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Note

New solvent mixtures for thin-layer chromatography of aflatoxin M_1

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The identification of aflatoxin M_1 is generally accomplished by thin-layer chromatography (TLC) with detection under UV light. Separation of aflatoxin M_1 from interfering substances on chromatoplates depends on the solvent mixture used for development. The following solvent mixtures are most often used: (1) chloroform–*n*-propanol[3]; (2) chloroform–acetone–*n*-propanol[3]; (3) chloroform–acetone–*n*-amyl alcohol[4]; (4) chloroform–acetone–isopropanol[1]; and (5) chloroform–acetone[5]. In milk and milk products, the separation of aflatoxin M_1 from interfering substances using these solvents is not satisfactory. Some blue interferring spots have the same R_F as aflatoxin M_1. Therefore it was decided to construct a new solvent mixture to improve the detection of aflatoxin M_1.

EXPERIMENTAL

The milk extract was prepared from 42 samples of powdered milk according to methods described previously[1,5–8]. All of the extracts were collected in one vial and kept as the stock solution.

Radial chromatography[9] (as shown in Fig. 1) was used to select an appropriate developing solvent. The following six spots were applied on chromatoplates (for each solvent) by use of 20 μl of liquid for each spot: two spots of aflatoxin M_1 standard; two spots of milk extract; and two spots of milk extract containing the internal standard. To one spot of each pair was applied 1 ml of one of 20 different solvents (Table I). The other spots were left as controls. These experiments showed which solvents were the best developers of the investigated substances, as well as for the separation of interfering substances from aflatoxin M_1. On this basis 20 different solvent mixtures were used for the separation of aflatoxin M_1 on chromatoplates coated with silica gel GHR. The five most promising mixtures are shown in Table II and Fig. 2.

RESULTS AND DISCUSSION

The results are presented in Table I and Fig. 1. Among the 20 different solvents,

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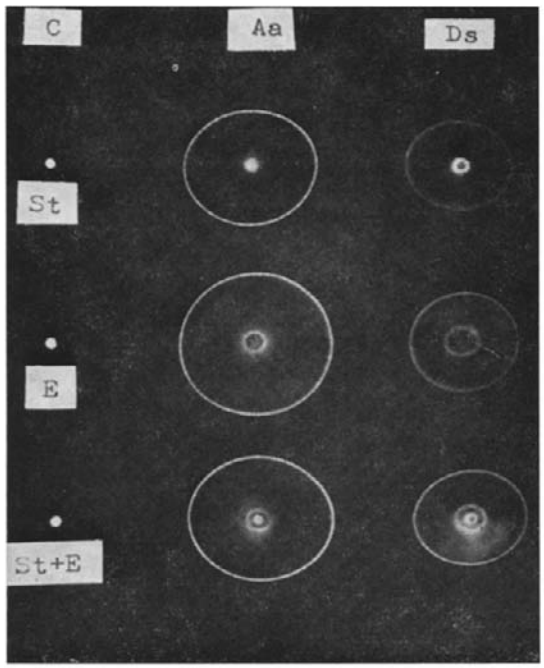

Fig. 1. Selection of solvent for the resolution of aflatoxin M_1 from milk extract. St = aflatoxin M_1 internal standard; E = milk extract; St + E = milk extract with aflatoxin M_1 internal standard; C = control (three undeveloped spots of milk extract, aflatoxin M_1 and milk extract plus aflatoxin M_1; Aa = acetic acid (three developed spots of milk extract, aflatoxin M_1 and milk extract plus aflatoxin M_1); Ds = developing solvent No. 2 in Table II (three developed spots of milk extracts, aflatoxin M_1 and milk extract plus aflatoxin M_1).

TABLE I

SELECTION OF SOLVENTS FOR THE SEPARATION OF MILK EXTRACTS FROM THE AFLATOXIN M_1 STANDARD

— = Immobile (start line); + = weakly mobile; ++ = intermediate mobile; +++ = strongly mobile (front line).

No.	*Solvent*	*Dielectric constant**	*Milk extract*	*Aflatoxin M_1 standard*
1	*n*-Hexane	1.9	—	—
2	*n*-Heptane	1.9	—	—
3	Light petroleum (b.p. 60–80°)	2.0	—	—
4	Carbon tetrachloride	2.2	—	—
5	Dioxan	2.2	—	—
6	Benzene	2.3	—	—
7	Toluene	2.4	—	—
8	Diethyl ether	4.3	++	+
9	Chloroform	4.8	+	+
10	Ethyl acetate	6.0	+++	++
11	Acetic acid	6.1	++	—
12	Pyridine	12.3	++	++
13	*n*-Amyl alcohol	13.9	+	+
14	Isopropanol	18.3	+	+
15	*n*-Propanol	20.1	++	++
16	Acetone	20.7	++	+++
17	Ethanol	24.3	++	+++
18	Methanol	32.6	+++	+++
19	Acetonitrile	37.0	++	+++
20	Water	78.5	+	+

* Data from ref. 9.

