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Note

Aflatoxin detection by high-speed liquid chromatography and mass spectrometry

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Of the various chemical carcinogens known today, aflatoxins, the metabolites of *Aspergillus flavus* Link, are among the most potent inducers of tumours. These toxins and other metabolites produced by the fungi pose a potential public health hazard as contaminants of food and feed[1]. Assessment of this hazard requires methodology for detecting fungal toxins. Pons and Goldblatt[2] have made an exhaustive review of physicochemical assay methods. Most of the methods used for the analysis of aflatoxins employ column chromatography or thin-layer chromatography for separation and densitometry, UV or fluorescence spectroscopy for quantitation. However, it has been demonstrated that photodegradation products of aflatoxins also exhibit a high degree of fluorescence[3]. Also, the total sample size that can be handled is low and the amount of time involved for preparative work is excessive for the existing methods. In addition, the necessity for positive confirmation of the isolates still exists. Usual confirmatory techniques involve visual comparison of the relative R_F values with that of the authentic standards and biological assay for activity.

In the method described in this report, aflatoxins were extracted using organic solvents and separated by high-speed liquid chromatography (HSLC). Mass spectrometry (MS) was employed for confirmation. A preliminary account of this work has been reported[4].

MATERIALS AND METHODS

A Varian Series 4000 liquid chromatograph was used. The column employed was a 1 m × 1.8 mm stainless-steel tube packed with silica gel (Sil-X, Nester-Faust) and maintained at room temperature. The eluents used were isopropyl ether–tetrahydrofuran (88:12) or diethyl ether–cyclohexane (75:25). The detector was a UV photometer monitoring absorption at 254 nm. The column pressure was adjusted (250 p.s.i.g.) so that a flow-rate of 24 ml/h was maintained. After obtaining a steady baseline, the sample was injected on to the column and the change in absorbance recorded.

A Finnigan 1015 mass spectrometer was employed for confirmation of the peaks eluted from the liquid chromatograph. Samples were concentrated in a glass capillary and introducted into the ionization chamber via the solid probe. The ionizer was operated at 70 eV and spectra were recorded at a probe temperature of 180–200°.

Samples were prepared for analysis[5,6] by extracting 25 g of coarsely ground *Aspergillus flavus* infested grain samples for 3 min in a blender with 200 ml of 15% aqueous acetonitrile. To 100 ml of the extract was added 50 ml of water and 20 ml of 20% lead acetate solution and the total volume was adjusted to 200 ml with water. This was mixed thoroughly with 4–5 g of Celite® and filtered through Whatman No. 4 paper. One hundred-sixty milliliters of the filtrate were shaken with 50 ml of methylene dichloride and the lower phase was dried by filtering twice with anhydrous sodium sulfate. The extract was concentrated *in vacuo* to a small volume and was saved until analyzed.

Samples from the crude extract as well as reference standards were injected onto the HSLC and the eluents representing the peaks were collected for MS analysis.

RESULTS AND DISCUSSION

Analysis of the crude extracts by HSLC using the ether solvent system revealed the presence of two peaks (Pk 1 and Pk 2) as shown in Fig. 1. The retention times of

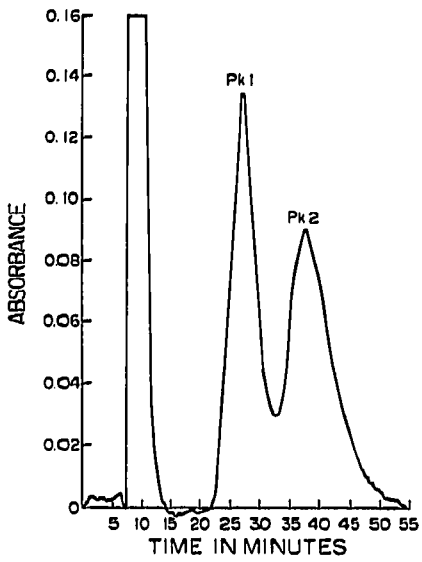

Fig. 1. Chromatogram of aflatoxin B_1 (Pk 1) and G_1 (Pk 2). Column, 1 m × 1.8 mm stainless steel; packing, silica gel (Sil-X); solvents, isopropyl ether–tetrahydrofuran (88:12); flow-rate, 24 ml/h; inlet pressure, 250 p.s.i.g.

