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Journal of Chromatography A, 1135 (2006) 241–244
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Determination of aflatoxins in Chinese medicinal herbs by high-performance liquid chromatography using immunoaffinity column cleanup Improvement of recovery

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Received 21 July 2006; received in revised form 9 October 2006; accepted 11 October 2006

Abstract

Although analytical methods are available for the determination of aflatoxins in medicinal herbs, none of them can be applied satisfactorily to all sample matrices. The difficulty arises from the complex chemical composition of the herbs. Recovery is generally low by using immunoaffinity column cleanup due to the acidity of the water extractive leading to a weakened binding affinity. As a solvent for dilution and neutralization, phosphate buffer saline is useful for certain herbs but not for others that have high acidity. The problem can be solved by using 0.1 M phosphate buffer, which has a higher buffering capacity and eliminates sodium chloride. The modified method was validated by the analysis of a certified reference material and shown to be useful for the determination of aflatoxins in herbal samples of high acidity.

Keywords: Aflatoxins; Herbal medicine; Immunoaffinity column

1. Introduction

Aflatoxins are a family of structurally related mycotoxins produced by *Aspergillus* fungi such as *A. flavus*, *A. parasiticus*, *A. nomius*, and *A. niger*. The most important members are aflatoxins B_1, B_2, G_1 and G_2, as well as two metabolic products, M_1 and M_2. They are potent carcinogens to humans. Earlier studies of the International Agency for Research on Cancer (IARC) have defined aflatoxins as Group 1 carcinogens to humans [1]. Recent studies reaffirmed the carcinogenic effect of these compounds [2].

Aflatoxins are most commonly associated with peanuts [3], maize [4], cottonseed [5], etc. They have also been found in meats and milk obtained from animals fed with contaminated feeds [6,7] and medicinal herbs [8,9]. Aflatoxin contamination in medicinal herbs has become a worldwide concern as the consumption of herbal products significantly increases in recent years.

The determination of aflatoxins using HPLC methods has been studied extensively in various food matrices such as peanuts, maize, milk and cheese [10–12]. More recently, methods were also developed to determine aflatoxins in medicinal herbs [9,13–15]. We have analyzed a large number of Chinese medicinal herbs for aflatoxin contamination and our results showed that the recovery of aflatoxins was low in some of the herbal samples when immunoaffinity column was used as a cleanup step. The present study aims to identify the problem and provide a solution to improve the recovery for the determination of aflatoxins B_1, B_2, G_1 and G_2 in Chinese herbal medicines.

2. Experimental

2.1. Chemicals

Aflatoxin standards were purchase from Sigma–Aldrich (St. Louis, MO, USA). Immunoaffinity column (AflaTest P) was supplied by VICAM (Watertown, MA, USA). Other chemicals and solvents were of analytical or HPLC grade. Deionized water was purified by the Milli-Q-Plus ultra-pure water system (Millipore, Billerica, MA, USA).

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doi:10.1016/j.chroma.2006.10.025

2.2. Instrumentation

Aflatoxins were analyzed using an HPLC system consisting of degasser, autosampler, diode-array detector, fluorescence detector (Agilent-1100 Series, Palo Alto, CA, USA) equipped with a post-column derivatization chamber (Pickering Laboratories, Mountain View, CA, USA). An Alltima C_{18} column (5 μm, 250 mm × 4.6 mm) was used for the separation and the signals were recorded by ChemStations computer software. The pH value was measured by a pH meter (Schott, Mainz, Germany). Centrifuge was carried out by using Eppendorf Centrifuge 5810R (Hambury, Germany).

2.3. Sample preparation and immunoaffinity column cleanup

The method was modified from AOAC Official Method 991.31 [16]. Briefly, the powdered sample (5 g) was mixed with sodium chloride (1 g), dissolved in 25 mL of 70% (v/v) methanol in a sealed centrifuge tube, and sonicated for 30 min, followed by centrifugation at 18,000 × *g* for 10 min. Five millilitre of the supernatant was diluted with 40 mL of either deionized water, phosphate buffer saline (PBS) (pH 7.4), or 0.1 M phosphate buffer (pH 8.0). The solution was centrifuged at 18,000 × *g* for 10 min. The supernatant was filtered through a 0.45 μm regenerated cellulose filter (Sartorius, Goettingen, Germany). The filtrate (40 mL) was then passed through an immunoaffinity column (AflaTest P) at a flow rate of about 3 mL/min, washed with water (10 mL) at the same flow rate and flushed with air (10 mL). Final elution was accomplished by adding methanol (1.5 mL) onto the column and flushed with air. The eluate was made up to 2 mL with water.

