Thin-layer chromatographic separation of preservatives

The increasing use of preserving agents in foodstuffs, both the overall use and the number of agents in use, brings in its wake the necessity for laboratories working in the field to be able to carry out qualitative and quantitative analyses with standard apparatus and under normal working conditions.

Recent advances have been made in this field by COPIUS-PEEREBOOM AND BEEKES[1], who separated several agents using thin-layer chromatography, and COVELLO AND SCHETTINO[2], who employed a thin layer of silica gel for the separation of a similar series of preservatives. The development length used by COPIUS-PEEREBOOM, however, was about 20 cm, which meant that standard plates (20 × 20 cm) could not be employed. The work of COVELLO AND SCHETTINO, on the other hand, did not give a complete separation and the method of detection using sublimation is not suitable for routine analysis.

SALO[3] separated *p*-hydroxybenzoic acid and thirteen of its esters on a mixture of acetylated cellulose and polyamide utilising Shell Sol A–glacial acetic acid (50:50, v/v) as solvent. LUECK[4] effected a separation of benzoic and sorbic acids after prior bromination.

The aim of the present work was to effect a separation of nine commonly used preserving agents using a standard technique. By employing a 1:1 mixture of Kieselgel G and Kieselguhr G (Merck)[1,2,5–7] and developing twice to a length of 15 cm with a petroleum ether–chloroform–formic acid (10:4:1, v/v/v) solvent mixture, a complete separation was obtained. All the agents under analysis were visible under U.V. (366 nm) after adding the fluorescent indicator Ultraphor W.T. (BASF) (0.02 %).

Experimental

A 1:1 mixture of Kieselgel G and Kieselguhr G was employed as adsorbent. Fifteen grams of each material were mixed with 60 ml of a 0.02 % solution of Ultraphor W.T. in water. A thickness of 0.25 mm of adsorbent was utilised on standard (20 × 20 cm) plates. The plates were dried in a warm air current for 10 min before activation by heating at 110° for 30 min.

The acetic acid concentration in the solvent mixture employed by COPIUS-PEEREBOOM AND BEEKES was raised slightly and this gave an increased separation.

TABLE I

No.	*Preservative*	*No. on photograph*	R_F	*Quantity spotted on the plate* (γ)
1	Benzoic acid	2	0.77 — 0.86	100
2	Sorbic acid	3	0.72 — 0.80	25
3	Salicylic acid	1	0.63 — 0.74	25
4	Dehydroacetic acid	9	0.57 — 0.66	50
5	Bromoacetic acid	6	0.41 — 0.44	25
6	Propyl-*p*-hydroxybenzoate	4	0.29 — 0.31	25
7	Ethyl-*p*-hydroxybenzoate	5	0.24 — 0.26	25
8	Methyl-*p*-hydroxybenzoate	7	0.19 — 0.20	25
9	*p*-Hydroxybenzoic acid	M lower spot	0.10 — 0.11	25

Substitution of acetic acid by formic acid and ether by $CHCl_3$ gave preferable results in our hands, and the eluting solvent finally employed was petroleum ether–$CHCl_3$–formic acid (100:40:10, v/v/v). We eluted twice on the same plate to a length of 15 cm for each elution.

We found that temperature control during elution was of prime importance. At 24° salicylic acid and dehydroacetic acid were not separated. At 22°, however, all nine preserving agents under study were plainly distinguishable from one another (see Fig. 1 and Table I).

A further refinement in our technique was the method of sample application. Depositing the extracts of preserving agents in a streak rather than in the more customary spot gave a clearer separation.

All nine preserving agents used were visible for several minutes under U.V. irradiation (366 nm) ("Fluotest"—original Hanau) (see Fig. 1). The use of Leuchtpigment ZS Super (Riedel de Haën) as a fluorescence indicator at 254 nm was not

