Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 128 (2014) 583–590

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Enhanced spectrofluorimetric determination of aflatoxin M_1 in liquid milk after magnetic solid phase extraction

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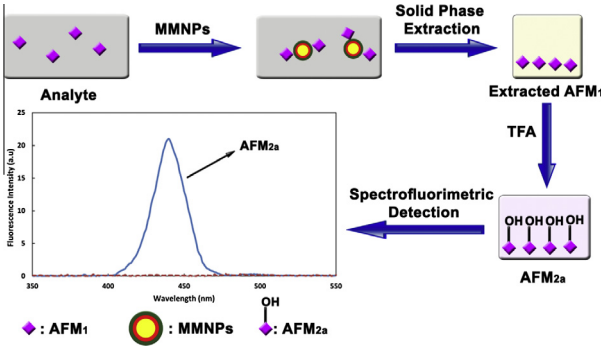
HIGHLIGHTS

- Magnetic solid phase extraction-spectrofluorimetry method is used for AFM_1 detection.
- The proposed method is suitable for determination of AFM_1 in liquid milk.
- Free antibody nanoparticles are used for separation and preconcentration of AFM_1.
- Trifluoroacetic acid was used as derivatization reagent.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 20 February 2014
Accepted 23 February 2014
Available online 4 March 2014

Keywords:
Aflatoxin M_1
Modified magnetic nanoparticles (MMNPs)
Enhanced spectrofluorimetric detection
Solid phase extraction (SPE)

ABSTRACT

A simple and sensitive method using magnetic solid phase extraction (MSPE) followed by spectrofluorimetric detection has been developed for separation and determination of aflatoxin M_1 (AFM_1) in liquid milk. The method is based on the extraction of AFM_1 on the modified magnetic nanoparticles (MMNPs) and subsequent derivatization of extracted AFM_1 to AFM_1 hemi-acetal derivative (AFM_{2a}) by reaction with trifluoroacetic acid (TFA) for spectrofluorimetric detection. Magnetic nanoparticles (MNPs) coated by 3-(trimethoxysilyl)-1-propantiol (TMSPT) and modified with 2-amino-5-mercapto-1,3,4-thiadiazole (AMT) were used as adsorbent in MSPE procedure. Influential parameters affecting the extraction efficiency were investigated and optimized. Under the optimum conditions the calibration curve for AFM_1 determination showed good linearity in the range 0.030–10.0 μg L^{-1} (R^2 = 0.9991). The repeatability and reproducibility (RSD%) for 0.050 μg L^{-1} of AFM_1 were 4.5% and 5.3%, respectively and limit of detection limit (S/N = 3) was estimated to be 0.010 μg L^{-1}. The developed method was successfully applied for extraction of AFM_1 from spiked liquid milk and natural contaminated liquid milk. The good spiked recoveries ranging from 91.6% to 96.1% were obtained. The results demonstrated that the developed method is simple, inexpensive, accurate and remarkably free from interference effects.

Introduction

Aflatoxins (AFs) are typically found as secondary metabolites of *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. AFs frequently contaminate cereal crops, such as corn, beans, peanuts, and dried fruit [1]. Among different AFs, AFB_1 is the most frequent produced mycotoxin, which is the most powerful carcinogen in mammals. When AFB_1 is ingested by cows, it is converted into its monohydroxylated metabolites, which has been designated as AFM_1 [2,3]. AFM_1 or milk toxin exhibits a high level of genotoxic activity and certainly represents a health hazard because of its

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http://dx.doi.org/10.1016/j.saa.2014.02.108
1386-1425/

possible accumulation and linkage to DNA. The toxicity of AFM_1 was initially classified as a Group 2B agent, but it has now moved to Group 1 by International Agency for Research on Cancer (IARC) [4]. European Community legislation limits the concentration of AFM_1 for milk and processed milk products intended for adults at 0.050 μg kg^{-1} [5] and for milk intended for infants or baby-food production at 0.025 μg kg^{-1} [6]. AFM_1 is relatively stable during pasteurizations, storage, and preparation of various dairy products [7]. Due to the widespread consumption of milk and dairy products, presence of AFM_1 in these products has become a worldwide concern. Therefore, accurate evaluation of AFM_1 in milk is of great interest.

A number of analytical methods have been reported for determination of AFM_1, such as ELISA [8,9], TLC [10], LC–MS [11], HPLC–FD [12,13], UHPLC–MS–MS [14], fluorimetry [15,16], electrochemical methods [17,18] and immunoassay methods [19–21]. Among these methods, spectrofluorimety can be considered as a valuable method because of its simplicity, sensitivity, relative selectivity, low cost, and less time consuming [22–24]. However, there are a few reports about the application of direct spectrofluorimetric determination of AFM_1 [15,25]. In spectrofluorimetric assay, chemical derivatization has been widely appreciated due to increasing of selectivity and sensitivity.

Generally, determination of AFM_1 in real samples requires a clean-up or enrichment technique. Immunoaffinity column (IAC), C18, carbograph-4 and multifunctional cleanup column were reported to have preferable purification effect for AFM_1 clean-up in different dairy products [12,13,15,26–28]. IAC is the most common clean-up method which allows a highly selective separation of analyte from a complex matrix. However, it is time consuming, tedious, relatively expensive and commercially available as a single-use format [29]. Moreover, its collection efficiency has failed to provide complete satisfaction for users. Recently, new SPE technique based on the use of magnetic nanoparticles (MNPs), called magnetic solid phase extraction (MSPE) has been introduced for separation and preconcentration of organic and inorganic species from complex matrices. In MSPE, the contact area between the adsorbent and the analyte is large enough to ensure a fast mass transfer, which is beneficial to guarantee high extraction efficiency for this method. MNPs have been extensively used as adsorbent in MSPE due to its super paramagnetism, high magnetic saturation, low toxicity, simple preparation process and low price. The stability and selectivity of the MNPs can be significantly improved by the modification of the surface of the adsorbent with special functional groups.

