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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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.

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

chromatography-tandem mass spectrometry

1. Introduction

Zearalenone (ZEA) [1] 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 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,

[1] ***Abbreviations:*** α-ZAL, α-zearalanol; α-ZOL, α-zearalenol; β-ZAL, β-zearalanol; β-ZOL, β-zearalenol; ELISA, enzyme-linked immunosorbent assay; LOD, limit of detection; LOQ, limit of quantification; LC, liquid chromatography; M-SPE, magnetic solid-phase extraction; ME, matrix effect; MWCNT, multi-walled carbon nanotube; MNP, magnetic nanoparticle; 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.

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 (Maofeng 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, Zougagh, & Ríos, 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., 10–30μm 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

153 solutions. The stock solutions were stored at –20◦C in the dark.

154 A total of 20 maize samples (250g each) were randomly collected from different
155 supermarkets in Shanghai, China. All samples were ground into powders, passed
156 through a 2 mm sieve, maintained in sealed bags in dark at room temperature.

157 *2.2 Preparation of MWCNT-MNPs*

158 The MWCNT-MNPs were synthesized according to Zhang and Shi (2012) with
159 some modifications: firstly, 300 mg of MWCNTs were added into 250 mL of water in
160 a 500 mL three-necked flask, and ultrasonicated for 1 h to enable the particles to be
161 well dispersed. Then, $FeCl_3 \cdot 6H_2O$ (810 mg) and $FeCl_2 \cdot 4H_2O$ (300 mg) were added
162 and the flask was kept in a magnetic stirring thermostatic water bath. Half an hour
163 later, 1 mol/L NaOH solution was slowly added to bring the pH to approximately 12,
164 and the reaction was allowed to proceed for another 2 h. During the entire reaction
165 process, the temperature was set at 60 °C and the flask was kept under nitrogen gas
166 protection. After cooling to room temperature, the black precipitates (MWCNT-MNPs)
167 were magnetically collected, washed with water at least three times, dried at 80 °C
168 and ground into powder for use. The synthesized materials were characterized using a
169 JEM-1230 transmission electron microscope (TEM; JEOL Ltd., Tokyo, Japan)
170 operated at 80 kV, under high vacuum (10^{-5} Pa), at room temperature.

171 *2.3 Sample pretreatment*

172 *2.3.1 Optimization of M-SPE procedure for sample pretreatment*

173 To achieve the optimal performance of the M-SPE procedure with MWCNT-MNPs

as adsorbents, several parameters were investigated including desorption solvent, adsorption solution, adsorption time, MWCNT-MNPs amount by using spiked sample extractions (50 ng/mL for each analyte).

2.3.2 Sample preparation

Each sample (2.0g) was accurately weighed into a 50 mL centrifuge tube. After maceration with 10mL of acetonitrile/water (84/16, v/v) for 5 min, the sample was ultrasonicated for 40 min and then centrifuged at 4000 g for 5 min. An aliquot (5 mL) of supernatant was collected for M-SPE purification. The supernatant was first dried by nitrogen gas at 50°C, and re-dissolved with 5 mL of acetonitrile/water (5/95, v/v). Then, 20 mg of MWCNT-MNPs were added. The mixture was vortexed for 3 min to enable the targeted mycotoxins to interact with and be adsorbed on MWCNT-MNPs. Afterwards, a magnet was placed under the centrifuge tube so that MWCNT-MNPs were magnetically collected and the supernatant was poured off. Then, the mycotoxins were desorbed with 10 mL of acetone containing 0.5% formic acid by ultrasonication for 5 min. The MWCNT-MNPs were magnetically gathered again and the desorption solution was collected, dried under a soft stream of nitrogen gas at 50 °C, re-dissolved in 1 mL of acetonitrile, passed through a 0.22 μm nylon filter and ready for analysis by UHPLC-MS/MS. A general scheme for M-SPE is shown in Fig.1.

