Food Control 79 (2017) 177–184

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Multi-walled carbon nanotubes-based magnetic solid-phase extraction for the determination of zearalenone and its derivatives in maize by ultra-high performance liquid chromatography-tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 10 January 2017
Received in revised form
25 March 2017
Accepted 27 March 2017
Available online 29 March 2017

Keywords:
Magnetic solid-phase extraction
Multi-walled carbon nanotubes
Maize
Zearalenone and its derivatives
Ultra-high performance liquid chromatography-tandem mass spectrometry

ABSTRACT

A simple and rapid magnetic solid-phase extraction (M-SPE) procedure using multi-walled carbon nanotube-magnetic nanoparticles (MWCNT-MNPs) as sorbents was established for purification of zearalenone (ZEA), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) in maize. The main parameters affecting the clean-up efficiency were thoroughly investigated, and high purification efficiencies for all analytes were obtained. The resulting MWCNT-MNP-ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method was validated for maize samples. The matrix effects were greatly minimized using the M-SPE approach, with signal suppression/enhancement values decreased from 69.9–127.6% to 92.1–103.8%. Consequently, complex matrix-matched calibration curves were not necessary and the calibrations constructed in acetonitrile could be applied for accurate quantification of the targeted mycotoxins in real samples. The average recoveries ranged from 75.8 to 104.1% and the inter- and intra-day precision values expressed as RSDs, were all lower than 14%. Limits of detection and quantification were in the range of 0.03–0.04 and 0.07–0.10 μg/kg, respectively. The analytical performance of the developed method was also successfully evaluated with maize samples, and this method was proved to be a powerful tool for monitoring ZEA and its derivatives in maize.

1. Introduction

Zearalenone (ZEA) and its derivatives, including α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL), are naturally occurring mycotoxins produced by *Fusarium* species (Desjardins, 2006; El-Kady & El-Maraghy, 1982; Glenn, 2007). These mycotoxins have been shown to possess estrogenic activity due to its competitive binding to the estrogen receptor, which consequently disrupts the reproductive system and causes abnormal fetal development in animals (Shier, Shier, Xie, & Mirocha, 2001). Besides the adverse hormonal effects, they have also been implicated in numerous mycotoxicosis of farm animals associated with hepatic and renal lesions in rodents

Abbreviations: α-ZAL, α-zearalanol; α-ZOL, α-zearalenol; β-ZAL, β-zearalanol; β-ZOL, β-zearalenol; ELISA, enzyme-linked immunosorbent assay; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; M-SPE, magnetic solid-phase extraction; ME, matrix effect; MNP, magnetic nanoparticle; MWCNT, multi-walled carbon nanotube; RSD, relative standard deviation; S/N, signal-to-noise ratio; SPE, solid-phase extraction; SSE, signal suppression/enhancement; TEM, transmission electron microscope; TLC, thin-layer chromatography; UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry; ZAN, zearalanone; ZEA, zearalenone.

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http://dx.doi.org/10.1016/j.foodcont.2017.03.044
0956-7135/

and the reduction of milk production in cows (M Dong et al., 2010; Maaroufi, Chekir, Creppy, Ellouz, & Bacha, 1996; Zinedine, Soriano, Molto, & Manes, 2007). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended a provisional maximum tolerable daily intake (PMTDI) of 0.5 μg/kg for ZEA. In previous studies (Ibáñez-Vea, González-Peñas, Lizarraga, & De Cerain, 2012; Iqbal, Asi, Jinap, & Rashid, 2014; Pleadin et al., 2012), ZEA and its derivatives have been frequently observed in a variety of cereal crops including maize, wheat, barley and cereal products, representing an important threat to food safety (Oliveira, Rocha, Sulyok, Krska, & Mallmann, 2016; Tralamazza, Bemvenuti, Zorzete, de Souza Garcia, & Corrêa, 2016). In order to protect consumer safety, legislative limits for ZEA in maize are set by the European Commission, which range from 20 to 400 μg/kg for a variety of products including refined maize oil (400 μg/kg), unprocessed maize (200–350 μg/kg dependent on milling procedure), maize intended for direct human consumption (100 μg/kg), processed maize based foods for infants and young children (20 μg/kg).

Established analytical methods for ZEA and its derivatives involve thin-layer chromatography (TLC) (Pleadin et al., 2012), enzyme-linked immunosorbent assay (ELISA) (Pleadin et al., 2012; Zhan, Huang, Chen, Li, & Xiong, 2016), biosensors (Välimaa, Kivistö, Leskinen, & Karp, 2010), liquid chromatography (LC) coupled with mass spectrometry (Han et al., 2011). TLC has been gradually substituted due to its poor separation efficiency and low sensitivity. ELISA can be provided as a frontline screening method but has limitations in used for legislative quantification because of the cross reactivity. Electrochemical biosensors are based on high affinity interactions between antigen and antibodies, and the lack of specific ligands for ZEA derivatives limits their application (Vidal et al., 2013). Comparatively, ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) coupling the optimal separation efficiency of UHPLC with the high sensitivity and selectivity of MS/MS, seems to be a promising approach for the simultaneous determination of ZEA and its derivatives (Arroyo-Manzanares, Huertas-Pérez, Gámiz-Gracia, & García-Campaña, 2015). The major disadvantages for this approach are matrix effects (MEs) in combination with the limited availability of internal standards for quantification. Complex matrix components may severely affect the ionization process and consequently the accuracy of the method (Stahnke, Kittlaus, Kempe, & Alder, 2012). Therefore, an appropriate clean-up procedure is required to minimize MEs and establish an accurate and sensitive UHPLC-MS/MS method.

