RESEARCH PAPER

Dispersive liquid-liquid microextraction coupled with magnetic nanoparticles for extraction of zearalenone in wheat samples

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ARTICLE INFO

Article History:
Received 21 November 2016
Accepted 26 December 2017
Published 15 January 2017

Keywords:
Dispersive liquid–liquid microextraction
Magnetic nanoparticles
micro-solid phase extraction
Wheat samples
Zearalenone

ABSTRACT

A new, sensitive and fast dispersive liquid-liquid microextraction (DLLME) coupled with micro-solid phase extraction (μ-SPE) was developed for determination of zearalenone (ZEN) in wheat samples. The DLLME was performed using acetonitrile/water (80:20 v/v) as the disperser solvent and 1-octanol as the extracting solvent. The acetonitrile/water (80:20 v/v) solvent was also used to extract ZEN from solid wheat matrix, and was directly applied as the disperser solvent for DLLME process. Additionally, hydrophobic oleic-acid-modified magnetic nanoparticles were used in μ-SPE approach to retrieve the analyte from the DLLME step. So, the method uses high surface area and strong magnetism properties of these nanoparticles to avoid time-consuming column-passing processes in traditional SPE. Main parameters affecting the extraction efficiency and signal enhancement were investigated and optimized. Under the optimum conditions, the calibration curve showed a good linearity in the range of 0.1-500 μg kg^{-1} (R^2=0.9996) with low detection limit of 83 ng g^{-1}. The intra-day and inter-day precisions (as RSD %) in the range of 2.6-4.3 % and high recoveries ranging from 91.6 to 99.1 % were obtained. The pre-concentration factor was 3. The method is simple, inexpensive, accurate and remarkably free from interference effects.

How to cite this article
Amoli-Diva M, Taherimaslak Z, Allahyari M, Pourghazi K. Dispersive liquid-liquid microextraction coupled with magnetic nanoparticles for extraction of zearalenone in wheat samples. Nanochem Res, 2017; 2(1):60-70. DOI: 10.22036/ncr.2017.01.006

INTRODUCTION

Zearalenone (ZEN) is a mycotoxin produced by Fusarium fungal species in particular *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium crookwellense* (Fig. 1) which occasionally contaminate agricultural commodities in the field and/or during storage [1]. ZEN mainly occurs in corn and wheat, and also in some food commodities frequently used in human and animal diets such as barley, rice and sorghum. Exposure to this toxin is associated with hyperestrogenism, such as infertility and other breeding issues in swine [2]. To minimize the risk

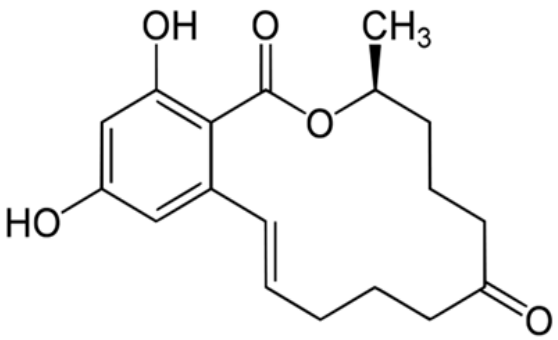

Fig. 1. The molecular structure of zearalenone.

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to humans and animals, the European Community Legislation has limited the concentration of ZEN at 20 and 100 μg kg^{-1} for cereal-based food intended for consumption by young children and infants and by adults, respectively [3]. Several analytical methods have been reported for determination of ZEN in different matrices, such as thin-layer chromatography (TLC) [4], enzyme-linked immunosorbent assay (ELISA) [5], high-performance liquid chromatography (HPLC) with different detection systems including fluorescence detection (FD) [6, 7], diode array detection (DAD) [8], and evaporative laser scattering detector (HPLC–ELSD) [2], ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) [9], high-performance liquid chromatography coupled with an molecularly imprinted optosensing material (MIOM) [10], and fluorescence resonance energy transfer (FRET) immunoassay [11]. Although some of these methods have shown good performances in terms of accuracy, precision, sensitivity and reproducibility, they are complicated, expensive and time-consuming techniques requiring involvement of skilled personnel. Thus, development of a simple, accurate and sensitive analytical technique for routine analysis of ZEN is desirable. Spectrofluorimetry can be considered as a valuable method because of its simplicity, sensitivity, relative selectivity, low cost, and less analysis time [12, 13]. Additionally, food matrices are often complex and determination of ZEN in real samples requires a pretreatment step for analyte enrichment and clean-up before analysis. Various pretreatment methods have been reported for extraction and clean-up of ZEN such as liquid-liquid microextraction [14] and solid phase extraction (SPE) using C_{18} cartridge [15], molecular imprinting polymer (MIP) [6, 16], and immunoaffinity column (IAC) [17]. IAC is the most common clean-up tool allowing a selective separation of analyte from a complex matrix [13]. However, IAC has some important disadvantages such as being time-consuming, not recyclable, relatively expensive, tedious and limited shelf-life [18, 19].

