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Note

Determination of aflatoxins by capillary column gas chromatography

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Aflatoxins (AFs) are a group of toxins produced by some *Aspergillus flavus* Link moulds[1]. These toxins are potent carcinogens in experimental animals and often contaminate various agricultural commodities such as maize and peanuts[2]. A variety of techniques have been used for the separation and identification of the four major naturally occurring AFs, namely aflatoxin B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2) (Fig. 1). The most widely used method has been thin-layer chromatography (TLC). High-performance liquid chromatography (HPLC) has been used since the late 1970s, but gas chromatography (GC), one of the most popular methods of analysis of various mycotoxins, has never been successfully applied to the analysis of mixtures of four aflatoxins. In 1981, Friedli[3] reported that AFB_1 could be analyzed without chemical derivatization by GC using a mass spectrometer as the detector (GC–MS). Subsequently, Trucksess *et al.*[4] and Rosen *et al.*[5] reported that AFB_1 or mixture of AFB_1 and AFB_2 in contaminated peanuts could be determined by GC–MS. We have now succeeded in determining four major AFs (B_1, B_2, G_1 and G_2) using GC with flame ionization detection (FID) with a capillary on-column injector and a fused-silica capillary column.

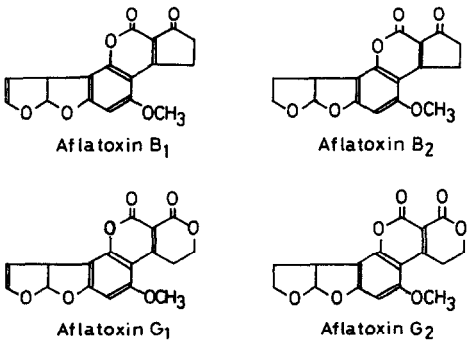

Fig. 1. Structures of aflatoxins.

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EXPERIMENTAL

Gas chromatography

A Shimadzu GC-15A gas chromatograph (Shimadzu, Kyoto, Japan) was used. This system consists of a GC-15A gas chromatograph with an OCI-9A capillary on-column injector, a flame ionization detector and a Chromatopac C-R4A reporting integrator. Fused-silica capillary columns (0.25 mm I.D.) containing a chemically bonded liquid stationary phase (0.25 μm) were purchased from J&W (CA, U.S.A.). The stationary phases were methylsilicone (DB-1) and 5% phenylmethylsilicone (DB-5) and the lengths of the columns were 3, 5, 10, 15 and 25 m. Helium was used as both the carrier gas and make-up gas.

Mass spectrometry

A Shimadzu GCMS QP1000 mass spectrometer was used for the confirmation of each aflatoxin. The ionization voltage was 70 eV, the ionization current was 60 μA and the ion source temperature was 250°C.

Chemicals and samples

Aflatoxin B_1, B_2, G_1 and G_2 were purchased from Makor Chemicals (Jerusalem, Israel) and were dissolved in benzene–acetonitrile (98:2), which was also used to prepare dilutions. Other chemicals and reagents were of analytical-reagent grade and were used without further purification. The AF-producing mould (unidentified) was cultivated in modified Czapek–Dox liquid medium at 27°C for 7 days. A volume of 1-l of this liquid medium contains 30 g of glucose, 3 g of sodium nitrate, 1 g of dipotassium phosphate, 0.5 g of magnesium sulphate, 0.5 g of potassium chloride, 0.01 g of iron(II) sulphate, 0.01 g of zinc sulphate and 0.005 g of copper(II) sulphate. After cultivation, 5 ml of medium were removed and to it were added 250 mg of sodium chloride and 5 ml of methanol. Subsequently, AFs were extracted twice with 3-ml volumes of chloroform. The chloroform extracts were combined and evaporated to dryness under a stream of nitrogen. The residue was dissolved in benzene–acetonitrile (98:2) and used for GC analysis.

RESULTS AND DISCUSSION

Analytical conditions

When the initial temperature of the column and injector was higher than 60°C, all four AF peaks became broad and the sensitivity decreased. Also, either the final temperature was low or the rate of heating was slow, causing an increase in the retention time and a decrease in sensitivity. Therefore, the initial and final temperatures were set at 50 and 300°C and the rate of heating was set at 15 or 20°C/min.

Two types of stationary phases were tested. The methylsilicone column (DB-1, 10 m) did not separate AFG_1 and AFG_2 and barely separated AFB_1 and AFB_2. As a result, the shape of the peaks was distorted. In contrast, a 5% phenylmethylsilicone column (DB-5, 10 m) distinctly separated AFB_1 and AFB_2 and also achieved a 50% separation between AFG_1 and AFG_2 (Fig. 2A). A longer column was used to improve the overall separation. Although the four AFs were completely separated on a 25-m DB-5 column, the sensitivity much lower for AFG_1 and AFG_2 than for AFB_1 and AFB_2.