2.4 UHPLC-MS/MS analysis

UHPLC was performed via a Waters Acquity UHPLC system (Waters, Milford, MA,

USA). Separation was achieved at 40 °C on a Poroshell EC-C18 column (100 mm × 3.0 mm, 2.7 μm) (Agilent, USA). The mobile phase consisted of (A) methanol and (B) water containing 5 mol/L ammonium acetate, and a linear gradient elution program was applied as follows: initial 50% A, 4 min 70% A, 6 min 75% A, 7 min 75% A, 7.2 min 50% A and hold on for another 1.8 min for equilibration, giving a total run time of 9 min. The mobile phase flow rate was 0.35 mL min^{-1} and the injection volume was 3 μL.

The separated compounds were analyzed by a Waters XEVO TQ-S mass spectrometer (Waters, Milford, MA, USA) with an electrospray ionization source operated in negative mode (ESI^-). The MS/MS conditions were set as follows: source temperature, 150 °C; desolvation temperature, 500 °C; cone gas flow, 30 L/h; desolvation gas flow, 1000 L/h. A multiple reaction monitoring (MRM) acquisition method was developed for the targeted analytes, and the conditions were optimized for each mycotoxin by direct infusion (Table S1, Supplementary Data). Data processing was performed by MassLynx v4.1 and Targetlynx (Waters).

2.5 Evaluation of the MEs

The stock solutions were diluted with acetonitrile and blank matrix, respectively, to yield a sequence of concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 ng mL^{-1}) for each analyte. Signal suppression/enhancement (SSE), calculated by comparing the slope of the calibration plot of the standards spiked in the matrix to that of the standards in acetonitrile, was used to evaluate the MEs.

2.6 Method validation

Mixed standard solutions of six analytes at 12 different concentrations (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 ng/mL) were prepared in acetonitrile. Calibration curves were constructed by plotting the responses versus analyte concentrations. The sensitivity was evaluated by determining limit of detection (LOD) and limit of quantification (LOQ), designed as the concentrations of the analytes that resulted in a signal-to-noise ratio (S/N) of 3 and 10 in matrix, respectively. The recovery, intra- and inter-day precision tests were all performed on non-contaminated samples. Fifteen portions (2 g) of the blank sample were spiked with low, intermediate and high concentration levels (5 μg/kg, 50 μg/kg and 200 μg/kg) of each standard, while three additional portions were used as control. All samples were pretreated as described in Section 2.3. The recovery was calculated by comparing the determined concentrations of the analyte with the theoretical values. The relative standard deviations (RSDs) at three concentration levels on the same day were used for evaluation of the intra-day precision, whereas the inter-day precision was assessed using values from five consecutive days.

3. Results and discussion

3.1 Optimization of UHPLC-MS/MS conditions

Three candidate columns with different lengths and particle sizes i.e. (1) Agilent Poroshell EC-C18 column (100 mm × 3.0 mm, 2.7 μm; Agilent, USA), (2) BEH C18 column (100 mm × 2.1 mm,1.7 μm; Waters, USA) and (3) HSS T3 column (100 mm

× 2.1 mm, 1.8 μm; Waters, USA) were compared. Column (1) was finally selected because the highest responses and best separation efficiency could be obtained for all mycotoxins (Fig. S1A, Supplementary material), and was further applied to analyze ZEA and its derivatives in spiked maize matrices (0.1–500 ng/mL). The results clearly showed that all targeted analytes could be completely separated from each other without any interference in maize matrices, and thus, Agilent Poroshell EC-C18 column (100 mm × 3.0 mm, 2.7 μm; Agilent, USA) was selected in the current research

Different mobile phases were also tested: (1) methanol-water, (2) methanol-water containing 0.1% formic acid, (3) methanol-water containing 5 mmol/L ammonium acetate, (4) methanol-water containing 0.1% formic acid and 5 mmol/L ammonium acetate and (5) acetonitrile-water containing 5 mmol/L ammonium acetate. The six analytes could be separated from each other under different mobile phases with retention times of approximately 4.45 min for β-ZAL, 4.80 min for β-ZOL, 5.35 min for α-ZAL, 5.60 min for α-ZOL, 5.76 min for ZAN and 5.98 min for ZEA, respectively. Among the five mobile phases investigated, the responses were the highest for all analytes when mobile phase (3) was applied (Supplementary material, Fig. S1B). Therefore, methanol-water containing 5 mmol/L ammonium acetate was finally chosen as the mobile phase.