Recently, dispersive liquid-liquid microextraction (DLLME) has been introduced as a single separation /preconcentration step which has many advantageous such as low solvent consumption, high preconcentration factor and low detection limit and found extensive applications as highlighted by several reviews [20-22]. The conventional DLLME with high density solvent is based on a ternary component solvent system in which an appropriate mixture of extracting and disperser solvents is rapidly injected into the aqueous sample. The extracting solvent is dispersed into the aqueous phase and target analytes were extracted into the fine droplets of extracting solvent [23]. After that, centrifugation is applied to sediment it from water samples. Then, the extracting solvent containing the analytes is withdrawn using a syringe and subjected to the instrumental analysis. However, this approach is restricted to the use of highly dense organic solvents (denser than water) which are known as toxic and non-environment-friendly solvents. Also, these solvents are limited to chlorinated solvents such as chlorobenzene, chloroform, tetrachloromethane and tetrachloroethane which limit wide applicability of DLLME. Therefore, DLLME with a low-density organic solvent, as an extractant, was developed to overcome these disadvantages [24, 25]. However, the method still requires additional processing steps apart from the mandatory centrifugation, including refrigeration to freeze the organic solvent, its manually retrieving to let it thaw, and the use of additional materials and apparatus such as surfactants or conical-bottom test tubes [26]. To overcome these drawbacks, DLLME coupled with μ-SPE (DLLME-μ-SPE) has been introduced for the determination of some analytes such as metal chelates, polycyclic aromatic hydrocarbons and 4-nonylphenol [27] or triazine herbicides from water sample [28].

Considering the importance of zearalenone in endangering human health and that wheat is the most widely consumed grain in many countries, the amount of zearalenone in wheat samples is investigated in this study.

In this study, a DLLME procedure using 1-octanol as the extraction solvent was applied to extract ZEN from the sample and a μ-SPE using hydrophobic oleic-acid-modified Fe_3O_4 nanoparticles as the adsorbent was applied to retrieve the analyte-containing extracting solvent for the fast extraction of ZEN. Since 1-octanol is a large alcohol with a non-polar hydrophobic chain, a hydrophobic interaction can occur between the solvent and the hydrophobic nanoparticles leading to a rapid partitioning of analyte on the surface of magnetic nanoparticles (MNPs). Separation was quickly carried out applying an external magnetic field that overcome the need for centrifugation, refrigeration to freeze, and manual collection of extractant or specialized apparatus. Then, a surfactant enhanced spectrofluorimetric determination based on the use of Triton X-100 micelle formation that was used for determination of ZEN. All the experimental parameters affecting

the two-step extraction procedure were investigated in details and the analytical characteristics of the method were evaluated. The method was successfully applied for determination of ZEN in wheat samples.

EXPERIMENTAL

Standards and materials

The standard solution of ZEN (10 mg L^{-1} in acetonitrile) and all HPLC-grade solvents such as acetone (Me_2CO), acetonitrile (MeCN), dichloromethane (CH_2Cl_2), methanol (MeOH), ethanol (EtOH), ethyl acetate ($C_4H_8O_2$), toluene (C_6H_5-CH_3), 1-heptanol ($C_7H_{16}O$), 1-octanol ($C_8H_{18}O$), 2-ethylhexanol ($C_8H_{18}O$), diethyl ether ($(C_2H_5)_2O$), and trichloroethane ($CHCl_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), Iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), sodium dodecyl sulfate (SDS), Triton X-100, hexadecyltrimethylammonium bromide (CTAB), oleic acid and other used chemicals were supplied by Merck (Darmstadt, Germany). Deionized water was used throughout the experiments.

As safety notes, all used laboratory glassware was treated with an aqueous solution of sodium hypochlorite (5%) before discarding to minimize health risks due to ZEN contamination.

Instrumentation

A Varian Cary Eclipse fluorescence spectrophotometer (Palo Alto, CA, USA) equipped with a xenon lamp was used for fluorescence spectra recording of ZEN with scan rate of 1200 nm min^{-1}. All measurements were performed using 10 mm quartz microcells at room temperature and spectra recording was carried out with slit widths of 5 nm. The excitation and emission wavelengths were 315 and 465 nm, respectively. The modified magnetic nanoparticles were characterized by a Hitachi H-800 (Tokyo, Japan) transmission electron microscope (TEM). Chemical interactions were studied using a Perkin Elmer Spectrum one Bv5.3.0 FT-IR spectrometer (Waltham, Massachusetts, US) in the range of 400-4000 cm^{-1} with KBr pellets. A Labinco BV L46 Vortex mixer (Breda, Netherlands) was used to mix and accelerate the reactions between reagents.