Pk 1 and Pk 2 corresponded to those obtained with authentic aflatoxins B_1 and G_1, respectively. As shown in Fig. 2 and 3, the mass spectra of the peaks corresponding to Pk 1 and Pk 2 were found to confirm the presence of both aflatoxins B_1 and G_1. The high-intensity ions at low mass numbers are probably due to solvent and sample contaminants. However, the ions at high mass numbers are those expected for the aflatoxins and the intensity of the molecular ions aids in the confirmatory identification by mass spectrometry[7].

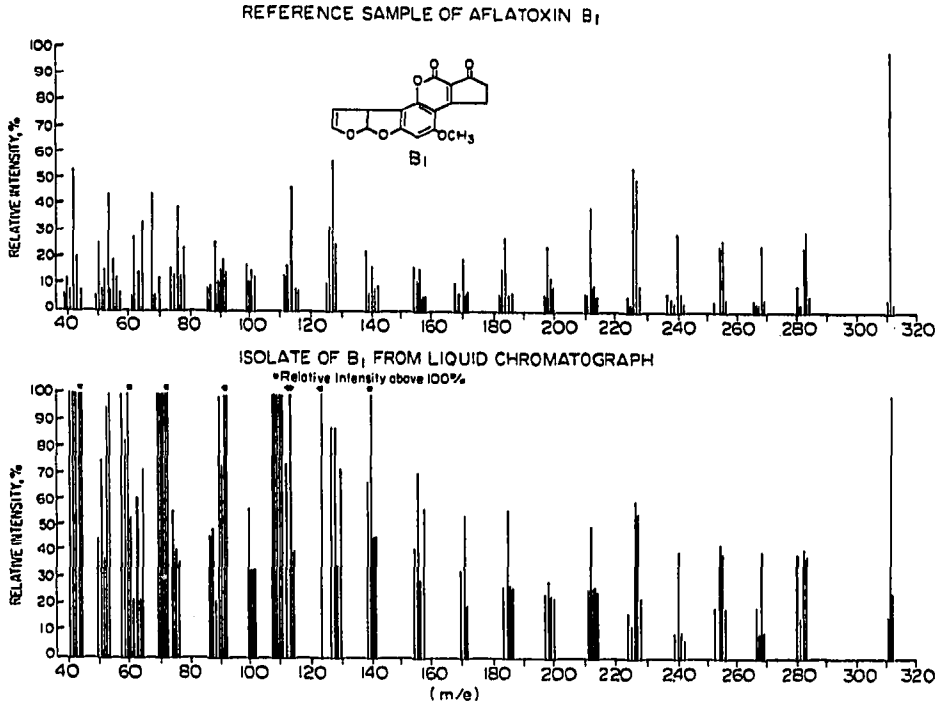

Fig. 2. Mass spectra of authentic aflatoxin B_1 (upper) and aflatoxin B_1 isolated from the liquid chromatograph (lower).

