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Note

Determination of aflatoxin M_1 in milk at the parts per trillion* level

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In view of the carcinogenic properties of aflatoxin M_1, its presence in milk should be restricted as far as possible[1]. Bovine fodders are usually contaminated with aflatoxin B_1 (ref. 2), which results in a concentration of 1–2% of aflatoxin M_1 in the milk[3,4]. It will be possible to accept tolerances of aflatoxin M_1 in milk only when tolerances for aflatoxin B_1 in fodder have been established. Although in the EEC the admittance of 20 ng/kg of aflatoxin B_1 in fodder has been discussed[5], which will result in about 0.1 μg/kg of aflatoxin M_1 in milk, toxicologists and hygienists will insist on decreasing this level in the milk.

Most of the methods published so far have a detection limit of the order of 0.04–0.1 μg/kg of aflatoxin M_1 (refs. 6–8). The final determination is carried out by thin-layer chromatography (TLC) by means of a densitometric measurement or visual estimation. Under our conditions, the visual detection limit for aflatoxin M_1 standard on the thin-layer plate is 0.2 ng. In order to obtain a very low detection limit, the extraction of a large amount of milk is necessary[6]. In our method, interfering impurities should be removed by a suitable clean-up procedure[9,10]. We use the property of aflatoxin M_1 not to be eluted from a silica gel column by dry diethyl ether.

PRELIMINARY PROCEDURE

A 60-ml volume of milk is heated in a glass-stoppered centrifuge tube for 15 min in a boiling water-bath. After cooling and diluting the milk with 50 ml of water, 10 ml of 10% (v/v) cadmium sulphate solution are added and mixed. After centrifuging, 100 ml of the clear solution (equivalent to about 50 ml of milk) are passed through a folded filter-paper into a second centrifuge tube. The solution is extracted twice with 50-ml volumes of chloroform and centrifuged again. The lower chloroform layer is passed through a filter containing anhydrous sodium sulphate into a round-bottomed flask and evaporated to dryness in a rotating vacuum evaporator. The residue is dissolved in 5 ml of dry diethyl ether and transferred on to a silica gel column (height 2.5 cm, I.D. 6.5 mm). The silica gel is previously deactivated with 3% of water. The flask and column are rinsed with 10 ml of dry diethyl ether. The aflatoxin M_1 is eluted with 10 ml of chloroform containing 3% of methanol.

* Throughout this article the American trillion (10^{12}) is meant.

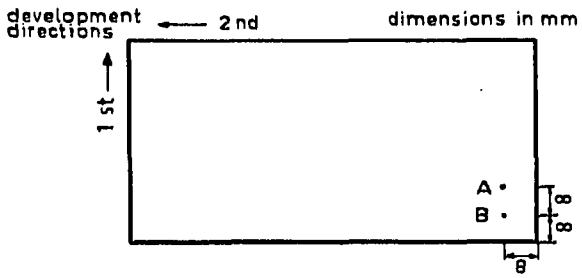

Fig. 1. Spotting in the screening procedure. Sample extract is applied at A and standard solution at B.

The eluate is evaporated to dryness and the residue is dissolved in 50 μl of chloroform. This solution is ready for TLC.

THIN-LAYER CHROMATOGRAPHY

Screening procedure

Plates of dimensions 5 × 6.5 cm are cut from Alufoil (Merck, Darmstadt, G.F.R.)[11]. A 20-μl volume of the sample extract is applied at A (see Fig. 1). The plate is first developed along its short axis with diethyl ether–methanol–water (94.5:4.5:1.5) in a saturated tank lined with filter-paper. The development time is 5–7 min. After drying for 10 min at room temperature, the standard aflatoxin M_1 is applied at point

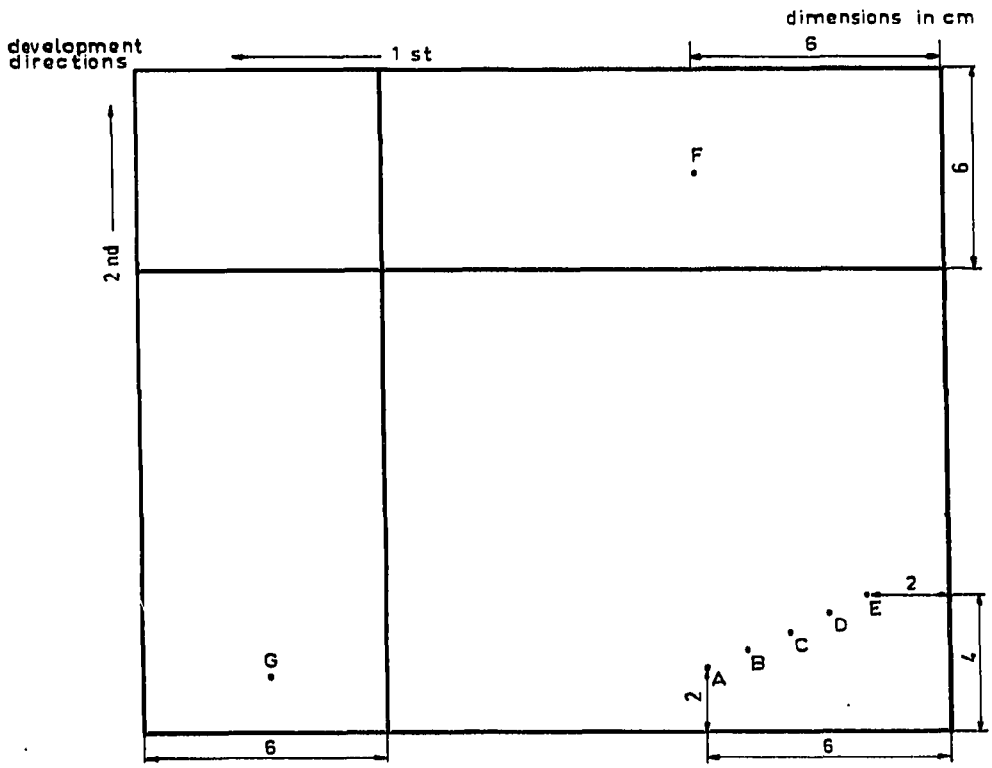

Fig. 2. Spotting of sample extract and standard solutions for quantitative determination.