3.2 Characterization of MWCNT-MNPs

TEM analysis was applied to collect information about the morphology and structural changes of the obtained composites as a measure of the reaction product as

a valuable and effective adsorption material for the M-SPE procedure. The TEM image of the synthesized MWCNT-MNPs is provided in Fig.1A. As shown in the microgram (100 nm scale), the black dots of nanoparticles (Fe_3O_4), synthesized using $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$, were attached uniformly onto the surface of the MWCNTs, indicating that the MWCNT-MNPs have been successfully synthesized and can be applied for the purification of target analytes in samples.

3.3 Optimization of the M-SPE procedure

To achieve optimal performance of M-SPE procedure with MWCNT-MNPs as adsorbents, several parameters were investigated including adsorption solution, adsorption time, MWCNT-MNPs amount and desorption solvent by using spiked sample extractions (50 ng/mL for each analyte).

3.3.1 Desorption solvent

First, three frequently used organic solvents, i.e., acetone, acetonitrile and methanol, were investigated as the desorption solvent (other conditions were the same as described in section 2.3.2). Unsatisfactory recoveries ranging from 43.0 to 83.4% were obtained (Fig. 2). Then, formic acid (0.5%) and ammonium hydroxide (0.5%) were added into the desorption solvents (5/995, formic acid or ammonium hydroxide/desorption solvent, v/v) to improve the recoveries. Compared to the original solvents, the desorption power of the solutions containing formic acid (0.5%) tremendously improved, resulting in higher recoveries for the targeted mycotoxins, whereas ammonia (0.5%) did not positively affect the desorption efficiency (Fig 2). This indicated that the adsorption performance of MWCNT-MNPs was strongly

influenced by the pH. The highest recoveries, in the range of 77.0–105.3%, were achieved when acetone containing 0.5% formic acid was used.

Further, the influence of the volumes of the desorption solvent in the range of 3 to 15 mL was assessed. The recoveries of all analytes rose when the volume increased from 3 up to 10 mL, but remained almost constant with further increase of the volume (10 to 15 mL) (Fig. 3A). Consequently, in the final method, 10 mL of the acetone containing 0.5% formic acid was used.

3.3.2 Adsorption solutions

To ensure the adsorption of all the target analytes on the MWCNT-MNPs, the effect of the acetonitrile content in extract (0, 2%, 5%, 10% and 20%) on the adsorption process was investigated. The results (Fig.3B) indicated that the adsorption efficiency of MWCNT-MNPs significantly increased with the decreasing percentages of acetonitrile in the adsorption solutions. When extracts containing 20% of acetonitrile were used, low recoveries (63.5 to 75.8%) were observed. Decreasing the percentage of acetonitrile from 20% to 10%, acceptable recoveries (73.0–93.2%) were obtained for most of the analytes except α-ZOL (68.9%). Satisfactory recoveries (76.5–103.5%) were obtained for all analytes when the acetonitrile content was 5% or lower. Consequently, it was decided to limit the acetonitrile content in adsorption solution to 5% in further experiments.

3.3.3 Adsorption time

The adsorption time in the range of 1-6 min was investigated. As shown in Fig. 3C, the recoveries dramatically increased for all analytes going from 1 to 3 min adsorption

time, they then remained constant between 3 to 6 min. To ensure efficient adsorption of all mycotoxins on the MWCNT-MNPs while keeping the operation time short, the adsorption time was set to 3 min.

3.3.4 MWCNT-MNPs amount

Different amounts of MWCNT-MNPs (10, 20, 30 and 40 mg) were compared (Fig. 3D). Satisfactory recoveries in the range of 77.9–105.3% were obtained for ZEA, β-ZOL, ZAN, α-ZAL and β-ZAL in the whole range of MWCNT-MNP amount investigated. On the other hand, α-ZOL was tightly adsorbed on the sorbent and could not be efficiently desorbed when 30 mg or 40 mg of MWCNT-MNPs were used, resulting in unsatisfactory recoveries, i.e.68.6% and 62.9%, respectively. When 10 mg of MWCNT-MNPs were used, the repeatability of the clean-up process was not good, with the RSDs higher than 20%. Therefore, the amount of sorbents was set to 20 mg.