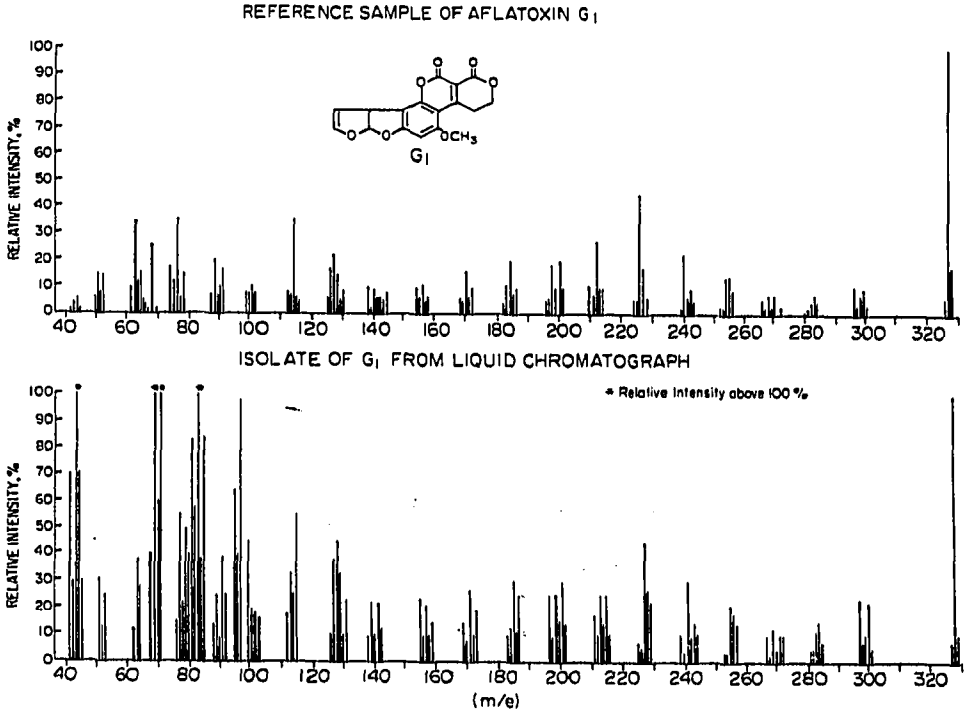

Fig. 3. Mass spectra of authentic aflatoxin G_1 (upper) and aflatoxin G_1 isolated from the liquid chromatograph (lower).

Further studies showed that the photodegradation products of aflatoxins had retention times similar to that of aflatoxin G_1 (Fig. 4). However, the presence of photodegradation products in Pk 2 could be detected by MS since these compounds show a characteristic ion at *m/e* 149 (ref. 3).

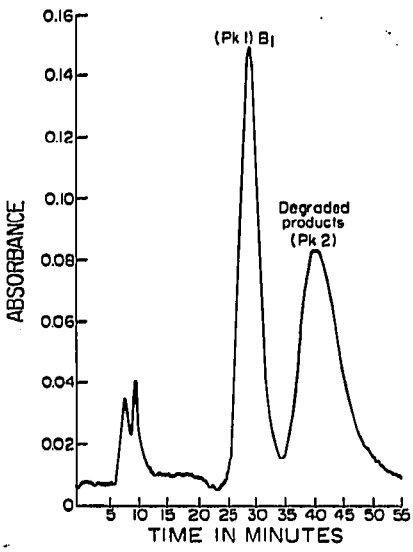

Fig. 4. Chromatogram of aflatoxin B_1 (Pk 1) and photochemical degradation products (Pk 2). Column, 1 m × 1.8 mm stainless steel; packing, silica gel (Sil-X); solvents, isopropyl ether–tetrahydrofuran (88:12); flow-rate, 24 ml/h; inlet pressure, 250 p.s.i.g.

The method described above is specific and sensitive. As little as 1 μg of aflatoxin B_1 or G_1 injected on to the column could be collected and analyzed by MS. The limiting factor in the sensitivity of the liquid chromatograph is the detector employed which operates at 254 nm. A detector operating at the absorption maxima of the aflatoxins (265 or 362 nm) may be expected to enhance the sensitivity. Alternatively, the use of a fluorescence detector, such as that recently described by Cassidy and Frei[8] would also increase the sensitivity of the liquid chromatograph. However, the mass spectrometer is adequately sensitive for confirmatory purposes because of the intensity of the molecular ions of the aflatoxins.

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