability for use in determining trace levels of multiple mycotoxins in real samples was demonstrated.

2. Experimental

2.1. Chemicals and reagents

Carboxylic multi-walled carbon nanotubes (C-MWCNTs), graphitized carboxyl multiwalled carbon nanotubes (C-GMWCNTs), multiwalled carbon nanotubes (MWCNTs), Graphene oxide (GO) and MHZR were obtained from Nanjing Jicang Nano Tech Co., Ltd. (Nanjing, China). Sodium dodecyl sulfate (SDS) was provided by ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). All reagents were purchased in high purity grade and used without further purification. For chromatographic analysis, purified water was obtained from Wahaha Group Ltd. (Hangzhou China). Methanol and acetonitrile were supplied by Sigma-Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Ethanol and acetone were obtained from Hangzhou Chemical Reagent Co., Ltd. (Hangzhou, China). Chloroform (analytical grade) was purchased from Quzhou Juhua Reagent Co. Ltd. (Quzhou, China). All mycotoxin standards were obtained from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). The standard solutions of FB1, AFB1, Ochratoxin B (OTB), T-2 toxin OTA were prepared in methanol and stored at −20 °C. Zearalenone (ZEN) was stored at the concentration of 50 μg/mL. The tested real samples including peach seed, milk powder, corn flour and beer were obtained from the local drugstore and market (Hangzhou, China).

2.2. Apparatus

A UHPLC 1290 system (Santa Clara, CA, USA) was connected to a 6530 tandem quadrupole-time-of-flight (Q-TOF) mass spectrometer (Agilent Technologies Inc., CA, USA), which was used in the analysis of multiple mycotoxins. Chromatographic separation was performed with an Eclipse Plus C8 RRHD column (2.1 × 50 mm, 1.8 μm) at a temperature of 30 °C. The binary gradient system, consisting of eluent A (water containing 0.1% formic acid) and eluent B (acetonitrile), was employed at a flow rate of 0.4 mL/min. Elution proceeded by means of linear gradient program as follows: 0–1 min, 10–40% B; 1–3 min, 40–65% B; 3–5 min, 65–80% B;5–6 min, 80–100% B; 6–9 min, 100–100% B. The total run time was 9 min and the sample injection volume was 2 μL.

Detection and qualitative analysis was carried out on a 6530 Q-TOF/MS system, which was operated in positive electrospray ionization mode. The MS conditions were as below: gas temperature, 350 °C; drying gas flow, 12 L/min; capillary voltage, 3.5 kV; nebuliser pressure, 45 psig; skimmer voltage, 65 V; fragmenter voltage, 165 V; octapole RF, 750 V. Data acquisition was performed with MassHunter Acquisition Software (version B 05.00).

To characterize the size and morphology of the MHZR, the surfaces features of MHZR was observed using scanning electron microscopy (SEM, HT7700, Hitachi, Tokyo, Japan). The morphology of MHZR was further investigated by transmission electron microscope (TEM, Zeiss, Oberkochen, Germany) operating at 100 kV.

BET analysis of MHZR is determined using the Quantachrome QUADRASORB SI series instrument, which is a USA high-performance surface area analyzer. The BET determination is measured with the nitrogen adsorption. Water and gases such as

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