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Determination of aflatoxins in food samples by automated on-line in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry

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ABSTRACT

A simple and sensitive automated method for determination of aflatoxins (B1, B2, G1, and G2) in nuts, cereals, dried fruits, and spices was developed consisting of in-tube solid-phase microextraction (SPME) coupled with liquid chromatography–mass spectrometry (LC–MS). Aflatoxins were separated within 8 min by high-performance liquid chromatography using a Zorbax Eclipse XDB-C8 column with methanol/acetonitrile (60/40, v/v): 5 mM ammonium formate (45:55) as the mobile phase. Electrospray ionization conditions in the positive ion mode were optimized for MS detection of aflatoxins. The pseudo-molecular ions $[M+H]^+$ were used to detect aflatoxins in selected ion monitoring (SIM) mode. The optimum in-tube SPME conditions were 25 draw/eject cycles of 40 μL of sample using a Supel-Q PLOT capillary column as an extraction device. The extracted aflatoxins were readily desorbed from the capillary by passage of the mobile phase, and no carryover was observed. Using the in-tube SPME LC–MS with SIM method, good linearity of the calibration curve ($r > 0.9994$) was obtained in the concentration range of 0.05–2.0 ng/mL using aflatoxin M1 as an internal standard, and the detection limits (S/N = 3) of aflatoxins were 2.1–2.8 pg/mL. The in-tube SPME method showed >23-fold higher sensitivity than the direct injection method (10 μL injection volume). The within-day and between-day precision (relative standard deviations) at the concentration of 1 ng/mL aflatoxin mixture were below 3.3% and 7.7% ($n = 5$), respectively. This method was applied successfully to analysis of food samples without interference peaks. The recoveries of aflatoxins spiked into nuts and cereals were >80%, and the relative standard deviations were <11.2%. Aflatoxins were detected at <10 ng/g in several commercial food samples.

1. Introduction

Aflatoxins, difuranocoumarin compounds (Fig. 1), are toxic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They were isolated and characterized after the death of more than 100,000 turkey poults (turkey X disease) was traced to the consumption of mold-contaminated peanut meal [1]. Thereafter, aflatoxins were found to contaminate a wide variety of agricultural products, including corn, rice, wheat, spices, and nuts.

Aflatoxins are associated with both toxicity and carcinogenicity in mammals such as humans and experimental animals including rats. The diseases caused by aflatoxin consumption are loosely called aflatoxicoses. The outbreak of aflatoxicosis in Kenya in 2004 was one of the most severe episodes of human aflatoxin poisoning, and over 100 of 317 patients died [2]. Acute aflatoxicosis results in death (LD_{50} for aflatoxin B1: 11.16 mg/kg in rats, p.o.), and chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions. Aflatoxin B1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains. The liver is the primary target organ and exposure to aflatoxins in the diet is considered an important risk factor for the development of primary hepatocellular carcinoma. The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 as a Group I human carcinogen [3]. Details of the occurrence and health effects of aflatoxins have been summarized in a number of reviews [1–7].

Thus, aflatoxin contamination is a worldwide problem with regard to food and feed safety, and many countries have instituted aflatoxin restrictions in foods. For example, the European Commission set the maximum level in foods to 2–5 ng/g and 4–20 ng/g for aflatoxin B1 and total aflatoxins, respectively [8]. In Japan, the maximum tolerated level for aflatoxin B1 must not be greater

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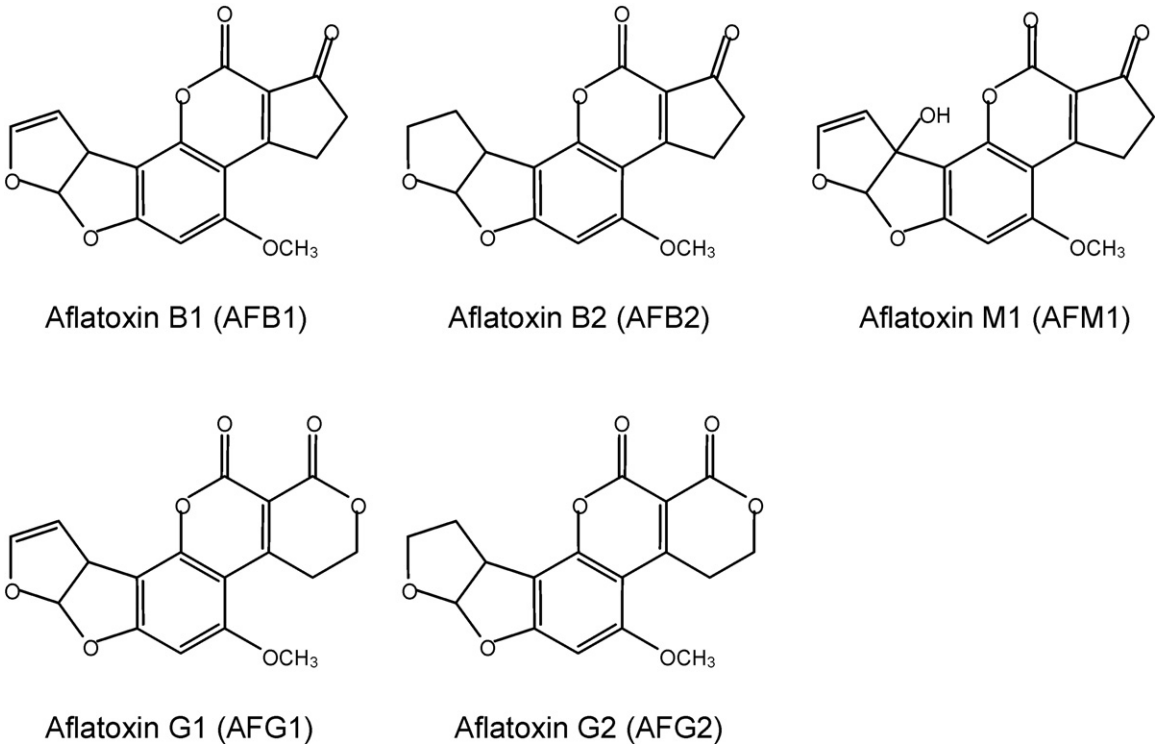

