Journal of Chromatography A, 1471 (2016) 51–59

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A facile method for the fabrication of magnetic molecularly imprinted stir-bars: A practical example with aflatoxins in baby foods

Mónica Díaz-Bao [1], Patricia Regal [*,1], Rocío Barreiro, Cristina A. Fente, Alberto Cepeda

Department of Analytical Chemistry, Nutrition and Bromatology, Universidade de Santiago de Compostela, 27002 Lugo, Spain

ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 13 August 2016
Accepted 8 October 2016
Available online 11 October 2016

Keywords:
Aflatoxin
MIP
Stir-bar
HPLC–MS/MS
Milk
Cereal

ABSTRACT

A fast and facile method for the fabrication of magnetic molecularly imprinted stir-bars (MMIP-SB) has been developed, using a combination of imprinting technology and magnetite. Magnetite was prepared in the laboratory from the raw and embedded into molecularly imprinted polymers through a process of bulk polymerization. This novel design was applied to the analysis of aflatoxins, one of the most important groups of mycotoxins in terms of occurrence and toxicity. In the context of food safety, molecularly imprinted polymers are a promising tool to achieve selective and accessible methods of extraction for different residues and contaminants. Considering the toxicity of aflatoxins, a dummy template was preferred for the synthesis of the imprinted polymers. A rapid and affordable extraction method for isolating five different aflatoxins that may be present in food was developed. The MMIP-SB was used as a conventional stir-bar and combined with high performance liquid chromatography and mass spectrometry for the determination of aflatoxin M_1 in milk powder (infant formulas) and aflatoxins B_1, B_2, G_1 and G_2 in cereal-based baby foods. The results showed an average recovery of 60%, 43, 40, 44 and 39%, respectively, and RSD below 10%. These in-house prepared stir-bars featured good stirring and extraction performance, and recognition abilities, offering a good alternative to more complicated.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Humans and animals are exposed to numerous chemicals risks during their life, including certain residues and contaminants in foodstuffs. Mycotoxins are natural substances produced as secondary metabolites by a wide variety of different species filamentous fungi such as *Aspergillus*, *Penicillum* and *Fusarium* [1]. These toxic compounds commonly enter the food chain through contaminated crops, mainly cereals, and their presence is highly dependent on environmental and storage conditions [2]. The situation becomes more worrying because several of these mycotoxins remain stable during food processing and can therefore reach the final products [3]. One of the most important classes of mycotoxins regarding their occurrence and toxicity is aflatoxins (AFs). These difuranocoumarin derivatives are produced by some *Aspergillus* species, namely *A. flavus* and *A. parasiticus* [4], or even by the rare *A. nomius* [5]. Although more than twenty AFs have been identified, only aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2) are classified as human's carcinogens [5]. Also aflatoxin M_1 (AFM_1), which is a major metabolite of AFB_1 in humans and animals, can be present in milk obtained from animals fed with feed contaminated with aflatoxin B_1. The exposure to these mycotoxins can cause chronic and acute toxic effects in consumers (carcinogenic, mutagenic, teratogenic and immunosuppressive) or even death [6]. In this respect, infants and children may be exposed to aflatoxins through different commercial products specially intended for them, such as formulas and cereal-based foodstuffs, or even through their mothers' milk [7–13]. Due to the inadequate manufacturing process, baby food and formula can be contaminated with microorganisms [10]. As a consequence, the European Commission has set the maximum levels of AFB_1 for processed cereal-based foods and baby foods for infants and young children at 0.1 $\mu g\,kg^{-1}$ [14]. The limit of AFM_1 in milk and infant formulas and follow-on formulas, including infant milk and follow-on milk, was set at 0.05 $\mu g\,kg^{-1}$ and 0.025 $\mu g\,kg^{-1}$, respectively [14].

Measures to minimize the exposure of consumers to AFs include the use of sensitive methods for their analysis in foods and feed, such as thin-layer chromatography (TLC), high performance chromatography (HPLC), liquid chromatography tandem mass spectrometry (LC–MS/MS) or immunoassays [13,15,16]. The most popular technique for determining AFs is HPLC with fluorescence or mass spectrometric detection [4,17]. In this context, solid-phase extraction (SPE), clean-up with organic solvents or

* Corresponding author.
E-mail address: patricia.regal@usc.es (P. Regal).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.chroma.2016.10.022
0021-9673/© 2016 Elsevier B.V. All rights reserved.

immunoaffinity columns (IAC) have been frequently applied to isolate the compounds from cereals and/or milk [4,18–21]. The official methods adopted by AOAC International are predominantly based on IAC clean-up, prior to LC with fluorescence detection [22–24]. There are clear advantages in combining IAC and LC–MS/MS, for instance high selectivity, low detection limits and separation of a wide range of mycotoxins with different physic-chemical properties. These immunoaffinity columns have even been employed in tandem to achieve multi-mycotoxin extractions at levels close to EU limits for adult and infant foods [19]. However, methods for the analysis of mycotoxins in baby food are scarce and there is currently a growing interest in the development of reliable detection systems for this purpose [7,8,10,19,25,26].