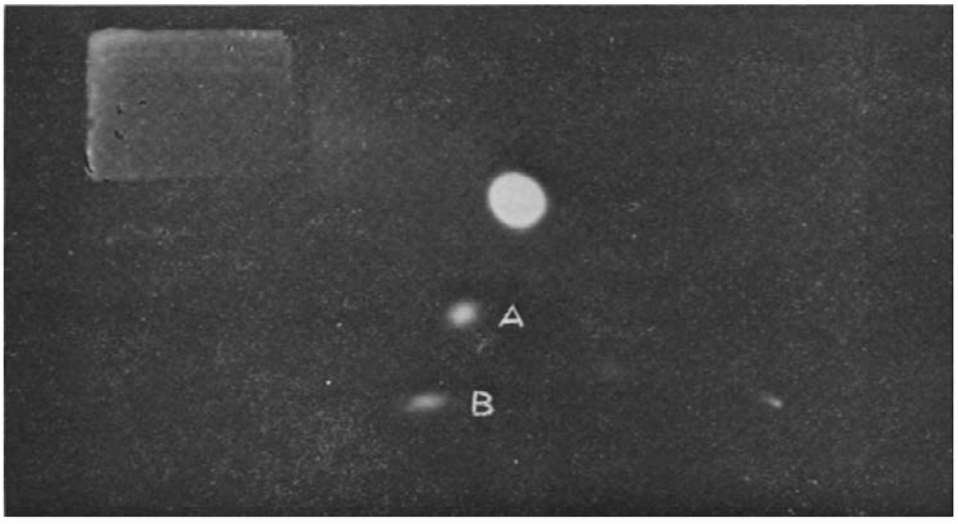

Fig. 3. Chromatogram obtained when 20 μl of extract is applied ($\approx$20 ml of milk). A = Aflatoxin M_1 from sample; B = aflatoxin M_1 standard.

B and the plate is developed along its long axis with chloroform–acetone–isopropanol (85:10:5) in an unsaturated tank. The development time is 15 min. After drying at room temperature, the plate is examined under UV light. The detection limit is 0.01 μg/kg.

Quantitative determination

Fertigplatte Kieselgel (Merck), 20 × 20 cm, are used. At A, B, C, D, E, F and G (see Fig. 2), the following amounts are applied: A, sample extract; B, 0.2 ng;

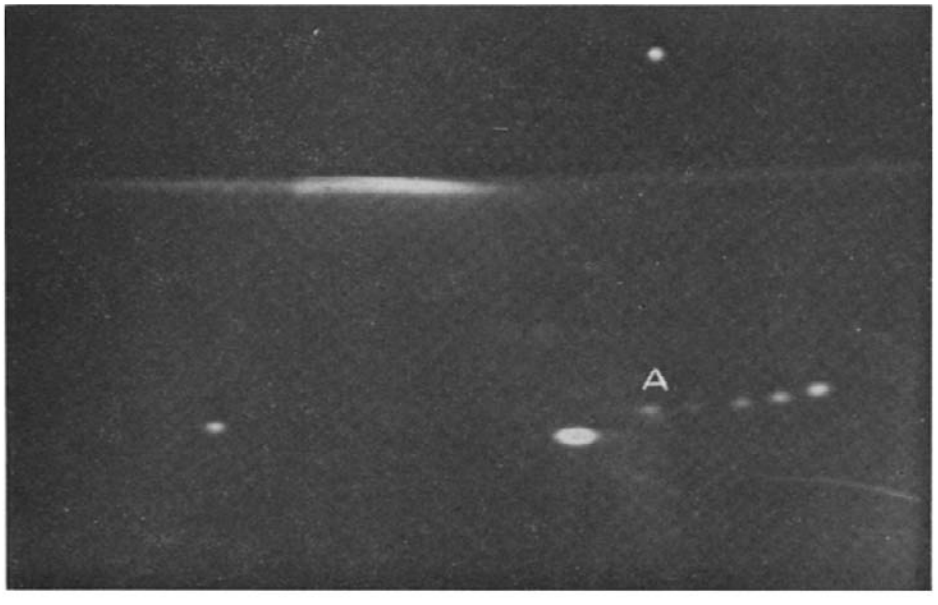

Fig. 4. Chromatogram obtained when 30 μl extract is applied ($\approx$30 ml of milk). A = Aflatoxin M_1 from sample.

C, 0.5 ng; D, 1 ng; E, 2 ng; F, 1 ng; and G, 1 ng of aflatoxin M_1. The developing solvents and conditions are the same as those used in the screening procedure.

When the total amount of sample is applied (50 μl), the detection limit is 4 ng/kg in milk. The plates are now suitable for visual quantification. A densitometric determination can also be used.

RESULTS

Fig. 3 shows an example of the TLC screening. An extract equivalent to 20 ml of milk was applied on the plate.

In Fig. 4, an extract of 30 ml of milk was applied. No aflatoxin M_1 was added to the milk. According to visual estimation, the amount of aflatoxin M_1 is approximately 0.02 μg/kg. When aflatoxin M_2 is present in sufficient amounts in the milk, its behaviour is identical with that of aflatoxin M_1. On the thin-layer chromatogram, aflatoxin M_2 is just separated from aflatoxin M_1.

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