3.4 Evaluation of the clean-up method

To characterize the established clean-up method, the visually observable features and the extent of MEs for each of the six mycotoxins for the sample extracts before and after M-SPE purification were assessed. As shown in Fig.4A, maize extract purified by M-SPE procedure was colorless and transparent, indicating that the established clean-up method could efficiently remove the pigments and impurities from the matrices so as to minimize the interferences in MS/MS analysis. The MEs data (Fig. 4B) were in good agreement with the visual appearance of the extracts. Satisfactory MEs data, ranging from 92.1 to 103.8% SSE, were observed for the purified extracts, while a conspicuous influence of the matrix components was

observed (69.9–127.6% SSE) for the crude extracts. Compared to the method reported by Moreno *et al.* (2016), the method described here presented some advantages. Since MEs were successfully eliminated using the proposed clean-up procedure, complex matrix-matched calibration curves were not necessary and calibrations constructed in acetonitrile could be used for accurate quantification of the targeted mycotoxins, which significantly reduced the labor and the amount of materials needed, and obviously resulted in high efficiency. This ultimately makes it possible to perform the analysis with ease, high sensitivity and reduced cost.

3.5 Method validation

Calibration curves of the six analytes in neat solvent are shown in Table 1. Good linear relationships with coefficients of determination (R^2) ≥ 0.993 were obtained over the range of 0.1–500 ng/mL for all targeted mycotoxins in acetonitrile. The LOD and LOQ values were in range of 0.03–0.04 μg/kg and 0.07–0.10 μg/kg, respectively. Satisfactory recoveries with mean values in the range of 75.8–104.1% were obtained (Table 2). The RSDs were in the range of 3.4–11.2% and 3.2–13.2% for the intra-day precision and for the inter-day precision, respectively. The validation data described above clearly indicated that the analytical method was accurate and repeatable, and could be applied for simultaneous analysis of ZEA and its derivatives in maize.

3.6 Application to maize samples

To further evaluate the applicability of the established method, a total of 50 maize samples were analyzed. As shown in Table S2 (Supplementary data), in 47 samples

ZEA and its derivatives were detected at concentration levels in the range of 0.10–3613.03 μg/kg. ZEA was the most prevalent mycotoxin with concentrations ranging from 0.18 to 3613.03 μg/kg. It is worth noting that ZEA contents in 4 samples exceeded the maximum regulatory limits set by the EU for maize intended for direct human consumption and if they were considered for consumption by young children then 9 samples exceeded the limit (Oliveira, et al., 2016), indicating a concern for food safety. α-ZOL and β-ZOL were also frequently detected (incidences of 38% and 44%) with the concentration levels ranging from 0.10 to 13.52 μg/kg and from 0.11 to 16.13 μg/kg, respectively. A total of 10 samples contained ZAN with the concentrations in the range of 0.13-37.60 μg/kg. α-ZAL and β-ZAL were detected, in trace amounts (0.71 and 0.45 μg/kg, respectively), in only one sample. MRM chromatograms of the six analytes in acetonitrile and in a contaminated maize sample (No.16) are shown in Fig. S2 (Supplementary data). To demonstrate the trueness of data generated with the new developed method, a comparison between the new developed method and the reference method in China (GB/T 23504-2009) was performed by determination of two positive samples (No. 6 and No. 15). The determination results obtained by the standard method and the current method were basically consistent (Table S3, supplementary data). Data from the present study confirmed previous reports (Oliveira, et al., 2016; Pleadin, et al., 2012) on the frequent contamination of maize with ZEA and its derivatives resulting in high potential health risks to humans and animals. These results demonstrated that analytical tools such as the method proposed in the present study for rapid and reliable

368 determination of ZEA and its derivatives in maize are essential, and could be used in
369 the future in support of the continuous monitoring efforts.