Synthesis of oleic-acid-modified MNPs

The Fe_3O_4 nanoparticles were prepared via a simple chemical co-precipitation method as previously reported [29] with slight modifications. $FeCl_3{\cdot}6H_2O$ (5. 8 g) and $FeCl_2{\cdot}4H_2O$ (2.1 g) were dissolved in 100 mL deionized water under nitrogen atmosphere with vigorous stirring at 85 °C. Then, 20 mL of aqueous ammonia solution (25% w/w) was added to the solution. The color of bulk solution changed from orange to black immediately. The magnetic precipitate was washed twice with deionized water and once with 0.02 mol L^{-1} sodium chloride solution. Then, oleic acid (1.0 g) was introduced and the reaction was kept at 80 °C for 3 h. Finally, the suspension was cooled to room temperature. The resulting nanoparticles were washed with deionized water (5×100 mL) and separated from the solution with the help of an external magnet. The oleic-acid-modified magnetite nanoparticles were stored in deionized water at a concentration of 80 mg mL^{-1}.

Real sample pretreatment

Wheat samples were purchased from a local market in Tehran (Iran) and stored at 4 °C until being used. For analysis, the samples were weighed and grinded to powder with a blender. Then, 25 g of thoroughly homogenized powder samples were extracted with 100 mL of a mixture of MeCN/H_2O (80:20, v/v) at high speed for 3 min. The extracts were filtered using a filter paper (Whatman No. 44) and then, directly processed (as the disperser solvent) by DLLME.

Analytical procedure

320 µL of 1-octanol was added to 3 mL of MeCN/H_2O (80:20, v/v) containing analyte, and the mixture was rapidly injected into a conical bottom vial containing 15 mL of deionized water. Then, the vial was sealed and swirled on a vortex agitator at 3500 rpm for 1 min (equilibration time). After that, 500 µL of the adsorbent (containing 40 mg of MNPs) were quickly added to the vial. The solution was vortexed for 3 min to facilitate interaction of organic solvent containing target analyte to the surface of oleic-acid-modified MNPs. Then, the magnetic adsorbent was collected using an external magnet and supernatant was removed. The analyte was eluted from the adsorbent by addition of 1 mL of MeCN for 4 min. After desorption, the eluent was separated by decantation and evaporated to dryness under nitrogen gas flow at room temperature. The dry residue was dissolved in 1 mL of 20 mM Triton X-100 in 0.1 M sodium phosphate buffer (pH 4), incubated for 5 min and used for taking fluorescence spectra.

RESULTS AND DISCUSSION

Characterization of the adsorbent

The size and morphology of oleic-acid-modified Fe_3O_4 nanoparticles were characterized by TEM images. As can be seen in Fig. 2, the modified nanoparticles had a uniform size distribution and most of the particles are quasi-spherical in shape with a mean diameter of approximately 10±1.2 nm. Chemical interaction between Fe_3O_4 nanoparticles and oleic acid was characterized by FT-IR spectroscopy. As can be seen in Fig. 3, the characteristic peak of Fe_3O_4 nanoparticles can be observed as a strong absorption band at 583 cm^{-1} corresponding to the Fe-O band of the bulk magnetite. This band can be observed in oleic-acid-modified MNPs spectrum too. The two sharp bands at 2923 and 2853 cm^{-1} were attributed to the asymmetric CH_2 stretch and the symmetric CH_2 stretch, respectively. It is worth noting that the C=O stretch band of the carboxyl group, which generally appears at 1700-1750 cm^{-1} was absent in the curve (b), spectrum of the oleic-acid-modified MNPs and there appeared two new bands at 1541 and 1630 cm^{-1}, which were characteristic of asymmetric ν_{as} (COO-) and symmetric ν_s (COO-) stretch, instead. These results reveal that oleic acid has been chemisorbed onto the Fe_3O_4 nanoparticles as a carboxylate and its hydrocarbon tail is free to interact with analyte containing 1-octanol solvent.

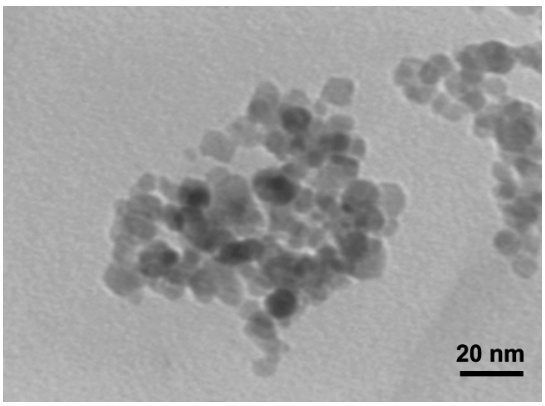

Fig. 2. TEM image of oleic-acid-modified MNPs.

