Journal of Chromatography B 1092 (2018) 228–236

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Determination of aflatoxin and zearalenone analogs in edible and medicinal herbs using a group-specific immunoaffinity column coupled to ultra-high-performance liquid chromatography with tandem mass spectrometry

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ARTICLE INFO

Keywords:
Aflatoxins
Zearalenone analogs
Edible and medicinal herbs
Group-specific
Immunoaffinity column
UPLC–MS/MS

ABSTRACT

Six aflatoxins (AFs; AF B_1, B_2, G_1, G_2, M_1 and M_2) and six zearalenone (ZEN) analogs (ZEN, zearalanone, α-zeralanol, β-zeralanol, α-zearalenol, and β-zearalenol) were simultaneously extracted from edible and medicinal herbs using a group-specific immunoaffinity column (IAC) and then identified by ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS). The IAC was prepared by coupling *N*-hydroxysuccinimide-activated Sepharose 4B Fast Flow gel with two group-specific monoclonal antibodies. The column capacities to six AFs and six ZEN analogs ranged from 100.2 ng to 167.1 ng and from 59.5 ng to 244.4 ng, respectively. The IAC–UPLC–MS/MS method was developed and validated with three different matrices (Chinese yam [*Dioscorea polystachya*], *Platycodon grandiflorum* and coix seed [Semen Coicis]). Recoveries of twelve analytes from edible and medicinal herbs were in the range of 64.7%–112.1%, with relative standard deviations below 13.7%. The limits of quantification were in the range from 0.08 μg kg^{-1} to 0.2 μg kg^{-1}. The method was proven to be sensitive and accurate, and suitable for the determination of real samples.

1. Introduction

Herbal medicines have been used for thousands of years in China, and some herbal medicines have been considered as daily food (known as edible and medicinal herbs) because of their health-promoting functions and disease treatment properties [1]. With the improvement of people's living standards, individuals increasingly care more about their health. To remain healthy, individuals have used many kinds of edible and medicinal herbs, such as Chinese yam, *Platycodon grandiflorum*, and coix seed, as alternative medicines. A survey conducted by World Health Organization showed that about 70%–80% of the world populations rely on non-conventional medicines comprised mainly of herbal sources in their primary healthcare [2]. Although many health benefits are present in edible and medicinal herbs, the safe consumption of these products has gained much concern because of contaminations in raw materials by aflatoxins (AFs) and zearalenone (ZEN) structural analogs during cultivating, harvesting, processing and storage [3].

AFs are produced by fungi belonging to *Aspergillus flavus* and *Aspergilllus parasiticus* under warm and moist conditions, which are designated as a group 1 carcinogenic compound by the International Agency for Research on Cancer [3]. AF B_1, B_2, G_1, G_2, M_1 and M_2 (Fig. 1; AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2, respectively) are the most ubiquitous members of AF family and have received increasing attention because of their great harm to the liver [4]. Recently, AFs have been widely detected in herbal medicines [5–8]. Han et al. reported the mean levels (incidence) of AFB_1, B_2, G_1 and G_2 in herbal medicines samples were 1.40 (68.8%), 1.27 (50.0%), 0.50 (43.8%) and 0.94 (43.8%) μg kg^{-1}; AFM_1 was also detected with maximum concentrations of 0.70 μg kg^{-1} [6]. ZEN has also been found in herbal medicines [9, 10], but has not been studied as extensively as AFs. Zhang et al. detected ZEN in coix seed, with levels ranging from 18.7 μg kg^{-1} to 211.4 μg kg^{-1}. ZEN is a naturally occurring nonsteroidal estrogenic mycotoxin produced by genus *Fusarium* [11], and its derivatives (Fig. 1; zearalanone [ZAN], α-zeralanol [α-ZAL], β-zeralanol [β-ZAL], α-zearalenol [α-ZOL], β-zearalenol [β-ZOL]) have also been characterized

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https://doi.org/10.1016/j.jchromb.2018.06.012
Received 20 April 2018; Received in revised form 4 June 2018; Accepted 6 June 2018
Available online 07 June 2018