370 **4. Conclusion**

371 MWCNT-MNPs were successfully prepared and used as M-SPE sorbents for
372 simultaneous purification of ZEA and its derivatives in maize. The established M-SPE
373 approach was demonstrated to be rapid, effective and efficient, and is therefore a
374 suitable alternative to the traditional SPE that is often tedious and time-consuming
375 due to the packing step and slow solvent follow rates. Coupled with UHPLC-MS/MS
376 detection, satisfactory sensitivities, linearities, recoveries and precisions were
377 obtained. When the validated method was applied to determine the natural occurrence
378 of mycotoxins in maize samples, up to 95% of the samples were found to be
379 contaminated with ZEA and its derivatives. The high incidence of this type of
380 mycotoxins in maize highlighted the importance of the current work, which provided
381 food safety authorities and researchers with a valuable tool for monitoring ZEA and
382 its derivatives in maize.

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384 **Acknowledgements**

385 This work was supported by the National Natural Science Foundation of China
386 [Grant No. 31671950], Shanghai Agriculture Commission Project [Grant No. 3-2,
387 2014), Shanghai Science and Technology Innovation Action Plan Project [Grant No.
388 15142201500].

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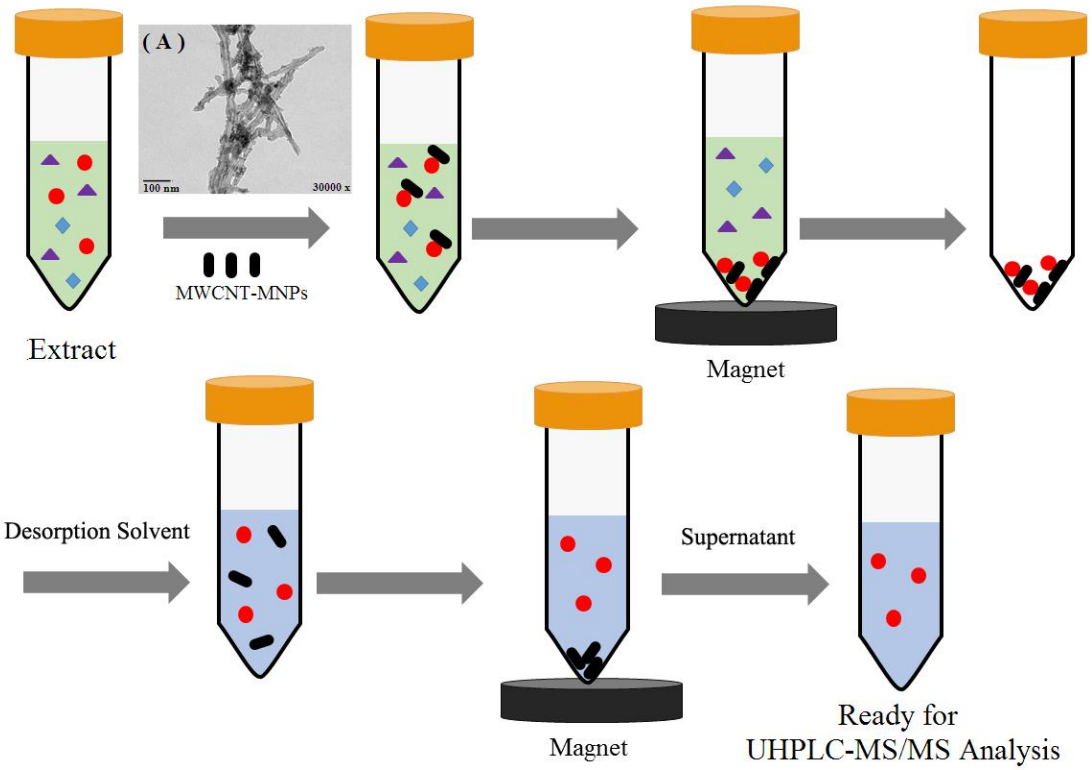
Figure captions

Fig. 1 Schematic diagrams of magnetic solid-phase extraction (M-SPE) procedure. (A) Transmission electron microscope (TEM) image of multi-walled carbon nanotubes-magnetic nanoparticles (MWCNT-MNPs).