Signal enhancement conditions

The fluorescence properties of ZEN are sensitive to the environment of toxin, and are modulated by solvent, pH, and water quenching phenomena. In fact, these properties enabled the development of more selective detection methods. For example, using surfactant in mycotoxin environment enables it to enhance its fluorescence intensity under certain conditions. Surfactants used for this study were selected based on their broad range of properties

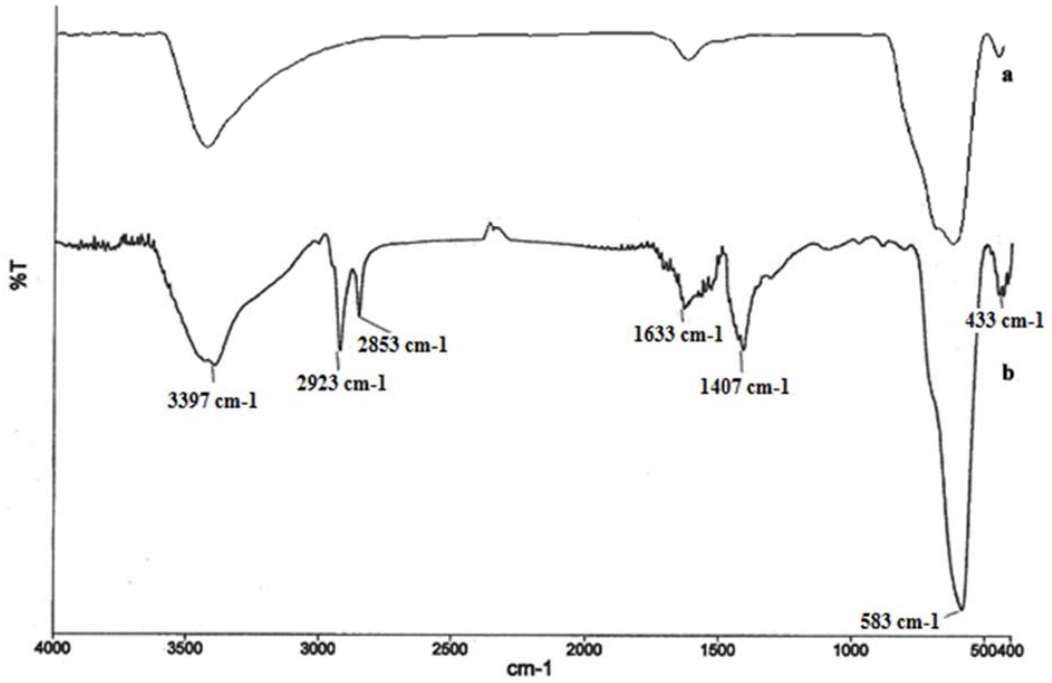

Fig. 3. FT-IR spectra of MNPs (a) and oleic-acid-modified MNPs (b).

and applications (such as their micelle formation behavior and charge) including ionic surfactants (anionic SDS and cationic CTAB) and the non-ionic Triton X-100. The emission spectra for ZEN in SDS, CTAB, and Trition X-100 are shown in Fig. 4. As can be seen, the non-ionic Triton X-100 and cationic CTAB provide significantly greater enhancement of fluorescence intensity of ZEN over SDS. In fact, the tail lengths of CTAB and Triton X-100 are significantly larger than SDS and the extent to which SDS micelles can encapsulate ZEN may be lower [1]. So, the larger micelles formed by CTAB and Triton X-100 can provide a better environment to encapsulate and restrict the intramolecular rotation of ZEN to boost emission. Furthermore, the effect of surfactant concentration on the fluorescence emission intensity of ZEN was studied in the range of 0.5-50 mM. The experimental results showed a significant fluorescence enhancement with increasing in surfactant concentration and reached the maximum in 20 mM which is above the critical micellar concentration (CMC) value of 2 mM for Triton X-100. In addition, the effect of pH on the fluorescence intensity of ZEN in the presence of surfactant was investigated in 0.1 M sodium phosphate buffer in the range of 2-8. The results (Fig. 5) reveal that ZEN in the presence of Triton X-100 exhibits a great fluorescence intensity enhancement even at high pH. It is interesting because other compound such as β-cyclodextrin which previously used for enhancing fluorescence signal of ZEN, quenched by high [30].