In recent decades, molecularly imprinted polymers (MIP) have become very popular and promising materials for extracting different analytes that are present in food [27]. Molecularly imprinted technology (MIT), in analytical chemistry, has gained considerable interest thanks to its ability to increase the selectivity of sample treatment processes [28]. Although the most common purification method for aflatoxins is immunoaffinity columns, their cost is usually high. Thus, alternative strategies to this classic clean-up approach are needed. At this point, MIP can offer a cheap, easy, affordable and highly versatile extractive solution. However, because of their high toxicity and cost, aflatoxin substitutes (dummy templates) which would give similar effect of specific interaction between an analyte and a stationary phase during the imprinting process, are preferred [29]. Template bleeding may be an additional problem of using imprinted polymers as purification techniques that may be solved with the use of dummy templates for MIP synthesis [30–33]. In this context, MIP sorbents in different formats have already been applied successfully for the separation of mycotoxins [34,35]. Their combination with different magnetic supports, the so-called magnetic molecularly imprinted polymers (MMIP), allows the laboratories to obtain automated methods in which the steps of classical extractions are performed with the aid of an external magnetic field. The coating of a layer of thin MIP film on solid supports such as filter membrane, silica glass fiber and magnetic nanoparticles would accelerate the dynamic adsorption and desorption process. In some cases, the analytes are extracted from the matrix into the polymer coating immobilized on a glass tube with a magnetic core [28]. Rapid molecular recognition equilibrium between adsorption and desorption could be established, since sampling is performed simultaneously with the self-stirring strategy [36]. The combination of MIPs with stir bar sorptive extraction technique (SBSE) can be especially useful when dealing with complex samples and can potentially bring huge benefits to sample preparation strategies [28]. In addition, the combination of magnetic properties with the advantages of MIP not only affords the selectivity for the target molecule but also provides the ability of one-step separation [37].

Various methods for the fabrication of magnetic molecularly imprinted stir-bars (MMIP-SB) have been reported in the literature, usually implying multiple preparative steps. In this work, a fast and facile method to obtain MMIP-SB has been developed, using a combination of magnetite synthesis and bulk polymerization. As a proof of principle that the novel stir-bar design works and it is applicable in real laboratory routines, it was applied to the analysis of five aflatoxins (B_1, B_2, G_1, G_2 and M_1) in different baby foods. Magnetite was embedded into a polymeric monolith which was molecularly imprinted using a dummy template. The structures of the five selected AFs and the dummy template are shown in Fig. 1. The magnetic bar showed great stirring ability and proper selectivity for the selected analytes, and it was combined with HPLC–MS/MS for the determination of AFM_1 in milk powder

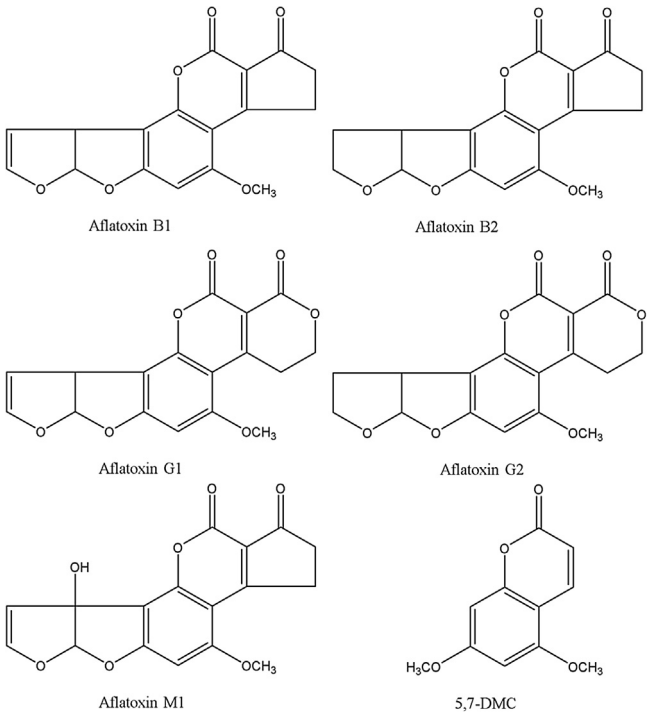

Fig. 1. Chemical structures of selected mycotoxins (aflatoxins) and dummy template.

(infant formulas) and AFB_1, AFB_2, AFG_1 and AFG_2 in cereal-based baby food, to demonstrate its applicability.

2. Experimental

2.1. Materials

The standards of aflatoxins B_1, B_2, G_1, G_2 and M_1 were obtained from Sigma-Aldrich Chemical Company (Madrid, Spain). They were diluted in methanol, resulting in the desired concentrations for each analysis. The chemicals to prepare magnetite $FeCl_2$, $FeCl_3$ and $NH_3{\cdot}H_2O$ (25%) were from Sigma-Aldrich Chemical Company (Madrid, Spain). The chemicals used for the polymers synthesis were 5,7-dimethoxycoumarin (DMC) as dummy template, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and the initiator 2,2′-azobis-(2-methyl-butyronitril) (AIMN), all from Sigma-Aldrich. MAA and EGDMA were freed from stabilizers by distillation under reduced pressure and AIMN was recrystallized from methanol prior to use. HPLC grade solvents were supplied by Merck (Madrid, Spain).

2.2. Samples

Baby food samples were obtained from local pharmacies. Samples were stored in a cool and dry place, as indicated by the manufacturer. Different baby formulas (n = 3) and cereal-based baby products (n = 4; wheat, corn, barley and rice-based) were analyzed.

2.3. Apparatus

The recoveries were calculated using LC–MS/MS. Separation was performed in an 1100 series HPLC system from Agilent Technologies (Minnesota, USA). A Luna 3 μm C18 (150 × 2 mm) column from Phenomenex (Torrance, CA, USA) was used. The mobile phase was water with 0.2% formic acid (A) mixed on a gradient mode with

Download English Version:

https://daneshyari.com/en/article/5135822

Download Persian Version:

https://daneshyari.com/article/5135822

Daneshyari.com