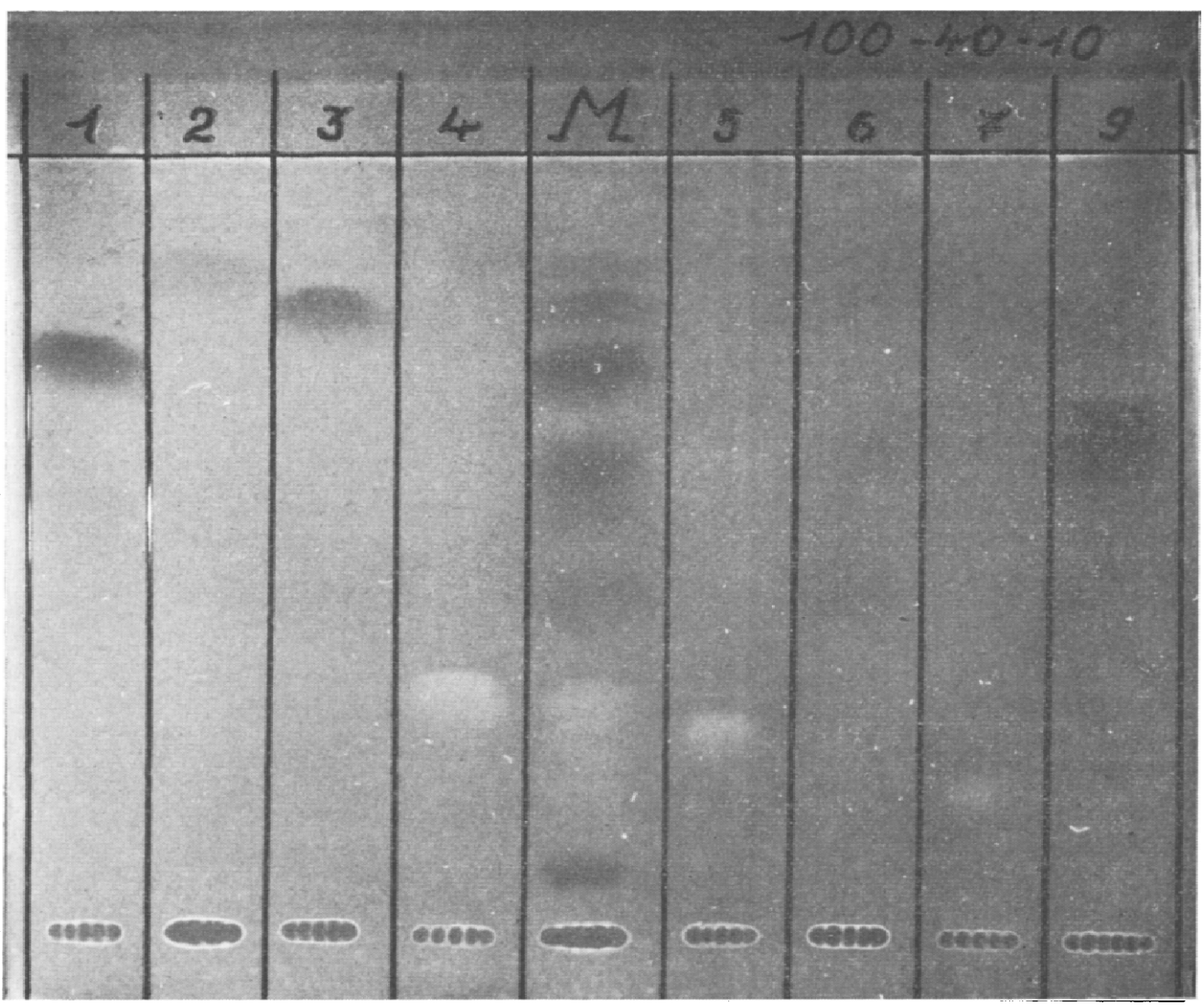

Fig. 1. Separation of preservatives by thin-layer chromatography. Conditions: 15 g Kieselgel G + 15 g Kieselguhr G (1:1) + 60 ml 0.02% Ultraphor; solvent: petroleum ether (25–70°, p.a.)–$CHCl_3$–HCOOH (100:40:10); elution twice for 15 cm; temperature maximum: 22°.

so successful due to the interference from Kieselguhr G. (Leuchtpigment ZS is recommended for DESAGA U.V. lamps.)

The above method has been used in the detection of preserving agents in a wide variety of foodstuffs and found to be successful on all occasions.

Detection reagents

1. Benzoic acid. (a) 4.5 ml H_2O_2 (30 %) + 4.5 ml H_2O + 1 ml saturated $MnSO_4$ solution; then (b) 0.3 % $FeSO_4$ aqueous solution.