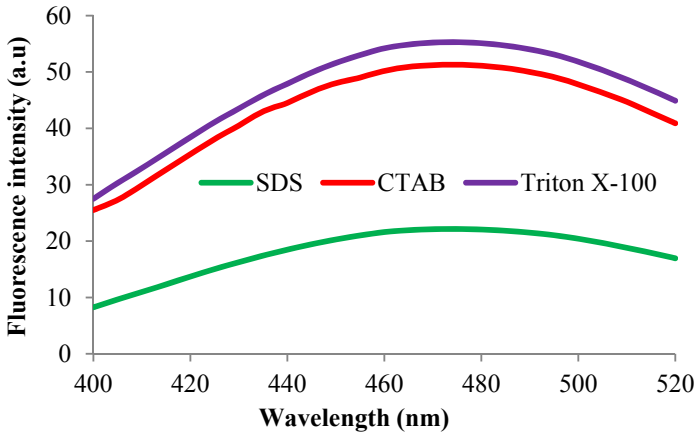

Fig. 4. Effect of surfactant type on the fluorescence intensity of ZEN. Conditions: dispersing solvent, 3 mL of MeCN/water (80:20 v/v); extraction solvent, 320 µL of 1-octanol; water volume, 15 mL, equilibration time, 60 s, adsorbent amount, 40 mg; adsorption time, 3 min; desorption time, 4 min, desorption solvent volume and type, 1 mL of MeCN.

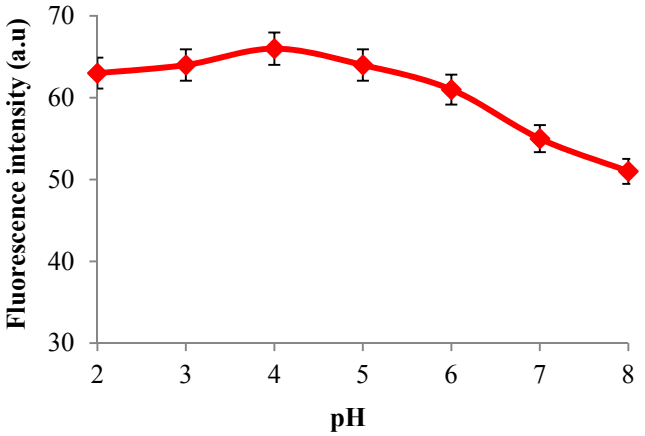

Fig. 5. Effect of pH on the fluorescence intensity of ZEN. Conditions: dispersive solvent, 3 mL of MeCN/water (80:20 v/v); extraction solvent, 320 µL of 1-octanol; water volume, 15 mL, equilibration time, 60 s, adsorbent amount, 40 mg; adsorption time, 3 min; desorption time, 4 min, desorption solvent volume and type, 1 mL of MeCN.

Optimization of the DLLME procedure

Selection of dispersing solvent

The solvent used to primary extract the analyte from solid matrix must then act as disperser solvent in DLLME process. Therefore, its selection must take into account both the properties required for analyte extraction from solid sample matrix and DLLME dispersant [31]. Generally, an aqueous mixture of MeCN is applied for extraction of ZEN from food samples in the literature [6, 10]. On the other hand, Me_2CO, MeCN and MeOH are the commonly used disperser solvents in DLLME method. Thus, the applicability of several solvents, including Me_2CO, MeOH, MeCN, EtOH, MeOH/water (80:20 v/v) and MeCN/water (80:20 v/v) was investigated in the preliminary experiments. The results revealed that the extraction efficiency achieved by MeCN/water (80:20 v/v) is higher than that for the other solvents (Fig. 6) and therefore, it was selected to act as both the extraction solvent for ZEN from wheat samples and as disperser solvent in DLLME for subsequent experiments.

Furthermore, the effect of disperser solvent volume on ZEN recovery was investigated in the range of 1-5 mL. The obtained results revealed that the extraction efficiency increases with increasing the volume of MeCN/water (80:20 v/v) up to 3 mL and then, decreases due to the increase in solubility of ZEN in aqueous phase and decrease in the distribution ratio. Accordingly, further studies were performed using 3 mL of MeCN/water (80:20 v/v).

Selection of extracting solvent

Selection of a suitable extracting solvent has great influence on optimization of DLLME process. In the present study, an extracting solvent should have several characteristics, such as good emulsification efficiency in the aqueous sample, high affinity for compounds of interest, low solubility in water, low density and low vapor pressure to prevent loss during agitation. Thus, the applicability of several low-density organic solvents, including ethyl acetate, toluene, 1-heptanol, 1-octanol, and 2-ethylhexanol were investigated in the preliminary experiments. A stable cloudy solution with good extraction efficiency was obtained with 1-octanol (Fig. 7).