Fig. 1. Structures of aflatoxins used in this study.

than 10 ng/g in foods. Therefore, a sensitive, selective, and simple method to determine the presence and contents of aflatoxins in food samples is required to evaluate the risks associated with human consumption of various agricultural products.

Analyses of aflatoxins have been carried out using thin layer chromatography (TLC) [9], high-performance liquid chromatography (HPLC) [10–15], liquid chromatography–mass spectrometry (LC–MS) [16,17], LC–MS–MS [18–24], and immunological methods [25–27]. The details of determination of aflatoxins in food samples have also been summarized in recent reviews [28–32]. TLC provides an economical method of screening for aflatoxins, and has an important role in developing countries for surveillance purposes and in control of regulatory limits [33]. HPLC methods coupled with fluorescence detection are sensitive and the most widely used methods, but most of these techniques require pre- or post-column derivatization because of weak native fluorescence. Enzyme-linked immunosorbent assay [25] and immunochromatography [26,27] provide rapid screening for total aflatoxin, but they may not be sufficiently reliable as quantitative methods for individual aflatoxins. On the other hand, LC–MS or LC–MS–MS methods are specific and sensitive, and their use is becoming increasingly widespread. Several methods use stable isotope-labeled aflatoxins as internal standards (IS) to improve recovery and matrix interference [22,29]. However, most of the above methods usually require sample preparation steps, such as extraction, concentration, and isolation, to enhance sensitivity and selectivity. For example, liquid–liquid extraction [18,24] with methanol/water or chloroform, column chromatography with silica gel or Florisil [9,11,17], solid-phase extraction (SPE) [12,14] with different adsorbent materials such as immunoaffinity columns [10,13,15,19,21,22,25], and matrix solid-phase dispersion [16,20,23] have been used as cleanup procedures. However, most of these sample preparation techniques are both complicated and time-consuming. Complicated pretreatment methods may introduce errors, and the use of large volumes of organic solvent poses a health hazard to those performing the analyses and contributes to environmental pollution. Therefore, it is important to develop an efficient sample pretreatment method, and automation will reduce both labor and costs. A routine analysis method will also facilitate the processing of large numbers of samples.

In-tube solid-phase microextraction (SPME) [34] using an open tubular fused-silica capillary column with an inner surface coating as the SPME device is simple and can be easily coupled on-line with HPLC and LC–MS. In-tube SPME allows convenient automation of the extraction process, which not only reduces the analysis time, but also provides better accuracy, precision, and sensitivity than manual off-line techniques. We have already developed an in-tube SPME method for determination of various compounds in food samples by coupling with HPLC [35,36] and LC–MS [37,38]. The details of the in-tube SPME technique and its applications have been summarized in some reviews [39–42]. The present study was performed to develop an automated on-line in-tube SPME/LC–MS method for determination of aflatoxins in food samples.

2. Experimental

2.1. Materials

Aflatoxins (AFB1, AFB2, AFG1 and AFG2) were purchased from Sigma–Aldrich (Saint Quentin Fallaviers, France) and dissolved in acetonitrile to make a stock solution at a concentration of 1 μg/mL. Aflatoxin M1 (AFM1) was used as an internal standard, because it is the hydroxylated metabolite of AFB1 and is not present in agricultural samples. AFM1 (10 μg/mL) was purchased from Supelco Japan (Tokyo, Japan) and diluted in acetonitrile to make a stock solution at a concentration of 1 μg/mL. These standard solutions were stored in amber screw-cap bottles at −30 °C and diluted to the required concentrations with pure water prior to use. LC–MS grade methanol, acetonitrile, and water used as mobile phases were purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were of analytical grade.