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Note

Rapid determination of aflatoxin M1 in milk and dairy products by high-performance liquid chromatography

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Aflatoxin M1 occurs as a metabolite of aflatoxin B1 in milk from cows fed on mould-contaminated feed. Two techniques are available for the determination of aflatoxin M1: thin-layer chromatography with direct semi-quantitative visual determination and high-performance liquid chromatography (HPLC) with quantitative spectrofluorimetric detection.

As the allowable level of aflatoxin M1 in milk, which was established as 500 ng/l (0.5 ppb) by the U.S. Food and Drugs Administration in 1977[1,2], has decreased to 50 ng/l for milk and 10 ng/l for dairy babyfoods in Switzerland[3] and around 30 ng/l in the EEC countries[4], the above techniques are becoming unsuitable. However, when working on pure aflatoxin M1 solutions, HPLC has sufficient sensitivity.

In order to determine aflatoxin M1 in milk by HPLC, a preliminary extraction and clean-up procedure was developed after comparison with previous methods, including purification on a column of silica[1,5–9], alumina[7], Sep-Pak[10], C_{18} Sep-Pak[11], Celite[7], Hyflo Supercel[10] or cellulose[12], precipitation with lead salts[6,12–14], cadmium salts[8] or sodium chloride[1], delipidation with a low-polarity solvent such as hexane[12] or diethyl ether[14] and extraction with chloroform[12], acetone[5,6,13], methanol–chloroform[15], or isopropanol–chloroform[7]. These techniques generally involve injection of the concentrated extract into a silica HPLC column[15] or, more often, a C_{18} grafted silica column[6,10,11,13]. Elution is usually achieved with either acetonitrile–water[9–11] or acetonitrile–methanol–water[13] and is followed by spectrofluorimetric detection[6,10,11,13,15].

The procedure presented here uses zinc hydroxide precipitation, followed by hexane clean-up. A subsequent vacuum concentrated, chloroform extract is injected on to a C_{18} grafted silica column and eluted with acetonitrile–methanol–water.

EXPERIMENTAL

Apparatus

A Kontron liquid chromatograph with an LC 410 pump and an SFM 23 fluorescence detector with 10-μl cuvette, operated at 365 nm (excitation) and with a 385 nm cut-off detection filter, a Rheodyne injector with a 20-μl loop and a LiChrosorb RP-18 (5 μm)(Merck, Darmstadt, G.F.R.), 25 cm × 4 mm I.D. column

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were used. Other apparatus consisted of a rotary vacuum evaporator, a water-bath and an ILA CX blender (20,000 rpm).

Reagents

Analytical-reagent grade sodium sulphate, sodium hydroxide (8% solution), zinc sulphate (28% solution), chloroform, methanol, hexane and acetonitrile were used.

Aflatoxin M1 (Sigma, St. Louis, MO, U.S.A.) was dissolved in chloroform to give a 10 μg/ml standard solution.

Procedure

The whole procedure described below requires 30 min.

Sample preparation: A 20-ml volume of liquid milk (or 2 g powdered milk) was diluted to 50 ml with doubly distilled water, or 2 g of cheese, was thoroughly blended with 50 ml of doubly distilled water at 40°C.

Deproteinization. To the above samples were added 14 ml of 28% zinc sulphate solution and 8 ml of 8% sodium hydroxide solution and the mixture was shaken gently and sieved through Whatman No. 1 filter-paper on a büchner funnel.

Purification. The filtrate, shaken twice with 50 ml of chloroform for 1 min in a blender, was poured into a separatory funnel. The chloroform extracts were dried over sodium sulphate and concentrated first in a rotary vacuum evaporator and then under a nitrogen flow in a 3-ml septum vial on a water-bath. The dry residue was dissolved into 100 μl of methanol.

HPLC. A 20-μl volume of the above methanol solution was injected on to the column and eluted with a mobile phase consisting of acetonitrile–methanol–water (20:5:75) at a flow-rate of 1 ml/min.

RESULTS AND DISCUSSION

Uncontaminated liquid milk samples were spiked with aflatoxin M1 using five samples per dose level. The recoveries are shown in Table I.

TABLE I

RECOVERY OF AFLATOXIN M1 FROM MILK

Aflatoxin M1 added (ng/l)	*Recovery ± standard deviation (%)*
10	89.8 ± 10.2
50	80.2 ± 2.8
100	83 ± 3.4

The method is accurate and reliable, giving a recovery of *ca.* 80% at the low ppb level. The clean-up step with zinc hydroxide yields a clear filtrate and no emulsion with hexane or chloroform. A slight modification of Gregory and Manley's mobile phase[13] allows for slight retardation of the aflatoxin M1 peak, with good separation from impurities (Figs. 1 and 2).

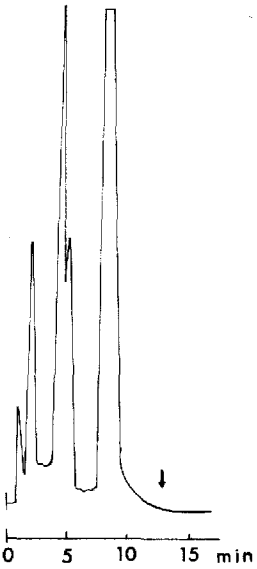

Fig. 1. Chromatogram of an aflatoxin M1-free milk.

Fig. 2. Chromatogram of an aflatoxin M1-fortified milk at 0.025 ppb.

CONCLUSION

The HPLC method described here allows, through a very efficient clean-up of the dairy sample, the rapid determination of aflatoxin M1 with a detection limit of 10 ng/l.

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