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Novel analytical procedure using a combination of hollow fiber supported liquid membrane and dispersive liquid–liquid microextraction for the determination of aflatoxins in soybean juice by high performance liquid chromatography – Fluorescence detector

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ABSTRACT

This study describes a combination between hollow fiber membrane and dispersive liquid–liquid microextraction for determination of aflatoxins in soybean juice by HPLC. The main advantage of this approach is the use of non-chlorinated solvent and small amounts of organic solvents. The optimum extraction conditions were 1-octanol as immobilized solvent; toluene and acetone at 1:5 ratio as extraction and disperser solvents (100 μL), NaCl at 2% of the sample volume and extraction time of 60 min. The optimal condition for the liquid desorption was 150 μL acetonitrile:water (50:50 v/v) and desorption time of 20 min. The linear range varied from 0.03 to 21 μg L^{-1}, with R^2 coefficients ranging from 0.9940 to 0.9995. The limits of detection and quantification ranged from 0.01 μg L^{-1} to 0.03 μg L^{-1} and from 0.03 μg L^{-1} to 0.1 μg L^{-1}, respectively. Recovery tests ranged from 72% to 117% and accuracy between 12% and 18%.

1. Introduction

Aflatoxins (AFLs) are mycotoxins produced mainly by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* fungi species; these substances are considered some of the most powerful and toxic existing in Nature. When ingested by humans or animals, AFLs have acute or chronic toxic effects with severe consequences (Binder, 2007; Guan et al., 2011; Hussein & Brasel, 2001; McKean et al., 2006; Moss, 1998). The main toxic effects are carcinogenicity and mutagenicity; because of these serious problems associated with the ingestion, International Agency for Research on Cancer (IARC) classifies these substances in Group 1, i.e. human carcinogen (Heinrich, 2003; Pereira, Fernandes, & Cunha, 2014). These mycotoxins are capable of contamination a large number of foods and feeds, such as raw materials, products and by-products. Among them are cereals, grains and seeds such as rice, corn, soybeans, wheat, oats, rye, sorghum, beans, groundnuts, oils, milk and others (Pereira et al., 2014). Because of this, food security agencies as the European Food Safety Authority (EFSA) and the European Union Commission (EC) regulated at 2 μg kg^{-1} for B1 and 4 μg kg^{-1} for the sum of all AFLs as the maximum allowed levels of AFLs for human food (EC., 2006, 2010). Although, there is no regulation for maximum residue limits of AFLs in matrices derivatives of soybean, such as juice, some studies have reported the determination of these contaminants in some soybean products (Beltrán et al., 2013; Xie et al., 2014).

Different analytical instrumentation are available, however the "gold standard" for aflatoxins determination is the high performance liquid chromatography (HPLC) followed by fluorescence (FD) or mass spectrometry (MS) (Amoli-Diva, Taherimaslak, Allahyari, Pourghazi, & Manafi, 2015). The HPLC–FD is widely used due to its great versatility in the analysis of complex matrices. In order to improve the detection limits, the native fluorescence of B1 and G1 has been enhanced by different pre-column or post-column derivatization procedures (Quinto, Spadaccino, Palermo, & Centonze, 2009). In the last years, the number of LC–MS/MS methods reported for multiclass analysis of mycotoxins in food has increased considerably due to the high selectivity and sensitivity reached (Beltrán et al., 2013). Currently, the introduction of ultra-high performance liquid chromatography (UHPLC) has provided additional advantages for determination of mycotoxins in foodstuffs (Varga et al., 2012).

On the other hand, in order to obtain low limits of detection and good selectivity of AFs in food matrices, the sample preparation step plays an important role prior to the instrumental analysis.

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For extraction and clean-up, different approaches have been proposed, the most common methodologies being solid–liquid extraction with methanol or acetonitrile and water followed by clean-up step by solid phase extraction (SPE) or immunoaffinity columns (IACs) (Arroyo-Manzanares, García-Campaña, & Gámiz-Gracia, 2013; Arroyo-Manzanares, Huertas-Pérez, Gámiz-Gracia, & García-Campaña, 2013). IA columns are commonly preferred when compared to SPE because of their better performances in terms of yield and quantification limits. However, the IA procedure requires a high expertise level and the use of expensive disposable cartridges (Quinto et al., 2009). Other extraction procedures such as microextraction techniques are emerging as a great advance in the sample preparation field. Most of these techniques were developed in the last decade of the 20th century and nowadays they are widely being used with some modifications and improvements. Among these techniques the hollow fiber liquid phase microextraction (HF-LPME) and dispersive liquid–liquid microextraction (DLLME) can be highlighted (Koesukwiwat, Sanguankaew, & Leepipatpiboon, 2014; Lai, Sun, Ruan, Zhang, & Liu, 2014; Pedersen-Bjergaard, Rasmussen, & Grønhaug Halvorsen, 2000).

