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Low-cost humic acid-bonded silica as an effective solid-phase extraction sorbent for convenient determination of aflatoxins in edible oils

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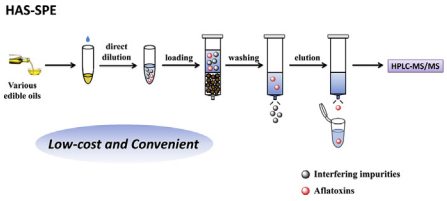
HIGHLIGHTS

- Low-cost humic acid-bound silica (HAS) as the solid-phase extraction (SPE) sorbent.
- Direct treatment diluted oil sample without liquid-liquid extraction.
- High recoveries for aflatoxins in various edible oils were achieved.
- Feeble matrix interference and accurate results comparable to IAC.

GRAPHICAL ABSTRACT



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ABSTRACT

Aflatoxins (AFs) are highly toxic, mutagenic, carcinogenic, and teratogenic secondary metabolites produced by the toxigenic fungi *Aspergillus flavus* and *Aspergillus parasiticus*. AFs tend to contaminate a wide range of foods which is a serious and recurring food safety problem worldwide. Currently, immunoaffinity chromatography (IAC) has become the most conventional sample clean-up method for determining AFs in foodstuffs. However, IAC method is limited in the large-scale food analysis because it requires the use of expensive disposable cartridges and the IA procedure is time-consuming. Herein, to achieve the cost-effective determination of AFs in edible oils, we developed a promising solid-phase extraction (SPE) method based on commercially available humic acid-bonded silica (HAS) sorbent, followed by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) analysis. In HAS-SPE, AFs can be captured by the HAS sorbent with both hydrophobic and hydrophilic interactions, whereas the oil matrix was captured only with the hydrophobic interactions. The oil matrix can be sufficiently washed off with isopropanol, while the AFs were still retained on the SPE packing, thus achieving selective extraction of AFs and clean-up of oil matrices. Under the optimal conditions of HAS-SPE, satisfactory recoveries ranging from 82% to 106% for four AFs (B_1, B_2, G_1, and G_2) were achieved in various oil matrices, containing blended oil, tea oil, rapeseed oil, peanut oil, sunflower seed oil, corn oil, blended olive oil, rice oil, soybean oil, and sesame oil. Only minor matrix effects ranging from 99% to 105% for four AFs were observed. Moreover, the LODs of AFs between 0.012 and 0.035 μg/kg completely meet the regulatory levels fixed by the EU, China or other countries. The methodology was further validated for assaying the naturally contaminated peanut oils, and consistent results between the HAS-SPE and the referenced IAC were obtained. In addition, HAS-SPE can directly treat diluted oil sample

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without liquid-liquid extraction and is automatable, thus making it simple and convenient for the large-scale determination of AFs in edible oils. Using this method, we successfully detected four AFs in the naturally contaminated peanut oils, which is, to the best of our knowledge, the first report about the determination of AFs in edible oils using HA-based SPE.

1. Introduction

Recently, serious concerns have been raised by consumers as well as by health professionals with respect to the presence of various toxigenic fungi or their secondary metabolites (as mycotoxins) in foods and feeds [1]. Aflatoxins (AFs) are one of the most typical groups of mycotoxins that are mainly produced by the toxigenic fungi *Aspergillus flavus* and *Aspergillus parasiticus* [2]. Until now, eighteen different types of AFs have been identified, wherein aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) are the four major members [2]. AFB_1 is the most toxic form, causing damage such as hepatitis, hemorrhage, edema, immunosuppression and hepatic carcinoma [3], and has been reported to be the most powerful natural carcinogen known [4]. Currently, widely growing reports and updated databases for foodstuffs reveal that contamination of human foods and animal feeds by AFs presents a serious and recurring food safety issue globally, thus ingestion of these contaminated foods and feeds may cause serious health damage to human and animals and incalculable economic losses [1,2,5].

AFs tend to contaminate the various oil-yielding seeds as well as the extracted oil [1,4] in warm and humid places that are optimal for the growth of molds and production of mycotoxins when foodstuff is grown, harvested, and finally stored [1,2,6]. Because AFs have an adverse effect on human health and the economy, the European Union (EU) Commission and more than 75 countries have gradually developed stringent laws/regulations for the maximum tolerable levels (limits) of AFs contamination in oilseeds and their derived products [1,4]. In China, the legal limits for AFB_1 are 20 μg/kg in peanut oil and corn oil and 10 μg/kg in other vegetable oils [7].