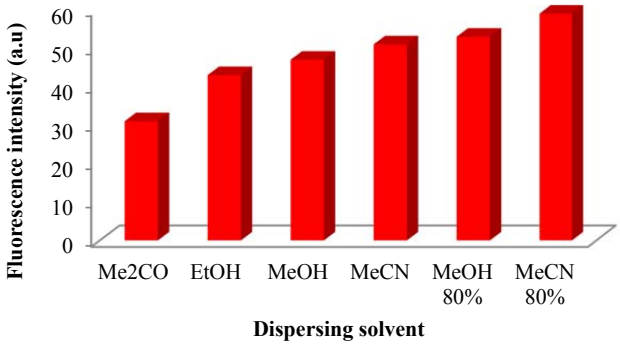

Fig. 6. Effect of dispersing solvent on the recovery of ZEN. Conditions: extraction solvent, 320 µL of 1-octanol; water volume, 15 mL, equilibration time, 60 s, adsorbent amount, 40 mg; adsorption time, 3 min; desorption time, 4 min, desorption solvent volume and type, 1 mL of MeCN.

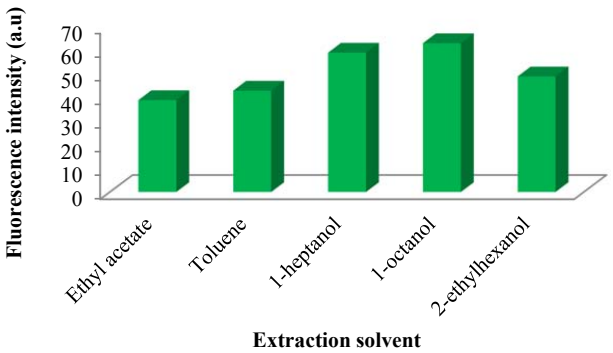

Fig. 7. Effect of extracting solvent on the recovery of ZEN. Conditions: dispersive solvent, 3 mL of MeCN/water (80:20 v/v); water volume, 15 mL, equilibration time, 60 s, adsorbent amount, 40 mg; adsorption time, 3 min; desorption time, 4 min, desorption solvent volume and type, 1 mL of MeCN.

The volume of extracting solvent is an important parameter which can affect the formation of cloudy state and efficiency of the extraction process. So, the effect of 1-octanol volume on the extraction of ZEN was investigated in the range of 250–350 μL. The results revealed that the fluorescence intensity of ZEN increases with increasing 1-octanol volume in the range of 250 to 320 μL. Decreasing in intensity with increasing in solvent volume above 320 μL is due to the dilution effects and decreasing in intensity in lower volume is due to the dissolution of organic phase in aqueous media. Therefore, 320 μL was selected as an optimum volume for further studies.

Effect of salt addition

Addition of salt to the sample may have several effects on the extraction efficiency of the analyte. Generally, salt addition can decrease solubility of target analyte in aqueous phase and promote analyte transfer toward the organic phase and thus improve the extraction efficiency (salting-out effect) [23]. Additionally , salt addition increases viscosity and density of the solution leading to reduce the efficiency of emulsification phenomenon due to lower solubility of extracting solvent in aqueous phase [32]. In this study, the effect of salt addition on the extraction efficiency of ZEN was investigated by addition of different amounts of NaCl (0-5 % w/v) to the samples. The results showed that the extraction efficiency of ZEN is not affected by the presence of NaCl in the range of 0-5 % w/v. Thus, the experiments were carried out without adding salts.

Effect of water volume

The analyte recovery was also affected by the water volume used in DLLME because it can influence the solubility of ZEN in the aqueous phase. The effect of water volume on the extraction efficiency of ZEN was investigated using different volumes in the range of 3-25 mL. The results indicated that the extraction efficiency is constant in the range of 5-18 mL. On the basis of the results, 15 mL was selected for the subsequent experiments.

Effect of equilibration time

The equilibration time is defined as the interval time from the occurrence of the cloudy state and just before addition of the hydrophobic magnetic nanoparticles. Equilibration time was investigated in the range of 0–300 s maintaining the rotational speed at the maximum level (3500 rpm) to maximize the energy transfer and reduce the mixing time. The results showed that the intensity of fluorescence signal versus extraction time is not affected remarkably and the mass transfer from sample solution to extracting solvent is very fast. Thus, the minimum time of 60 s was selected for the subsequent experiments.

Optimization of magnetic μ-SPE procedure

Effect of MNPs amount and adsorption time

The amount of hydrophobic MNPs is a key parameter to accomplish quantitative removal of the extraction solvent containing ZEN in DLLME. Thus, different amounts of oleic-acid-modified Fe_3O_4 were investigated in the range of 10-100 mg. The results showed that the extraction efficiency increases with increasing the amounts of adsorbent up to 40 mg and then levels off. Therefore, 40 mg was selected for the further experiments.

