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A note on the estimation of aflatoxins by thin-layer chromatography

All the chemical assay methods for aflatoxins in foodstuffs employ thin-layer chromatography (TLC)[1]; however, extracts from peanut products, maize, milk, etc., contain oil and pigments that frequently interfere with accurate estimates of the aflatoxin content. Column chromatographic clean-up procedures are usually employed to remove these interfering substances. An alternative TLC clean-up procedure is now reported that is simple and effective for the removal of these interfering substances.

Procedure and results

A primary extract of a particular foodstuff is made using any one of the appropriate published procedures. The weight of the total extract is determined by transferring it to a preweighed vial. A 10-μl volume of this oily extract is applied directly to a warm TLC plate (6 × 14 cm) coated with Camag D-5 silica gel. The oil is allowed to soak into the silica gel to form a small spot (5 mm in diameter). An aflatoxin standard is also spotted on the plate (see Fig. 1 for position). The TLC plate is first developed along the short axis using pure dry ether. When the solvent front has travelled to the edge of the plate (6 cm) the plate is dried in an oven (80° for 5 min). The plate is then developed along its long axis using the following solvent system: chloroform–trichloroethylene–*tert.*-butanol–formic acid (85:10:4:1).

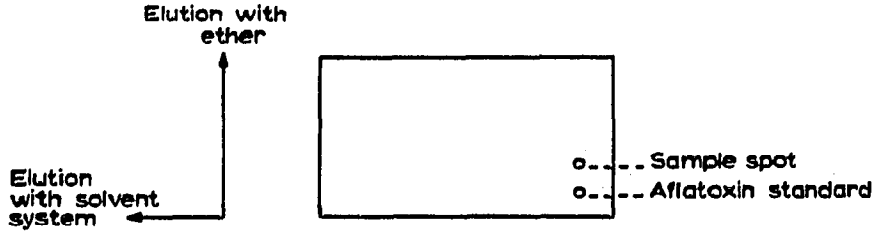

Fig. 1. The spotting position on a TLC plate and the direction of elution.

If the region of the aflatoxin in the TLC plate is not completely clear of interfering background, the TLC plate is again placed in ether and developed along its short axis. An accurate calculation of the aflatoxin content can now be made by using the weight of the 10-μl sample and the total sample weight after densitometric comparison with the standard.

Discussion

Standard solutions of aflatoxin B_1, B_2, G_1, G_2 and M_1, respectively, spotted on the plate at the outset of the analysis gave spots with an intensity equal to the same standard solutions spotted after the heat-treatment in the oven. Further, the initial development with ether did not cause any of the above aflatoxins to migrate more than 2 mm, irrespective of whether the aflatoxin was spotted directly on the plate or on the top of an oily extract. Two-dimensional chromatography for the separation of the aflatoxins has been reported[2] but it was not used as a clean-up procedure for extracts containing oil and interfering pigments.

TABLE I

SENSITIVITY OF TWO-DIMENSIONAL CHROMATOGRAPHY FOR THE ESTIMATION OF AFLATOXIN

Food sample	*Extraction procedure (ref.)*	*Total weight of oil (mg)*	*Lower limit[a] of detection (μg/kg)*
Maize flour	3	280–350	3–4
Peanut butter	4	460–580	4–6
Milk powder	5	600–750	6–8
Milk powder	6	460–920	5–9

[a] This calculation is based on the assumption that 0.005 μg of aflatoxin is the smallest amount that can be accurately estimated on TLC. Appropriate refinements for particular aflatoxins can readily be applied.

The advantage of the above method is that lengthy column chromatographic clean-up procedures are omitted. A disadvantage is that each sample requires a separate TLC plate. The sensitivity of the method depends on the weight of total sample. This varies with the nature of the food sample and the particular extraction procedure as seen in Table I, which lists typical results obtained with three different foodstuffs.

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