In the HF-LPME a supported liquid membrane (SLM) is created by means of the immobilization of an organic solvent in the porous wall of a hollow fiber, generally composed of polypropylene; at the same time a small volume of the same solvent is placed in the lumen of the hollow fiber. The analytes are extracted from the aqueous sample to the organic phase, present both in the porous wall and in the lumen of the hollow fiber allowing an excellent clean-up of the sample and high enrichment factors. However, this technique requires more extraction time. (Bardstu, Ho, Rasmussen, Pedersen-Bjergaard, & Jonsson, 2007; Ho, Pedersen-Bjergaard, & Rasmussen, 2002; Pedersen-Bjergaard & Rasmussen, 2008; Rezaee et al., 2006). A more recent development in liquid phase microextraction is the dispersive liquid–liquid microextraction (DLLME), which was introduced to the scientific community in 2006 (Rezaee, Yamini, & Faraji, 2010) and consists basically in using a few microliters of organic solvents, a mixture of disperser and extraction solvents, and enabling the achievement of high surface area between the sample and the extracting solvent which increases its efficiency. High extraction efficiency and capacity of pre-concentration as well as fast extraction are among the main features of the technique. However, this technique not is adequate for "dirty" samples. The DLLME is used for several applications in different fields of science; large number of works use this technique not only for the extraction of organic compounds but also for the extraction of metal species in aqueous matrices (Amoli-Diva et al., 2015). Currently, the DLLME have been combined in sequence with other sample preparation techniques such as vortex-assisted dispersive solid phase extraction (Amoli-Diva et al., 2015) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) (Arroyo-Manzanares and García-Campaña et al., 2013; Arroyo-Manzanares and Huertas-Pérez et al., 2013) for extraction of mycotoxins from different samples.

Therefore, the development of new extraction procedures which use small amount of non-chlorinated solvents, as well inexpensive and less laborious extraction steps, is a key topic for the analysis of aflatoxins in foodstuff. According to this, our study proposes a novel and efficient approach for sample preparation for the extraction of AFLs in soybean juice samples with separation/detection by HPLC-FD. In this study the simultaneous combination of two widespread microextraction techniques (DLLME and HF-LPME) were used. For the DLLME non-chlorinated solvents were used. On the other hand, for the HF-LPME the extraction was taken only in the pores of the membrane. The proposed sample preparation was called dispersive liquid–liquid microextraction supported by hollow fiber (HF-DLLME).The aflatoxins (AFLs) and soybean juice were the analytes and the matrix chosen to carry out this research.

2. Materials and methods

2.1. Reagents and materials

AFLs standard stock solutions containing 1 μg mL^{-1} (B1 and G1) and 0.3 μg mL^{-1} (B2 and G2) in methanol (Sigma Aldrich, Milwaukee, WI, USA) were used. Working solutions containing 25 and 100 μg L^{-1} were obtained by diluting the stock solutions in methanol. Aflatoxins underwent light degradation, and then standard solutions were kept in amber vials. Since aflatoxins are subject to light degradation, the work solutions need to be protected adequately from daylight. All solutions were kept stored in a refrigerator at −8 °C with protection against light incidence until the analysis was performed. Therefore, all the procedures were carried out in subdued light and protected from direct UV light (Cho et al., 2008). Methanol and acetonitrile (JT Baker, Center Valley, PA USA) both HPLC grade were used as mobile phase and dispersants for method optimization. Acetone and n-octanol (Sigma–Aldrich, Milwaukee, WI, USA) were used as the disperser and the extraction solvent, respectively. Toluene, n-hexane and chloroform (Tedia, Fairfield, OH, USA) were used as extraction solvents. In this work sodium chloride P.A. (Vetec, Química Fina, Duque de Caxias, RJ, Brazil), anhydride trifluoroacetic (TFAA) (Sigma–Aldrich, Milwaukee, WI, USA) and ultra-purified water (Mega Purity, Billerica, USA) were used. Polyethylene inserts of 150 μL and vials of 2 and 4 mL (Supelco, Bellefonte, PA, USA) were also used. Microsyringes models 1701N and 1701RN with volume of 100 and 250 μL (Hamilton, Reno, Nevada, USA), polypropylene membranes Accurel Q3/2 with 600 μm of d.i., wall thickness of 200 μm and 0.2 μm of pore size (Membrane, Wuppertal, Germany), and thermostatic bath (Microquimica Ind. e Com Ldta, Palhoça, Santa Catarina, Brazil) were used.

2.2. Instruments

A Shimadzu Prominence LC 20AT series HPLC system (Shimadzu, Kyoto, Japan) equipped with fluorescence detector RF 20A series with a loop of 20 μL and manual injection Rheodyne 7725i (Rohnert Park, CA, USA) were used in this study. A C18 column (Phenomenex Kinetex, 250 mm × 4.6 mm × 5 μm) in reverse phase mode was used. The chromatographic conditions were: mobile phase water:methanol:acetonitrile (55:30:15) with isocratic flow rate of 0.8 mL min^{-1}, total analysis time of 15 min and injection volume of 20 μL fixed. The fluorescence detector was set to analyze the excitation wavelength of 360 nm and the emission wavelength of 440 nm. In the chromatographic analysis a guard column SecurityGuard™ ULTRA C_{18} (Phenomenex, Torrence, CA, USA) was also used. The chromatographic data were evaluated with LCSolution software (Shimadzu, Kyoto, Japan).

2.3. Sample preparation

Packages containing 200 mL of soybean juice with apple flavor were purchased in supermarkets in Florianópolis, Santa Catarina – Brazil. These packages were stored in a refrigerator at 4 °C until the analysis thereof. Prior to it the containers were shaken for 10 s for homogenization and a 4 mL aliquot was transferred to a vial containing a magnetic stir bar. After these procedures the samples were carried to the HF-DLLME.