To realize the effect of adsorption time on the recovery of the analyte, it was investigated in the range of 1-7 min. The experimental results indicated that 3 min is sufficient for achieving an appropriate adsorption of the analyte and using for the next experiments.

Desorption conditions

An ideal elution solvent should be strong enough to elute all the target compounds from the sorbent. In this study, the effect of several organic solvents including Me_2CO, EtOH, MeOH, MeCN and $CHCl_3$ on desorption efficiency of ZEN was investigated. As can be seen from Fig. 8, the best result was found for MeCN as the desorbing solvent.

Furthermore, the effect of desorbing solvent volume on the recovery of ZEN was investigated in the range of 0.5-5 mL and the maximum recovery was obtained with volumes higher than 1 mL. Therefore, 1 mL of acetonitrile was selected. In addition, the effect of desorption time was investigated in the range of 1-10 min. A duration time of 4 min was appeared to be sufficient for complete desorption. Since, modified nanoparticles can be easily and rapidly collected from the solution using an external magnetic field, the analysis time greatly reduces compared to the conventional SPE methods.

Selectivity studies

The effect of interferences on the recovery of ZEN was studied by co-existing of other mycotoxins that may exist in cereal samples, including aflatoxins

(B_1, B_2, G_1, and G_2), deoxynivalenol (DON), and ochratoxin (OTA) alone and in mixture under the optimum conditions. The obtained results (Table 1) revealed that ZEN recoveries are not significantly affected by the presence of the interferences, indicating good selectivity of the method for determination of ZEN in wheat samples.

Analytical parameters

Under the optimum experimental conditions, the calibration curve was linear over the concentration range of 0.1-500 µg Kg^{-1} with R^2 of 0.9996. Solutions for the construction of calibration curve were prepared by spiking appropriate amounts of ZEN working solutions to the non-contaminated wheat sample and subjected to the proposed DLLME-µ-SPE procedure following the enhanced fluorescence measurements. The limit of detection (LOD=3.3S_b/m, where S_b is the standard deviation of ten replicate measurements of blank solution and m is the slope of the calibration curve) was found to be 83 ng g^{-1}. The precision of the method was evaluated as RSD% through investigation of intra-day and inter-day precisions. The intra-day precision was evaluated using five replicates measurements of two spiked samples with the concentration of 5 and 50 µg Kg^{-1} in the same day and the inter-day precision was evaluated using five replicate measurements of spiked samples at the same concentration levels in five consecutive days. The results, summarized in Table 2, indicate good precision of the proposed method. Adsorption capacity of the adsorbent was determined by the static method. For this purpose, 40 mg of hydrophobic adsorbent was equilibrated with 18 mL of solution containing dispersed analyte after DLLME step, at different concentration levels at the optimum conditions. After 10 min, the mixture was filtered and the supernatant were analyzed. The results showed that the amount of analyte adsorbed per unit mass of the adsorbent was increased linearly with concentration of ZEN and then was reached to a plateau value (adsorption capacity value), representing the saturation of active surface

Table 1. Effect of mycotoxins interferences on the extraction efficiency of 5 µg kg^{-1} of ZEN.

Interference	Concentration (µg kg^{-1})	Recovery ± RSD (%)
Aflatoxin	10	93.7 ± 1.9
OTA	5	95.7 ± 1.7
DON	50	97.1 ± 2.4
Mixture	Total	91.3 ± 3.8

Table 2. The characteristic data of the proposed method.

Parameters	value
Dynamic range (µg L^{-1})	0.1-500
Correlation coefficient (R^2)	0.9996
Intra-day precision (RSD%, n=5)	3.9[a]
	2.6[b]
Inter-day precision (RSD%, n=5)	4.3[a]
	3.6[b]
Limit of detection (3.3S_b/m [c], ng L^{-1})	83

[a] For 5 µg L^{-1} of ZEN; [b] For 50 µg L^{-1} of ZEN
[c] S_b is the standard deviation for ten blank measurements and m is the slope of the calibration curve.