TABLE II

DEVELOPING SOLVENTS FOR TLC OF MILK EXTRACTS AND AFLATOXIN B_1 AND M_1 STANDARDS

No.	*Solvent*	*Proportion*	*Resolution of aflatoxins B_1 and M_1*	*Separation of aflatoxins from fluorescent substances*	
				B_1	M_1
1	*n*-Hexane	10			
	Light petroleum	10			
	Benzene	10			
	Chloroform	40	+	+	−
	Acetone	10			
	Acetonitrile	10			
	Acetic acid	10			
2	*n*-Hexane	10			
	Light petroleum	10			
	Benzene	10			
	Chloroform	20	+	+	+
	Acetone	10			
	Acetonitrile	10			
	Acetic acid	30			
3	Chloroform	85			
	Acetone	10	+	+	+
	n-Propanol	5			
4	*n*-Hexane	10			
	Light petroleum	10			
	Benzene	10	+	−	+
	Chloroform	55			
	Acetone	10			
	n-Propanol	5			
5	*n*-Hexane	10			
	Light petroleum	10			
	Benzene	10	+	+	+
	Chloroform	55			
	Acetone	10			
	n-Amyl alcohol	5			

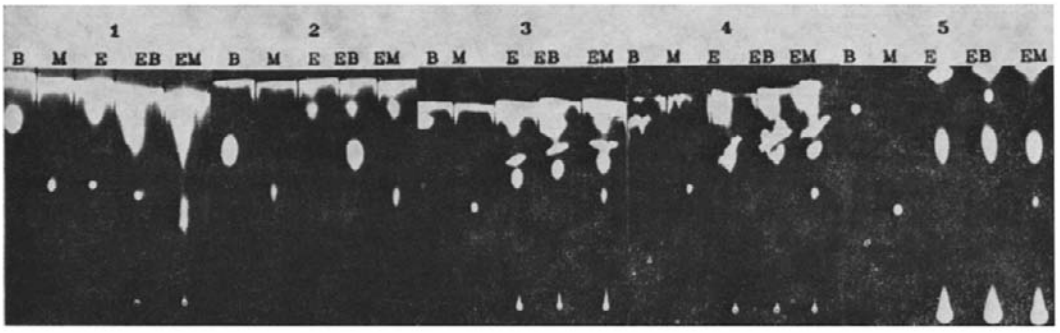

Fig. 2. Chromatoplates with aflatoxin B_1, M_1 and milk extract developed in different developing solvents. B = Aflatoxin B_1; M = aflatoxin M_1; E = milk extract; EB = milk extract with aflatoxin B_1 internal standard; EM = milk extract with aflatoxin M_1 internal standard; 1–5 = number of solvent in Table II.

seven separate aflatoxin M_1 from interfering substances only slightly (diethyl ether, ethyl acetate, water, dioxane, acetone, ethanol and acetonitrile) and one (acetic acid) significantly well. Four solvents (diethyl ether, ethyl acetate, acetic acid and water) developed the interfering substances and four (dioxan, acetone, ethanol and acetonitrile) were good developers for aflatoxin M_1. On the basis of polarity, the solvents might be divided into two groups: (a) weakly polar (diethyl ether, ethyl acetate and acetic acid) and (b) intermediate polar (acetone, ethanol and acetonitrile). Their dielectric constants are 4.3, 6.0 and 6.1 (a) and 20.7, 24.3 and 37.5 (b), respectively.

The HEBCA[2] solvent mixture was used as a base for the experiments because it had been established for the separation of aflatoxins in food extracts. In this mixture, hexane (H) petroleum ether (E) and benzene (B) were used for the separation of lipids, and chloroform (C) with acetone (A) for the development and resolution of aflatoxins.

In the milk extracts, some interfering substances had the same R_F value and blue colour as aflatoxin M_1 when the HEBCA mixture was used for the development of TLC plates. Also, aflatoxin M_1 was too close to the start line. Therefore, two additional solvents, acetic acid and acetonitrile, were used to improve the separation of aflatoxin M_1 from the other substances. The new developing solvent mixture $HEBCAA_cA_a$ (No. 2 in Table II) had the following composition: hexane–light-petroleum (b.p. 60–80°)–benzene–chloroform–acetone–acetonitrile–acetic acid (1:1:1:2:1:1:3). The mixture was estimated as the best because it changed the colour of interfering substances from blue to pale green as well as improving the resolution and separation of aflatoxin M_1 significantly.

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