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Note

Aflatoxin separation by high-pressure liquid chromatography

R. C. GARNER

Department of Experimental Pathology and Cancer Research, University of Leeds, 171 Woodhouse Lane, Leeds 2, Yorkshire (Great Britain)

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The aflatoxins, mould metabolites of *Aspergillus flavus*, are found as human food contaminants in many areas of the world[1]. One of these metabolites, aflatoxin B_1, is the most potent liver carcinogen known for the rat[2] and has been associated with the high human liver cancer incidence in certain parts of Africa[3]. A human health hazard is posed by these compounds, not only in those countries where these mycotoxins are produced but also in countries of the Western world which might import aflatoxin-contaminated food.

It is important therefore to devise rapid and sensitive ways to assay human foods for this mycotoxin contamination. Methods presently used rely extensively on thin-layer chromatography (TLC) of solvent extracts from foods and comparison of R_F values with standard reference compounds[4]. Such techniques are only semi-quantitative, there being considerable variation in results from laboratory to laboratory[5]. This report sets out a method for complete separation of the four naturally occurring aflatoxins using a commercial high-pressure liquid chromatograph which is quantitative and which is almost as sensitive as TLC methods.

MATERIALS AND METHODS

A commercial high-pressure liquid chromatograph was used (Siemens Model S100, Karlsruhe, G.F.R.) coupled to a Zeiss PM2 variable-wavelength spectrophotometer set at 362 nm. Ten-microlitre samples were applied through a high-pressure syringe injection system onto a 25-cm × 3-mm-I.D. stainless-steel column packed with silica gel (Merckogel Si 150, 5–10 μm particle size from Merck, Darmstadt, G.F.R. or Partisil-5, 6-μm mean particle size, Reeve-Angel, London, Great Britain). The aflatoxin samples were eluted with 0.3% v/v methanol in water-saturated dichloromethane (hand shaken for 2 min) at a pressure of 3750 lbs./sq. in. and a flow-rate of 2.6 ml/min.

Aflatoxins B_1, B_2, G_1 and G_2 (Makor Chemicals, Jerusalem, Israel) were dissolved in dichloromethane and their concentration determined from the optical density at 360 nm using published extinction coefficients. Aflatoxin B_{2a} was prepared using the method of Büchi *et al.*[6]. A crude [^{14}C]aflatoxin preparation in dichloromethane was obtained by dichloromethane extraction of an *Aspergillus parasiticus* incubate grown in sodium [^{14}C]acetate medium as described previously[7].

RESULTS AND DISCUSSION

In general, high-pressure liquid chromatographic (HPLC) separations using silica gel require slightly less polar solvents than do separations by TLC. With chloroform as an eluent and Merckogel Si 150 as packing material the four aflatoxins eluted from the column in the void volume; on the other hand, dichloromethane failed to elute any of the aflatoxins from the column. If water-saturated dichloromethane was used[8] as an eluting solvent, separation of the four aflatoxins was obtained but with extremely long retention times (30 min or more). These times could be reduced by adding methanol to the eluting solvent. Addition of 0.3% v/v methanol to the water-saturated dichloromethane gave good separations with reasonable retention times. Of the two silica gel packing materials used, Partisil-5 gave the better resolution between aflatoxins B_2 and G_1 and was therefore used for further study.

Fig. 1 shows the separation of the four naturally occurring aflatoxins. Good resolution of the four compounds was obtained with no overlap of peaks. This is in contrast to other reports of HPLC of the aflatoxins[9,10]. As little as 10 ng of aflatoxin B_1 applied in a sample volume of 10 μl could be detected with this instrument, this being of comparable sensitivity to fluorescence methods of detecting aflatoxins by TLC. Application of a crude dichloromethane extract of an *Aspergillus parasiticus* mould incubation to the column shows three of the four aflatoxins have been produced and that the major component is aflatoxin B_1, (Fig. 2). Aflation B_{2a}, a water addition product of aflatoxin B_1, was not eluted with the methanol–dichloromethane solvent system used.

Previous reports[9,10] have shown a limited separation of the four naturally occurring aflatoxins by HPLC and the authors have stated that the method may only have a limited use compared to TLC procedures. The data reported here show that resolution can be at least as good as, if not better, than by TLC procedures but has the distinct advantage that amounts of compound can be calculated on the basis of peak area. Retention times are extremely reproducible and each run is over within 10 min. The method has the further advantage that up to 200 μl of solution containing

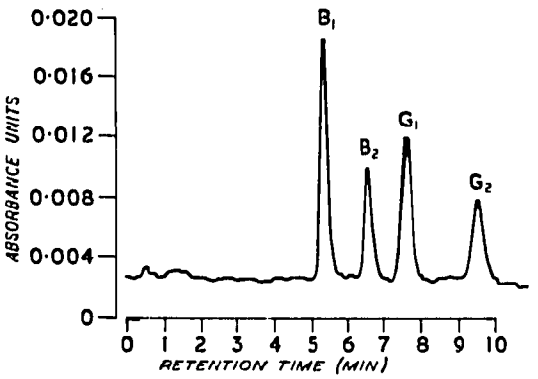

Fig. 1. Chromatogram of the four naturally occurring aflatoxins. Column, 25 cm × 3 mm stainless steel; packing, silica gel (Partisil-5); solvent, 0.3% v/v methanol in water-saturated dichloromethane; flow-rate, 2.6 ml/min; pressure 3750 p.s.i. 10 ng of each aflatoxin applied in a total sample volume of 10 μl.

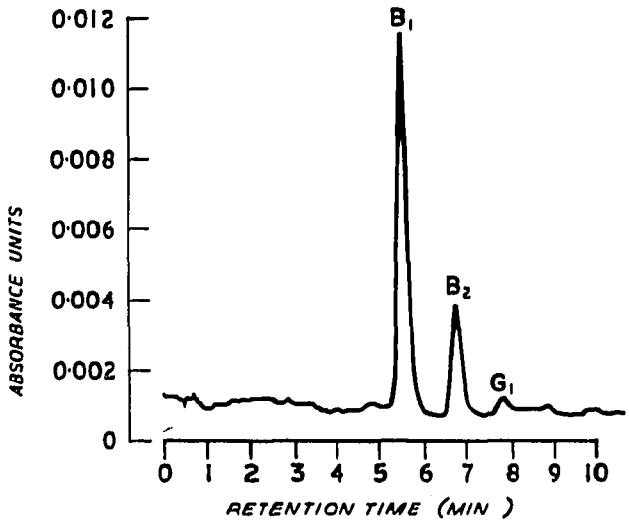

Fig. 2. Chromatogram of a mould extract of *Aspergillus parasiticus*. Details, as in Fig. 1.

the sample can be applied when only low concentrations are available and that the method can be made preparative. This particular instrument has an automatic injection device coupled with a fraction collector which can be set to collect fractions on the basis of retention time or peak height. If one had a sample with very low concentrations of aflatoxin, then repeated samples could be injected and fractions collected at the known retention times of standard aflatoxin solutions.

The method should therefore be valuable for the quantitative assay of aflatoxins in foods and also for the study of aflatoxin metabolism, an area in which our laboratory is concerned[11].

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