2. Sorbic acid. (a) 5 ml 0.5 % $K_2Cr_2O_7$ + 5 ml 0.3 *N* H_2SO_4; followed by (b) saturated thiobarbituric acid solution.

3. Salicylic acid. 0.1 % $FeCl_3$ in water.

4. Dehydroacetic acid. 3 % $TiCl_3$ aqueous solution; or 0.1 % $FeCl_3$ aqueous solution.

5. Bromoacetic acid[8] (see under next section). The following mixture (a) 3 vol. phenol red (24 mg phenol red in 2.4 ml 0.1 *N* NaOH made up to 100 ml with acetone) and 1 vol of a CH_3COONa solution (6 g CH_3COONa + 3 ml CH_3COOH + water to 100 ml) is sprayed on the chromatogram and followed by a spray of (b) chloramine T solution (25 mg chloramine T in 15 ml of water–acetone, 1:1).

6–9. The esters and the free p-hydroxybenzoic acid[5]. Millon's reagent: 1 part of Hg by weight + 2 parts of fuming HNO_3 + 2 vol. water.

Detection

Following the separation, the various components of the mixture were confirmed using suitable reagents for each constituent. The order of use of the reagents is as indicated in the list above.

Small amounts of the reagent were sprayed on the plate, exposing only the part under scrutiny. If this was insufficient to give an unequivocable decision, the plate was heated and re-sprayed, small amounts of reagent again being employed.

Benzoic acid. Nearly all the detection reactions described in the literature employ H_2O_2 as an oxidising agent followed by $FeCl_3$ to give a colour[1,2,5–7]. KRÖLLER[9], however, added a saturated $MnSO_4$ solution to the peroxide to give a catalytic effect. He also used a mixture of $FeCl_3$ and $FeSO_4$ on paper chromatograms. In our hands a 0.3 % $FeSO_4 \cdot 7H_2O$ solution gave the best results. After initial spraying with this reagent the plate was redried for 3 min and resprayed. The reagent is oxidised on the plate and gives a light brown spot on a white background. Redrying and respraying with aqueous $FeSO_4$ gives a white spot which turns brown on drying.

Sorbic acid. After oxidising with 0.5 % $K_2Cr_2O_7$ solution the plate was dried and sprayed with a saturated solution of thiobarbituric acid. On further drying a pink spot on a white background is revealed. The dilute solution of $K_2Cr_2O_7$ employed by SCHMIDT[10] is preferred to the 10 % solution used by COPIUS-PEEREBOOM AND BEEKES[1], the yellow background being given by the latter solution making identification more difficult.

PEKKARINEN AND PORKKA[6] mention interference with this detection by various oxidation products in rancid fats, when carried out on the extract directly.

Salicylic acid. This gives a brown-violet spot after spraying with a dilute aqueous 0.1 % $FeCl_3$ solution. Another possibility is to spray with the Millon's reagent[5], the latter giving a yellow-orange spot.

Dehydroacetic acid. An aqueous 3 % $TiCl_3$ solution[1] gives a purple blue colour. We also used dinitrophenylhydrazine as a chromogenic agent. The difference between the yellow spot and the yellow background of the detection agent was not clear. We prefer a very dilute aqueous 0.1 % $FeCl_3$ solution which gives a yellow spot on a white background.

Bromoacetic acid. The plate is exposed to ammonia vapour for 10 min and heated afterwards for 10 min to eliminate the excess ammonia. After spraying with a mixture of phenol red and CH_3COONa the spot was immediately revealed with chloramine T. A blue spot on a white or a yellow background resulted[8].

Propyl, ethyl and methyl-p-hydroxybenzoate. The best detecting reagent was Millon's reagent. The three esters and the free acid give a red or red-brown colour after spraying with Millon's reagent and heating. If after the first spray the spots were not clear, the plate was sprayed for a second time and heated again for a few minutes at 100°.

p-Hydroxybenzoic acid. On account of the position of the esters and the acid on the chromatogram the most simple detection agent is Millon's reagent. We also used diazotized *p*-nitroaniline to detect the acid to give a yellow spot which turned to orange on exposure to ammonia vapours.

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