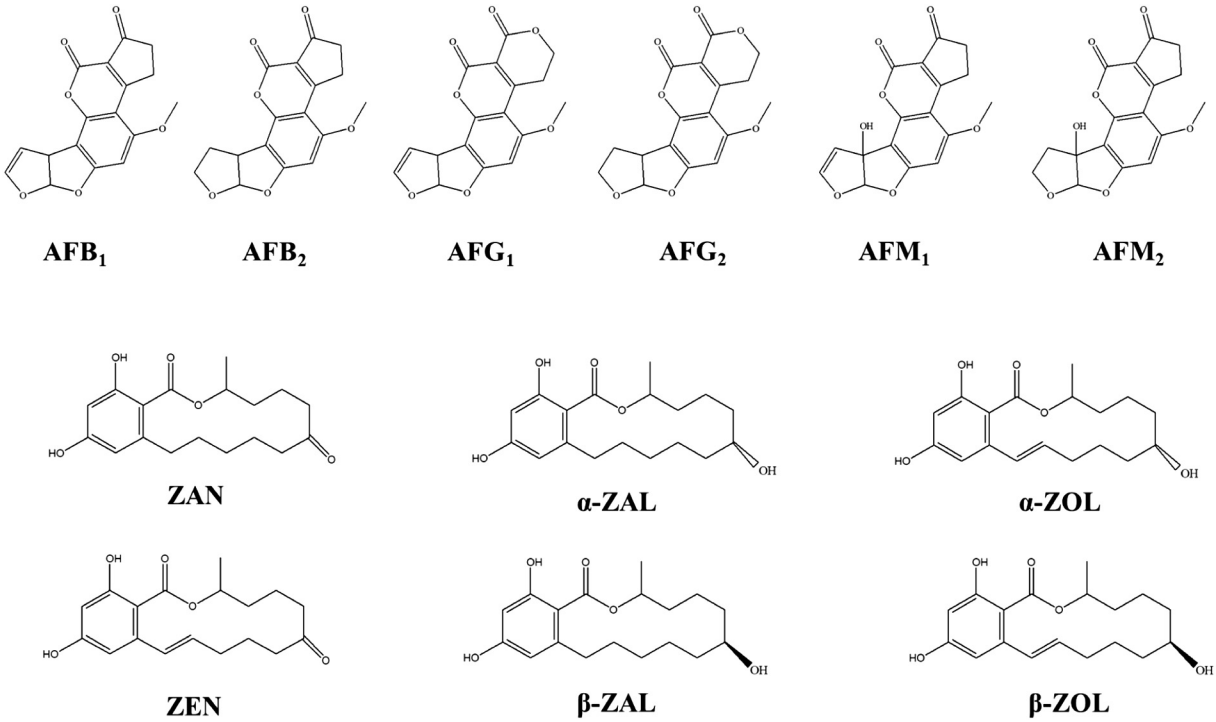

Fig. 1. Chemical structures of the AFs and ZEN structural analogs.

with estrogenic effects [12]. Therefore, to ensure consumers' health and minimize economic losses from contaminated herbal medicines, sensitive and accurate analytical methods are urgently needed for the detection of AF and ZEN analogs concentrations in herbal medicines.

To date, the most common analytical methods include liquid chromatography electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) [6, 7, 9, 13, 14] or LC coupled with fluorescence detection (FLD) [5, 8, 10, 15]. However, LC–ESI-MS/MS is more preferred because of its excellent selectivity and sensitivity. The complexities of herbal medicine matrices considerably challenge the attainment of accurate data under detection by MS/MS. Therefore, the clean-up performance of analytical methods is of great importance during the analytical process. The current purifying technologies for determination of AFs or ZEN are mainly based on solid phase extraction (SPE) [6], quick, easy, cheap, effective, rugged, and safe (QuEChERS) method [14] and immunoaffinty column (IAC) [5, 7, 8, 10]. SPE is easy to perform and solvent conserving, but retainment of analytes is based on nonspecific binding, which can lead to the coextraction of analytes and matrix impurities. QuEChERS offers a less expensive and time-saving approach to determine target analytes. However, its strong matrix effect is a central issue that cannot be ignored. IAC is a powerful purification technique that relies on the specific recognition between the antibody and complementary analytes [16]. Compared with SPE and QuEChERS, IAC is most frequently used because of its high specificity and efficiency. However, most studies have only detected AFs or ZEN with commercial IACs [7, 10, 17], the IACs may not be suitable for all the herbal medicines, and none has been used to determine two classes of analytes. The application of the screening and determination of two different types of mycotoxin has been largely restricted by the high cost of commercial IACs. Thus, a cost-saving IAC that can purify two classes of mycotoxins is needed for research. An IAC preparation that uses two group-specific monoclonal antibodies (Mabs) has not been reported. Such lack may be due to the difficulty in obtaining ZEN group-specific Mabs and the requirement for steady preparative techniques for further developing a multi-mycotoxin IAC.

In this paper, a novel IAC was prepared using a recently obtained Mab against AFs (named 5H3) [18] and a previously obtained Mab against ZEN analogs (named 3D4) [19], 5H3 recognizes six AFs (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 and AFM_2), and whereas 3D4 recognizes six ZEN analogs (ZEN, ZAN, α-ZAL, β-ZAL, α-ZOL and β-ZOL), respectively. As far as we know, this study is the first report on a multiple mycotoxins IAC for the simultaneous determination of AFs and ZEN analogs in three kinds of edible and medicinal herbs, including Chinese yam, *Platycodon grandiflorum* and coix seed. Finally, the developed IAC-LC-MS/MS method proved to be sufficiently sensitive and accurate and hence successfully used in real sample detection.

2. Materials and methods

2.1. Reagents and materials

The AFs Mab (5H3) [18] and the ZENs Mab (3D4) [19] were previously produced by our laboratory. AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, ZEN, ZAN, α-ZAL, β-ZAL, α-ZOL and β-ZOL standards were purchased from Sigma–Aldrich (St. Louis, MO). *N*-hydroxysuccinimide (NHS)-activated Sepharose 4B Fast Flow (FF) and polypropylene columns (3 mL) with polytetrafluoroethylene (PTFE) frits were purchased from Biocomma (Shenzhen, China). LC-MS grade acetonitrile (ACN) and methanol (MeOH) and were supplied by Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA). All other chemicals and solvents were of analytical grade orhigher.

Stock mixed standard solutions (10 mg mL^{-1}) were prepared by dissolving 10 mg of each standard substance in MeOH (10 mL) in amber glass vials. Working mixed standard solutions (10 μg mL^{-1}) were prepared by diluting the stock solutions with MeOH. The working solutions were stored at 4 °C. Phosphate buffered saline (PBS) was prepared by dissolving 8.8 g NaCl, 0.02 g KCl, 2.9 g $NaH_2PO_4{\cdot}2H_2O$ and 0.59 g $Na_2HPO_4{\cdot}12H_2O$ in 1 L of water, and the pH was adjusted to 7.4 with 1 M NaOH aqueous solution.

2.2. Samples

Edible and medicinal herb samples including Chinese yam,