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Ultra-high-pressure liquid chromatography–solid-phase clean-up for determining aflatoxins in Egyptian food commodities

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ABSTRACT

In this work, we determined the content of regulated aflatoxins (ATs) B1, B2, G1, and G2 in food commodities using solid-phase extraction (SPE) and ultra-high-pressure liquid chromatography with fluorescence detection without derivatization. We extracted ATs from the ground samples by mixing in NaCl and 80% (v/v) methanol. The sample was enriched and cleaned up by SPE technique using Bakerbond® C18 cartridges. The extract that we obtained was immediately analyzed using isocratic elution with a mobile phase consisting of acetonitrile, methanol and deionized water in a ratio of 64:18:18. Method validation was carried out by determining these ATs in a quality control material consisting of almond T02445QC and with the add-found test. The results provided satisfactory recovery within the range of 89.6–103.3%. Repeatability and intermediate precisions were assessed as RSD (%) which were found in the range of 1.1–11.3% and 1.5–12.0%, respectively. The limit of detection (S/N = 3) was 0.03, 0.02, 0.04, and 0.02 μg kg^{-1} for B1, B2, G1 and G2, respectively. Finally, the method was successfully applied to determine ATs in raw Egyptian food commodities, namely maize, popcorn, pistachio, corn, peanuts, chilli, wheat, green coffee and almond, and the corresponding RSD did not exceed 11%.

1. Introduction

Aflatoxins (ATs) are fungal secondary toxic metabolites that naturally contaminate food and feed (Fig. 1). They are produced by some *Aspergillus* moulds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Ali et al., 2005; Molina et al., 2009). *A. flavus* can produce only type B ATs; the other two fungi can produce type G ATs (Molina et al., 2009). These toxins are classified into B1, G1, B2 and G2 with a toxicity order of B1 > G1 > B2 > G2. Fungi can generally grow in wheat, rice, nuts, fruits and in living crops when stored for several days (Turner et al., 2009).

ATs can occur in both temperate and tropical regions around the world, depending on which fungal species are present. They can affect many food commodities including cereals, nuts, dried fruit, coffee, cocoa, spices, oil seeds, dried peas, beans, fruits, and, in particular, apples. The human food chain may contain ATs via meat or other animal products such as eggs, milk and cheese as a result of livestock eating contaminated feed. Not only are these ATs genotypically specific, but they could also be produced by one or more fungal species (Logrieco and Visconti, 2004), and in some cases, one species can form more than one AT (Frisvad, 1994).

AT compounds have a diverse range of both acute and chronic toxic effects (Chu, 1992; Betina, 1989). However, toxicology of ATs is a challenging and complex issue. Susceptibility to ATs varies across species and between persons depending largely on the fraction of the dose directed into the various possible pathways. Harmful "biological" exposure can result from the activity of the epoxide moiety which can react with proteins and DNA (Williams et al., 2004).

From a mycological perspective, there are qualitative and quantitative differences in the toxigenic abilities displayed by several strains within each aflatoxigenic species. About half of *A. flavus* strains can produce ATs of more than 106 μg kg^{-1} (Filazi and Sireli, 2013). The AT type B1 is the most well-known potent natural carcinogen, and it is usually the major product of *Aspergillus* strains (Kostarelou et al., 2014).

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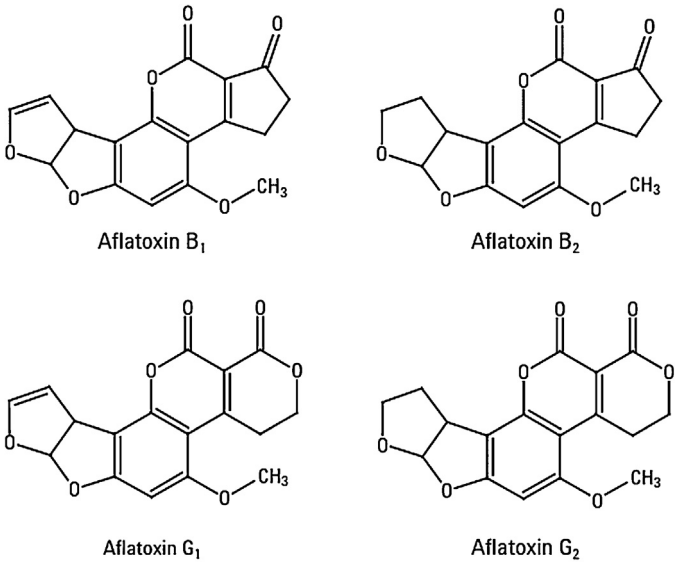

Fig. 1. Chemical structure of the studied aflatoxins B1, G1, B2 and G2.

Several techniques have been reported for the determination of Ats, such as electrochemical immune sensors (Tan et al., 2009), ion-mobility spectrometry (Sheibani et al., 2008), fluorescence spectroscopy (Nasir and Jolley, 2002), thin layer chromatography (TLC) (Stroka et al., 2000), gas chromatography (GC) (Goto et al., 1988), liquid chromatography (LC) (Sobolev and Dorner, 2002), liquid chromatography–mass spectrometry (Zhu et al., 2013), high performance liquid chromatography–fluorescence detection (Škrbić et al., 2014), and ultra-high-pressure liquid chromatography (UPLC) with UV detection (Fu et al., 2008).