Fig. 2 Recoveries of zearalenone (ZEA), α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL) in maize extracts purified by the M-SPE procedure using acetone, acetonitrile and methanol with 0.5% formic acid or 0.5% aqueous ammonia as the desorption solvent. Acceptable recoveries lay within the two dashed lines (70–120%). [a] represents the pure organic solvents; [b] represents the organic solvents with 0.5% formic acid; [c] represents the organic solvents with 0.5% aqueous ammonia.

Fig. 3 Recovery data for the investigated mycotoxins as a function of the major parameters affecting the purification efficiency of M-SPE procedure. (A) desorption solution volume, (B) the acetonitrile content in adsorption solution, (C) adsorption time, and (D) MWCNT-MNPs amount.

Fig. 4 Visually observable features (A) and matrix effects data (B) for six mycotoxins purified or not purified by magnetic solid-phase extraction (M-SPE) procedure. The tolerance level of matrix effects is in the range delineated by the two dashed lines (80–120%).



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548 **Fig. 1**

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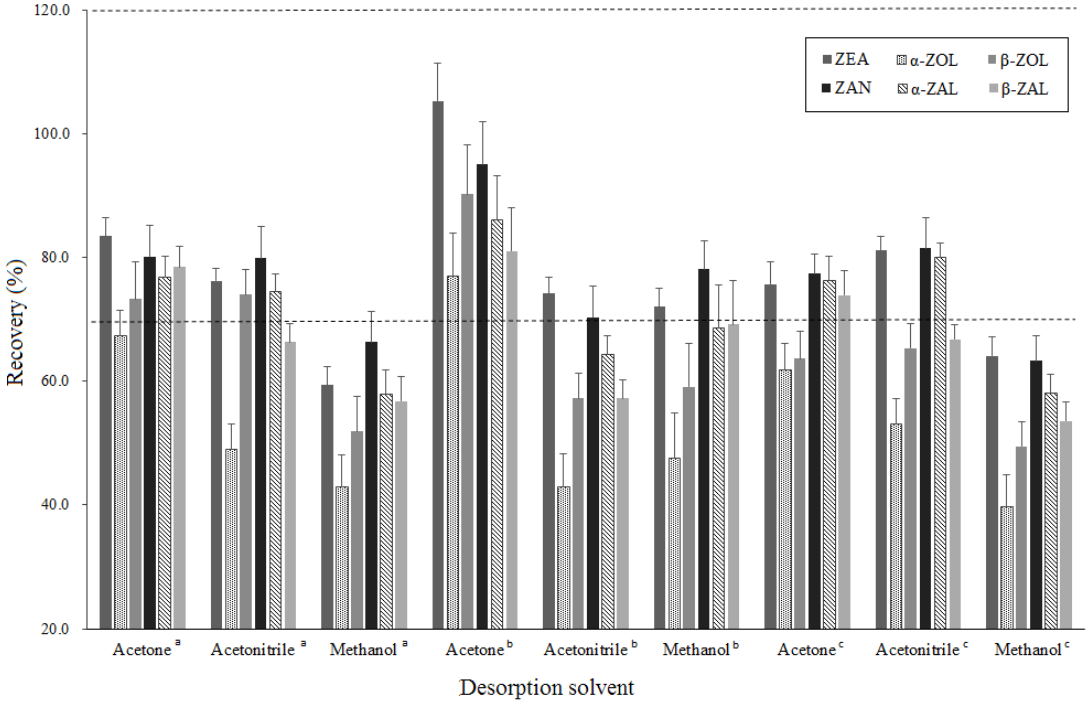