Frequently used approaches for clean-up of ZEA and its derivatives are liquid-liquid extraction, solid-phase extraction (SPE), molecularly imprinted polymers, and solid-phase microextraction. SPE-based clean-up procedures offer a number of important advantages, including low organic solvent consumption, high enrichment factor and rapid phase separation (Pyrzynska, Kubiak, & Wysocka, 2016), and have thus been widely used for purification of different mycotoxin-containing extracts of agricultural products (Giménez et al., 2013; Lucci, Derrien, Alix, Perollier, & Bayoudh, 2010; Zollner, Jodlbauer, & Lindner, 1999). Despite the effectiveness of purification, the conventional SPE encompasses loading, washing and elution steps with slow flow rate, which makes this clean-up procedure time-consuming and labor-intensive. These tedious steps are regarded as bottlenecks for high throughput mycotoxin analysis. In recent years, magnetic solid-phase extraction (M-SPE) has attracted the interest of researchers as a new alternative mode of SPE for sample pretreatment (Geng, Ding, Chen, Li, & Lin, 2012; Yazdinezhad, Ballesteros-Gómez, Lunar, & Rubio, 2013; Yilmaz, Alosmanov, & Soylak, 2015). Compared to conventional SPE, M-SPE is free from tedious process of packing columns and demands smaller volume of sample and solvents for extraction and desorption, yielding comparable recoveries of the analytes (Vasconcelos & Fernandes, 2017), and thus has been used in ZEA and its derivatives purification in several types of food (Gonzalez-Salamo, Socas-Rodriguez, Hernandez-Borges, & Rodriguez-Delgado, 2017; Moreno, Zougagh, & Ríos, 2016).

Multi-walled carbon nanotubes (MWCNTs) have become one of the most frequently used constructive nano-materials due to their unique electronic, mechanical, and chemical properties. Previous studies have demonstrated that MWCNTs possess unique features of notable purification and enrichment efficiency as sorbents for heavy metals (Kosa, Al-Zhrani, & Salam, 2012), pesticide residues (Qin et al., 2015) and type A trichothecenes (Dong et al., 2015). Magnetic MWCNT composites are hybrids of magnetic nanoparticles (MNPs) and MWCNTs. These composites can be simply synthesized and integrate the unique physical and chemical properties of MWCNTs with the paramagnetic property of MNPs, enabling them to be valuable adsorption materials in the M-SPE procedure. The magnetic MWCNT composites have been applied in combination with chromatographic techniques, for the determination of diverse types of environmental pollutants (pesticide and drug residues, heavy metals and bisphenol A, etc.) (Jiao et al., 2012; Tarigh & Shemirani, 2013; Xu et al., 2013). With regard to ZEA and its derivatives, a MNP-MWCNT-nano$C_{18}SiO_2$ composite was synthesized and applied for purification of ZEA and its derivatives. Although this material presented several advantages, the procedure for the synthesis of MNP-MWCNT-nano$C_{18}SiO_2$ composite was very complicated. Moreover, the matrix effects could not be eliminated by this material and complex matrix-matched calibration curves are still necessary for accurate quantification (Moreno et al., 2016).

In the present study, a simple, rapid and reliable M-SPE procedure using magnetic MWCNTs as sorbents for the simultaneous purification and enrichment of ZEA and its derivatives was developed. The procedure was implemented for maize and the resulting clean extracts were then analyzed by UHPLC-MS/MS. The established method was extensively validated according to the Commission Decision 2002/657/EC, and was then successfully applied to monitor the occurrence of ZEA and its derivatives in real-life maize samples collected in China.

2. Material and methods

2.1. Chemicals and materials

The MWCNTs (8 nm i.d., 10–30 μm length, 500 m^2/g) were purchased from XF Nano Materials Tech Co. Ltd. (Nanjing, Jiangsu, China). All organic solvents, acids, alkalis and salts were HPLC or analytical grade. Acetonitrile, methanol and acetone were purchased from Merck (Darmstadt, Germany). Ammonium acetate, formic acid, concentrated ammonium hydroxide, sodium hydroxide (NaOH), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) were provided by Aladdin Co. (Shanghai, China). Water used throughout the study was purified using a Milli-Q system (Milli-pore, Billerica, MA, USA). The standards of ZEA, α-ZOL, β-ZOL, ZAN, α-ZAL and β-ZAL were obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in acetonitrile to prepare 10 μg/mL of stock solutions. The stock solutions were stored at −20 °C in the dark.

A total of 50 maize samples (250 g each) were randomly collected from different supermarkets in Shanghai, China. All samples were ground into powders, passed through a 2 mm sieve, maintained in sealed bags in dark at room temperature.