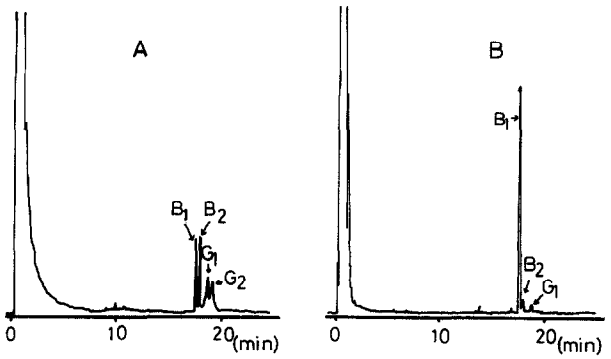

Fig. 2. Gas chromatograms of aflatoxins. (A) Aflatoxin standards: AFB_1, 5 ng; AFB_2, 5 ng; AFG_1, 10 ng; AFG_2, 10 ng. (B) Extract from culture medium.

Determination of the relationship between column length and the sensitivity for AFG_1 and AFG_2

Four columns of different length were used. The relationship between column length and the ratio of the peak areas between the AFB group and the AFG group was calculated by the following method. Each injection contained 25 ng of AFB_1 and AFB_2 and 50 ng of AFG_1 and AFG_2:

$$\text{Ratio of peak area} = \frac{\text{peak area of } AFG_1 + \text{peak area of } AFG_2}{\text{peak area of } AFB_1 + \text{peak area of } AFB_2}$$

As shown in Fig. 3, the ratio of the area attributed to the AFG group decreased depending on the length of column. After considering both sensitivity and separation, a column length of 15 m was selected for practical applications of the method.

Determination of aflatoxins

As shown in Fig. 4, the limit of quantification (signal-to-noise ratio = 5) of each AF was 1 ng; the linear range of this quantitative analysis was from 1 to 50 ng.

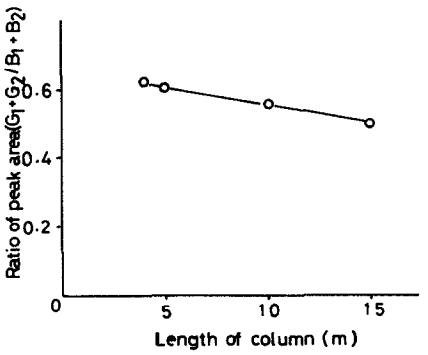

Fig. 3. Ratio of peak areas of the AFB group to the AFG group.

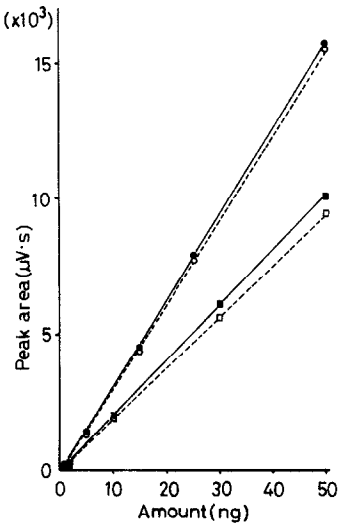

Fig. 4. Calibration graphs for aflatoxins using gas chromatography. ● = AFB_1; ○ = AFB_2; ■ = AFG_1; □ = AGF_2.

The analysis was highly reproducible (Table I) at 2 ng for AFB_1 and AFB_2 and at 4 ng for AFG_1 and AFG_2.

Determination of aflatoxins in culture medium

Extracts of aflatoxigenic mould cultivated in liquid medium were analysed by the method described above (Fig. 2B). AFB_1, AFB_2 and AFG_1 were quantitatively detected, but AFG_2 was not, which agreed with the results obtained using either TLC or HPLC for quantitation. The molecular ions of AFB_1 (*m/z* 312), AFB_2 (*m/z* 314) and AFG_1 (*m/z* 328) were detected at the corresponding retention times in the samples analysed by GC–MS.

Clearly all four major AFs (B_1, B_2, G_1 and G_2), and not only AFB_1 and AFB_2[5], can be determined by GC with FID. The sensitivity of the method is not as high as that of other methods[6,7]. A possible reason for the low sensitivity is the presence of oxygen in the molecule[8]. However, GC does have the advantage of immediate compound identification by using MS detection.

TABLE I

REPRODUCIBILITY OF AFLATOXIN DETERMINATION

Results (n = 7) obtained using a 15-m column with temperature programming from 50 to 300°C at 15°C/min.

	AFB_1 (2 ng)	*AFB_2 (2 ng)*	*AFG_1 (4 ng)*	*AFG_2 (4 ng)*
Average result (ng)	1.98	1.94	3.96	3.93
S.D. (ng)	0.0637	0.0832	0.237	0.149

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REFERENCES

1 R. Allcroft and R. B. A. Carnaghan, *Vet. Res.*, 74 (1962) 863.
2 J. C. Young and R. G. Fuicher, *Cereal Foods World*, 29 (1984) 725.
3 F. Friedl, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 495.
4 M. W. Trucksess, W. C. Brumley and S. Nesheim, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 973.
5 R. T. Rosen, J. D. Rosen and V. P. DiProssino, *J. Agric. Food Chem.*, 32 (1984) 276.
6 K. Y. Lee, C. F. Poole and A. Zlatkis, *Anal. Chem.*, 52 (1980) 837.
7 M. Manabe, T. Goto and S. Matsuura, *Agric. Biol. Chem.*, 42 (1978) 2003.
8 J. C. Sternberg, W. S. Gallaway and D. T. L. Jones, *Gas Chromatography*, Academic Press, New York 1962.