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551 **Fig. 2**

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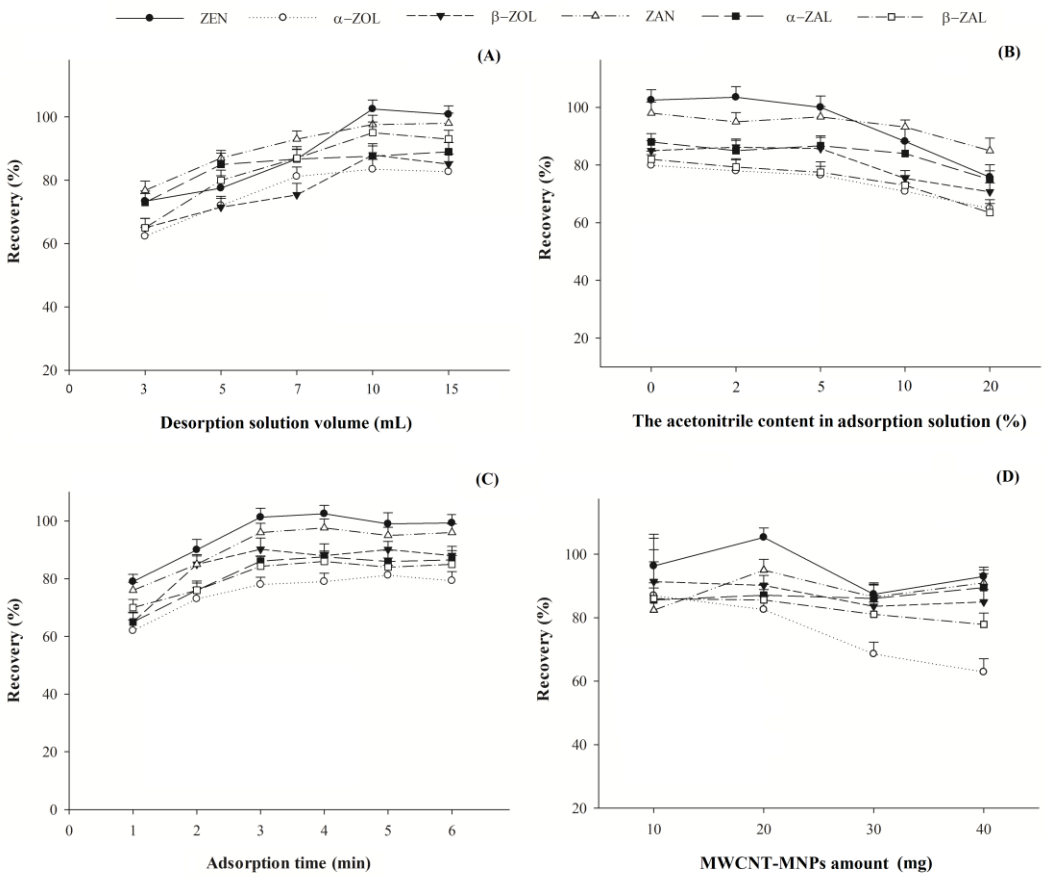