High-performance liquid chromatography (HPLC) combined with fluorescence detection is the most-used technique, but in many cases, it requires further derivatization (Cavaliere et al., 2006). However, quantification by LC, TLC and HPLC is the most widely used method of research and routine analysis (Vosough et al., 2010). Recently, many analytical methods have been developed for the determination of ATs in food and feed, in particular, immunochemical and liquid chromatography coupled with mass spectrometry (LC–MS/MS) methods (Berthiller et al., 2014).

Although the HPLC technique can offer better sensitivity, high dynamic range, versatility and soft ionization conditions, it is usually coupled with UV absorption, fluorescence, mass spectrometry, and amperometric detection. In case of fluorescence detection, either with pre-column or post-column, a derivatization step is always necessary to improve fluorescence properties (Elizalde-Gonzalez et al., 1998).

Furthermore, HPLC with diode array detector (DAD) and second order iterative algorithm are useful tools for quantifying ATs after cleaning up the sample with SPE (Manetta et al., 2005). In spite of the excellent sensitivity of this technique, it often requires skilled operators, extensive sample pre-treatment and expensive equipment (Sapsford et al., 2006).

UPLC involves the use of columns packed with smaller particles, lower flow rate and an increased speed in gradient separation than normal HPLC. Using columns packed with porous particles of diameter sub 2 μm increases the number of resolved peaks per unit time, and allows superior resolution and sensitivity (Singh and Singh, 2010). The resolving power of UPLC is not compromised even at high elution speed, making it suitable for fast separations, and quantitative and qualitative analyses (Du et al., 2010).

In Egypt, the level of ATs in food commodities is a critical issue, especially when exporting agricultural products. Therefore, the development of accurate, rapid and reliable methods for determination of ATs is a challenge. In this work, we validated and applied SPE with UPLC for the fast determination of ATs in several agricultural products.

2. Experimental

2.1. Chemicals and equipments

Unless otherwise stated, all chemicals and reagents used in this work were of analytical reagent grade. AT chemicals of purity greater than 99% purity were supplied by Aldrich (Aldrich Chemical Company Inc., St Louis, MO). Standard solution (2 mg mL^{-1}) was prepared by dissolving an appropriate amount from each AT in LC grade methanol (Sigma-Aldrich, Lyon, France). Then it was stored at 4 °C in the dark. Working solutions were immediately prepared by diluting from the standard solution with 100% (v/v) methanol followed by sequential dilution with 50% (v/v) methanol and 1% (v/v) acetic acid (Sigma-Aldrich Corporation, St Louis, MO, USA).

Isopropanol (Riedel-de Haen AG, Seelze, Germany) and acetonitrile (Carlo Erba Reagenti, Val de Reuil, France) were filtered through a 0.2 μm cellulose acetate membrane (Sterlitech Corporation, Kent, WA, USA). A Milli-Q water purification system (Millipore Corporation, Saint-Quentin, France) was used to get deionized water. Micropipettes with volumes from 0.1 to 1000 μL were procured from Gilson medical electronics (Villiers le Bel, France). A Sartorius electronic balance (Sartorius Corporation, Edgewood, NY, USA) was used for accurate weighing purposes. A Waring blender of a commercial type (Waring Commercial Co., Stamford, CT, USA) was employed for grinding the solid samples.

2.2. UPLC procedure

Acquity ultra-performance UPLC™ system H-class with a fluorescence (FLR) detector (Waters Company, Milford, MA, USA) was used for determination of ATs. Chromatographic separation was carried out at 30 °C using BEH C18 column (2.1 mm × 100 mm) purchased from YMC (Kyoto, Japan) with a particle size of 1.7 μm. The excitation wavelength was fixed at 365 nm. The emission wavelength was 429 nm for B1and B2 and was 455 nm for G1and G2. Empower2 software (Waters, Milford, MA, USA) was used for system control and data processing.

Isocratic elution was carried out using a filtered and degassed mobile phase, consisting of water–methanol–acetonitrile (64:18:18), flowing at a flow rate of 0.4 mL min^{-1}. The column temperature and injection volume were adapted to 30 °C and 20 μL, respectively. Before injecting the sample, the injection needle was washed with 50% (v/v) methanol.

The weak and strong washings were performed using 1000 μL solution from water–methanol–acetonitrile (3:1:1) and 500 μL acetonitrile–isopropanol–water (5:1:1), respectively. A data rate-filter of 20 points s^{-1}, time constant of 0.3 s, and total analysis time of 6.0 min were employed.

2.3. Sample preparation

Nine food commodities, namely maize, popcorn, pistachio, corn, peanuts, chilli powder, wheat, green coffee, and almond, were received as raw samples from different regions in Egypt. An accurately weighed portion of 25 g of the dry sample was ground, then mixed with 5 g of NaCl and 100 mL of 80% (v/v) methanol. The mixture was ground in the blender for 2 min. Thereafter, it was filtered through a Whatman filter paper of 1.5 μm pore size and 11 cm diameter. An aliquot of 10 mL of the supernatant solution was vortexed with 40 mL deionized water in a falcon tube for 1 min and then shaken until homogeneity. Finally, a 10 mL aliquot from

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