2.4. HPLC separation and post-column derivatization

HPLC separation was achieved by an isocratic elution with a mobile phase consisting of water, methanol and acetonitrile (3:1:1, v/v) at 1 mL/min. Post-column derivatization reagent was prepared by dissolving 100 mg of iodine in 2 mL of methanol and diluted to 1 L with deionized water. The post-column reagent was delivered at a flow rate of 0.3 mL/min and the reaction coil was maintained at 70 °C. The eluate was monitored by a fluorescence detector set at excitation wavelength of 360 nm and emission wavelength of 450 nm (Fig. 1).

2.5. Quantitation and method validation

Calibration curves were constructed by plotting the peak area versus the concentration (0–10 μg/L) of aflatoxins dissolved in 70% methanol. The calibration curves of the four aflatoxins exhibited linearity with square of the correlation coefficients greater than 0.995. Relative standard derivation (RSD) of five replicate analyses of medical herbs spiked with aflatoxins was smaller that 5% which indicated the high repeatability of the method. Detection and quantitation limits of the aflatoxins were 0.06 μg/kg and 0.3 μg/kg, respectively. The content of aflatoxins in the test sample (*C*) was calculated by the following equation:

$$C\ (\mu g/kg) = A \times V \times D \times \frac{K}{W}$$

where *A* is the concentration of aflatoxins determined from the calibration curve (μg/L); *V* is final make-up volume of eluate (2 mL); *D* is a dilution factor (if necessary); *K* is a volume factor given by (45/40) × (25/5); *W* is the weight of sample used for the analysis (5 g).

The recovery was given by the ratio of aflatoxins calculated by above equation to the amount spiked to the medical herbs as the as aflatoxins were not detected in the matrix background. A certified reference material, Sunflower Seed (T0438, FAPAS, Sand Hutton, York, UK), was analyzed. The estimated contents of aflatoxins were compared with those specified by the supplier.

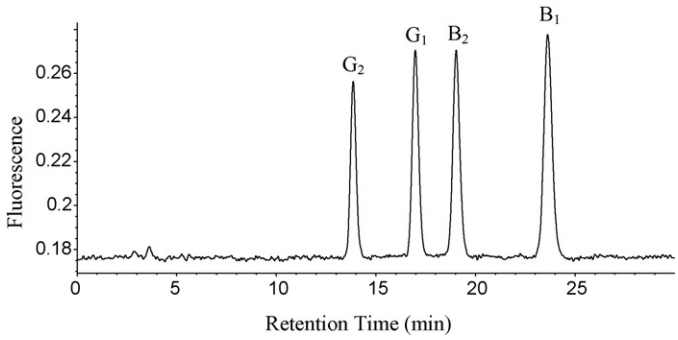

Fig. 1. A typical chromatogram for determination of aflatoxins B_1, B_2, G_1, and G_2 in medical herbs spike with the aflatoxins.

3. Results and discussion

Although there are published methods for the determination of aflatoxins in medicinal herbs [9,13–15], none of them can be applied to all sample matrices. The difficulty arises from the complex chemical ingredients of the herbs. As shown in Table 1, when the samples of medicinal herbs were extracted with 70% methanol and diluted with water, the pH values of the solutions were low. It is well known that the binding affinity of an immunoaffinity column can be affected by factors such as pH, salt concentration, and competitive ligands. Indeed, the recovery of aflatoxins, especially aflatoxin G_2, was decreased when the pH value of the buffer was below 4 or above 8 (Table 2).

Table 1
pH values of testing solutions of medicinal herbs using different dilution solvents

Dilution solvent	Medicinal herbs			
	Bulbus Fritillariae Thunbergii	Fructus Schisandrae Chinensis	Fructus Crataegi	Fructus Mume
Deionized water	4.1	3.0	3.0	2.8
PBS (pH 7.4)	6.8	3.5	3.6	3.1
0.1 M PB (pH 8.0)	7.9	6.7	7.1	6.2

Powdered samples of the medicinal herbs were extracted with 70% (v/v) methanol. After centrifugation, the supernatant was diluted with respective solvent. The pH value of the diluted solution was measured.