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554 **Fig. 3**

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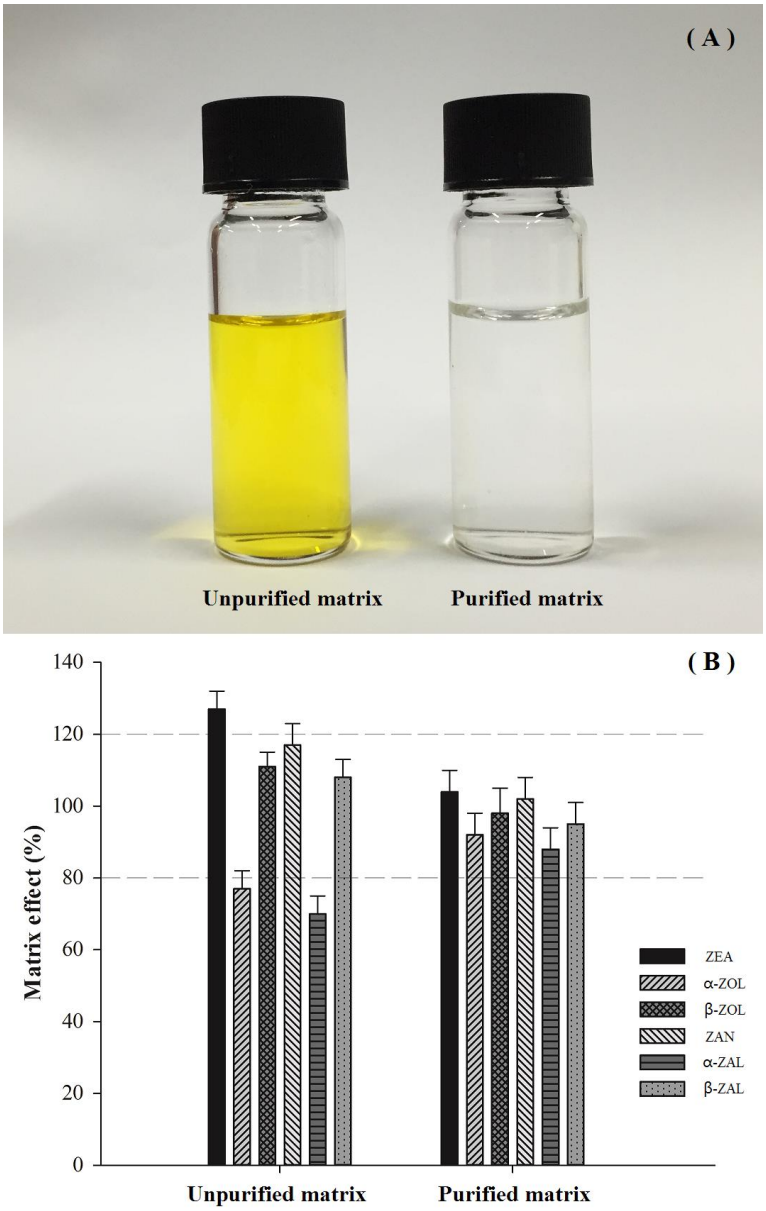

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557 **Fig. 4**

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563 **Table 1.** Calibration curves of ZEA and its derivatives in acetonitrile

Mycotoxin	Slope ($\bar{X}$ ± SD)	Intercept ($\bar{X}$ ± SD)	R^2	Linear range (ng/mL)	LOD [a] (μg/kg)	LOQ [b] (μg/kg)
ZEA	10130 ± 1020	2209 ± 240	0.998	0.1 - 500	0.03	0.07
α-ZOL	2915 ± 281	663 ± 301	0.995	0.1 - 500	0.04	0.10
β-ZOL	2248 ± 138	443 ± 109	0.996	0.1 - 500	0.04	0.09
ZAN	5304 ± 379	1847 ± 650	0.996	0.1 - 500	0.03	0.07
α-ZAL	2143 ± 152	345 ± 98	0.995	0.1 - 500	0.04	0.10
β-ZAL	1768 ± 89	932 ± 203	0.993	0.1 - 500	0.04	0.10

564 [a] Limit of detection (S/N = 3)

565 [b] Limit of quantification (S/N = 10)

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Table 2. Recovery, intra-day and inter-day precision data for ZEA and its derivatives in Maize

Mycotoxin	Spiked concentration levels (μg/kg)	Recovery $\bar{X}$ ± SD (%)	Intra-day precision (RSD, %)	Inter-day precision (RSD, %)
ZEA	5	100.5 ± 6.9	6.8	7.5
	50	101.2 ± 6.2	6.1	11.5
	200	104.1 ± 4.9	4.7	9.8
α-ZOL	5	81.1 ± 4.3	5.3	5.2
	50	77.8 ± 4.9	6.2	10.6
	200	80.5 ± 4.6	5.6	6.0
β-ZOL	5	80.2 ± 3.9	4.8	5.6
	50	81.3 ± 4.1	5.0	9.1
	200	80.5 ± 9.0	11.2	13.2
ZAN	5	94.0 ± 4.6	4.9	8.3
	50	92.5 ± 4.8	5.2	6.1
	200	94.8 ± 3.5	3.7	3.2
α-ZAL	5	77.6 ± 3.6	4.6	9.0
	50	81.9 ± 3.0	3.7	4.1
	200	79.8 ± 2.7	3.4	4.9
β-ZAL	5	75.8 ± 4.0	5.2	12.6
	50	78.0 ± 4.8	6.1	7.5
	200	76.9 ± 3.2	4.2	9.0

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