The aim of this study was to investigate the applicability of the MSPE with modified magnetic nanoparticles (MMNPs) for enhanced spectrofluorimetric determination of AFM_1 in milk. The method is based on the separation of AFM_1 by MSPE procedure and subsequent derivatization of separated AFM_1 to AFM_1 hemiacetal derivative (AFM_{2a}) by reaction with TFA and final spectrofluorimetric determination. To the best of our knowledge, this is the first report about application of an antibody free adsorbent for separation and enhanced spectrofluorimetric determination of AFM_1 in milk. All the experimental parameters affecting the extraction were investigated in details and the analytical characteristics of the method were evaluated. The method was demonstrated to be applicable for the analysis of AFM_1 in liquid milk samples.

Experimental

Standards and materials

The standard solution of AFM_1 (500 μg L^{-1} in acetonitrile), TFA and all HPLC-grade solvents such as acetone (Me_2CO), acetonitrile (MeCN), dichloromethane (CH_2Cl_2), methanol (MeOH) and water (H_2O) were purchased from Sigma–Aldrich (St. Louis, MO, USA). $FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, 3-(trimethoxysilyl)-1-propantiol (TMSPT), 2-amino-5-mercapto-1,3,4-thiadiazole (AMT) and other used chemicals were supplied by Merck (Darmstadt, Germany). Phosphate buffered saline (PBS, pH = 7.4) was prepared by dissolving 0.20 g KCl, 0.20 g KH_2PO_4, 1.16 g Na_2HPO_4 and 8.00 g NaCl in 1 L water. The purity of the used organic solvents was checked via fluorescence prior to use. As safety notes, all used laboratory glassware were treated with an aqueous solution of sodium hypochlorite (5%) before the discarding to minimize health risks due to AFM_1 contamination.

Instrumentation

The fluorescence measurements were performed using a Cary Eclipse Fluorescence Spectrophotometer (Varian, USA) equipped with a xenon lamp. All measurements were performed in 10 mm quartz microcells, at room temperature. Spectra recording were carried out in fluorescence scan mode with the slit widths of 5 nm. Chromatographic analysis was performed by Waters HPLC system which consists of a Waters 474 fluorescence detector, a post column derivatization reactor and a Waters C18 column. The modified magnetic nanoparticles were characterized by an S-4160 scanning electron microscope (SEM) (Hitachi, Japan), APD2000 X-ray Diffractometer (XRD) (Italstructures, Italy) and FT-IR Spectrometer (Perkin Elmer, spectrum version 10.01.00, USA). A permanent magnet of Nd–Fe–B (100 mm × 50 mm × 40 mm, Model N48, China) was used for magnetic separation. Ultrasonic bath (Uc-150 Sturdy Industrial CO LTD, Taiwan) was used in modification step. An Eppendorf 5810 centrifuge was used for centrifugation. A pH-meter (Corning, Model 140, Switzerland) with a double junction glass electrode was used to check the pH of the solutions.

Synthesis of modified magnetic nanoparticles

The magnetic nanoparticles (MNPs) were prepared via improved chemical co-precipitation method [30] and modified according to Mashhadizadeh method [31]. $FeCl_3{\cdot}6H_2O$ (11.68 g) and $FeCl_2{\cdot}4H_2O$ (4.30 g) were dissolved in 200 mL deionized water under nitrogen atmosphere with vigorous stirring at 85 °C. Then, 20 mL of 30% aqueous ammonia was added to the solution. The color of bulk solution changed from orange to black immediately. The magnetic precipitates were washed twice with deionized water and once with 0.02 mol L^{-1} sodium chloride solution. Then, 20 mL of prepared magnetic suspension was placed in a 250 mL round-bottom flask and allowed to settle. The supernatant was removed and coating of MNPs with 3-(trimethoxysilyl)-1-propanthiol (TMSPT) was carried with addition of an aqueous solution of TMSPT (10%, v/v, 80 mL), followed by glycerol (60 mL). The mixture was then stirred and heated at 85 °C for 2 h under a nitrogen atmosphere. After cooling to room temperature, the suspension was washed sequentially with deionized water (3 × 200 mL), methanol (3 × 100 mL), and deionized water (5 × 200 mL). The TMSPT-MNPs composite was stored in deionized water at a concentration of 40 g L^{-1}. TMSPT-MNPs prepared as described above (25 mL) were washed with methanol (2 × 100 mL) and then homogeneously dispersed into 150 mL of 1.0% aqueous solution of AMT. The solution was transferred to a 500 mL beaker and ultrasonicated for 2 h. After that, the resulting modified nanoparticles (AMT–TMSPT-MNPs) were washed three times with deionized water and twice with methanol and then dried in a vacuum oven at 45 °C for 2 h.

Analytical procedure

Sample preparation and MSPE procedure

Liquid milk was accurately weighed (10 ± 0.1 g) into 50 mL centrifuge tube and centrifuged (4000 rpm) for 15 min. After