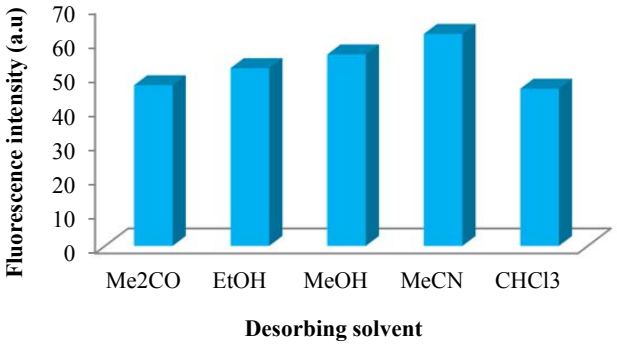

Fig. 8 Effect of desorption solvent type on the recovery of ZEN. Conditions: dispersive solvent volume and type, 3 mL of MeCN/water (80:20 v/v), extraction solvent, 320 µL of 1-octanol, water volume, 15 mL, equilibration time, 60 s, adsorbent amount, 50 mg; adsorption time, 3 min; desorption time, 4 min, desorption solvent volume, 1 mL.

of the hydrophobic adsorbent. The maximum adsorption capacity of the adsorbent for ZEN was found to be 573 μg g^{-1}.

Real sample analysis

To evaluate the applicability of the proposed method in real matrices, it was applied to the determination of ZEN in wheat samples. Recovery studies were carried out by spiking the samples with different amounts of ZEN. The obtained results (Table 3) showed that the recovery values were in the range of 91.6 to 99.1 %. The acceptable recoveries demonstrate that the matrix of wheat sample does not effect on the extraction efficiency. Further examination of accuracy was performed by comparison of the results obtained from the proposed method and the AOAC standard method (IAC-HPLC-FD) for determination of ZEN in two contaminated wheat samples. The results are summarized in Table 4. The statistical t-test analysis of the results showed that there are no significant differences between data obtained by the two methods at 95% confidence level. Furthermore, a comparison of the analytical characteristics obtained by the proposed method and some other reported methods for determination of ZEN is presented in Table 5. As can be seen, the proposed method has distinct advantages in terms of low detection limit, wide linear range, ease of operation and simplicity.

CONCLUSION

In this study, a two-step extraction technique, namely DLLME coupled magnetic nanoparticles-based μ-SPE, followed by surfactant-enhanced spectrofluorimetric detection was developed for the extraction of ZEN in wheat samples. The proposed method demonstrates that an organic solvent with lower density than water can be used in DLLME without involving any additional

Table 3. Determination of ZEN in spiked wheat samples (n=3).

Sample	Spiked (μg kg^{-1})	Found (μg kg^{-1})	Recovery (%)
Sample 1	0	ND[a]	-
	10	9.431 ± 1.1	94.3
	50	48.35 ± 3.2	96.7
	300	297.3 ± 2.6	99.1
Sample 2	0	ND	-
	10	9.163 ± 1.4	91.6
	50	48.95 ± 2.7	97.9
	300	297.1 ± 2.5	99.0
Sample 3	0	ND	-
	10	9.22 ± 1.2	92.2
	50	46.94 ± 1.9	93. 8
	300	295.5 ± 2.1	98.5

[a]Not detected

Table 4. Comparison of ZEN analyses (mean ± SD, n=3) in contaminated wheat samples by the proposed and standard IAC-HPLC-FD method.

sample	Proposed method	[a] HPLC-FD IAC-
	ZEN (μg kg^{-1})	ZEN (μg kg^{-1})
Sample 1	2.37 ± 0.13	2.53 ± 0.09
Sample 2	2.11 ± 0.21	2.32 ± 0.14

[a] HPLC analysis by AOAC standard method.

Table 5. Comparison of the proposed method with some previously reported methods for the determination of ZEN.

Method	Matrix	LOD (μg kg^{-1})	Linear range (μg kg^{-1})	Recovery (%)	Ref.
QuEChERS[a]-HPLC	barley	1.56	0.1-10	83.6-91.5	[2]
MIP[b]-SPE-HPLC-FD[c]	corn, wheat	-	20–8800	82-87	[6]
MIP-SPE-HPLC-FD	wheat, barley, corn,	1.7-2.4	6–500	86-97	[16]
IAC-HPLC-FD	wheat, barley, maize	3.5-17.6	-	84.0-105.0	[33]
SPE-HPLC-DAD[d]	corn	0.7	0-400	90.0	[8]
DLLME-μ-SPE	wheat	0.083	0.1-500	91.6-99.1	This work

[a]Quick Easy Cheap Effective Rugged and Safe method; [b]Molecularly imprinted polymer
[c]Fluorescence detector; [d]Diode array detector

handling procedure and apparatus by applying the hydrophobic magnetic nanoparticles to retrieve the extracting solvent of DLLME. The method has many advantages including simplicity for extraction, minimum organic solvent consumption, excellent enrichment in a short period of time, good repeatability and reproducibility for determination of ZEN, low cost and high accuracy. The good recoveries from real samples and the inherent sensitivity and selectivity of spectrofluorimetric method showed that the present method could be effectively used for determination of ZEN in wheat samples.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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