Determination of AFs in edible oils is challenging owing to their low concentrations relative to the extremely high concentrations of endogenous triglycerides (commonly > 95%, by oil weight [8]) in the oil matrix [9] which can significantly interfere instrumental detection [10]. Currently, various analytical methods and strategies have been applied for the determination of AFs in foodstuff and animal feed, including thin-layer chromatography (TLC) [11], fluorescence immunoassay [12–14], portable glucometer-based immunoassay [15], infrared spectroscopy [16], various biosensor devices [17,18], high-performance liquid chromatography (HPLC) [19], and liquid chromatography–mass spectrometry (LC-MS) [20,21]. TLC is commonly used in routine analysis for screening of AF contaminated samples, but this method often gives false positive results owing to the fact that the reliability of this method is strongly dependent on the operator's experience [2]. The immunoreactions-based fluorescence detection is a rapid and sensitive technique for routine analysis of foodstuff that demands minimum sample clean-up [22], but the matrix effect is a significant problem in immunoassay studies and leads to inaccurate results [23]. Biosensor-based methods used competitive-based immunoassays biosensor devices for rapid detection of AFs [24], but these immunoreaction-based microarray biosensors usually require an expensive robot for spotting probes and a confocal fluorescence scanner or a CCD camera for obtaining the detectable signal [25–27], which may hamper their widespread application. By contrast, HPLC coupled with fluorescence detection (HPLC-FLD) after clean-up by immunoaffinity chromatography (IAC) has become the most frequently used method [4]. Moreover, the highly selective IAC method is regarded as the reference clean-up method because it allows excellent signal-to-noise ratios and sufficient elimination of matrix interferences [4]. However, the IA procedure is time-consuming and requires the use of expensive disposable cartridges [4]. As the recommended alternative to the more expensive IAC, the multifunctional column (MFC) can effectively remove unwanted interfering components in the clean-up of the extracts [28], and thus is also widely employed for clean-up of mycotoxins. However, in comparison with IAC, because of the lower selectivity, MFC clean-up may produce a more significant matrix effect for the determination of mycotoxins in some foodstuffs using liquid chromatography coupled to tandem mass spectrometry analysis (LC-MS/MS) [29,30]. Recently, due to superior selectivity and sensitivity of mass spectrometry, LC-MS/MS has gained increasing importance in the analysis of AFs [31], where many "dilute-and-shoot" methods have been developed that do not need a clean-up step [32]. When using these methods, the matrix effect can be compensated by matrix-matched calibration, internal standardisation or by taking into account the recovery, but some complicated matrices may give rise to a low and unsatisfactory detection sensitivity for ESI-MS [33]. Thus, it is still a challenge to exploit a cost-effective approach that can improve the simplicity, selectivity, and sensitivity of these analytical methods.

Solid-phase extraction (SPE) is a well-established sample pretreatment technique in pharmaceutical, biomedical and environmental fields because it requires less organic solvents and is time-saving [34]. It enables preconcentration and purification of extracts in one step without increasing the matrix content in the treated extract compared to some other sample pretreatment methods, such as liquid-liquid extraction (LLE) and the QuEChERS method [35]. Moreover, SPE can be automated, and thus is convenient for large-scale sample preparation. In general, the sorbent is a decisive factor of SPE because it decides the cost of cartridges and the determination performance [9]. Humic acid (HA) is a naturally-occurring organic mixture resulting from complicated microbial degradations of plants and animal debris [36]. As shown in Fig. 1, the proposed basic chemical structure of humic acids consists of an aromatic ring of di- or trihydroxyphenol type bridged by –O–, –CH–, –NH–, –N=, and other substantial groups such as carboxylic and phenolic functional groups which both contribute most to surface charge and reactivity of humic acids [36]. These hydrophobic frameworks and abundant hydrophilic groups enable HA to easily interact with metal ions, oxides and even some poisonous organic compounds through chelation, charge-transfer interactions, ion exchange reactions, hydrophobic interactions, and hydrophilic interactions [9,37,38]. As depicted in Fig. 1, the chemical structures of four AFs incorporate dihydrofuran and tetrahydrofuran moieties coupled to a substituted coumarin, thus AFs can be captured by HA with both hydrophobic and hydrophilic interaction mentioned above, whereas the hydrophobic oil matrix is captured only with hydrophobic interaction. Theoretically, the oil matrix can be selectively washed off from HA under hydrophobic conditions and