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Modified thin-layer chromatographic separation of preservatives

Our thin-layer chromatographic method for the separation of preservatives was tested in several laboraties in the Common Market countries. We used Ultraphor WT, proposed by COPIUS-PEEREBOOM AND BEEKES[2], as a fluorescent indicator at 366 nm. Several laboratories have encountered difficulties in visualising the spots at 366 nm with their UV lamps. In order to avoid these difficulties we changed the adsorbent mixture, and instead of silica gel and kieselguhr, we now use a mixture of Silica Gel GF_{254} and Cellulose MN 300 F_{254}, where the fluorescent indicators are already incorporated by the manufacturers. Bromoacetic acid is, however, not detectable under the new conditions, but it can be located just above the propyl ester of *p*-hydroxybenzoic acid with the proposed detection reagent for bromoacetic acid described below. Some laboratories also had difficulties with the detection of benzoic acid with the prescribed reagent. It was demonstrated that the spray conditions laid down were not respected, and that several operators over-sprayed the plates.

This modified method has now been in use for more than a year in the routine analysis of preservatives in all kinds of food. We prefer this method to other methods[3–5,8], as UV detection alone or detection with the proposed acid–base indicators lack specificity. For example, free fatty acids in salad dressings could be erroneously considered as benzoic acid, as they have nearly the same R_F value as this preservative. Another point is the toxicity of the Millon reagent, in which case the esters are hydrolysed on the plate with a NaOH solution and the phenols so obtained are detected by spraying with an aminoantipyrine solution.

Experimental and results

Adsorbent. In order to find the best silica gel : cellulose ratio for the separation of the preservatives under examination, we made several gradient plates. As Fig. 1 illustrates, the silica gel on the left-hand side gives a better separation of the free acids, while the cellulose side is better for the esters. Finally, we chose a mixture of 15 g Silica Gel GF_{254} and 7.5 g Cellulose MN 300 F_{254}. This mixture was thoroughly mixed with 70 ml water in an electric mixer and used for five 0.25-mm (20 × 20 cm) plates. The plates were dried in air and then activated by heating at 110° for 30 min.

Solvent. The substitution of cellulose in place of kieselguhr obliged us to change the solvent as well. As the mobile phase light petroleum (b.p. 40–60°)–carbon tetrachloride–chloroform–formic acid–acetic acid (50:40:20:8:2) was used under normal conditions (a temperature of *ca.* 22° and a relative humidity between 35% and 70%). The solvent mixture was shaken in a separating funnel, the two layers were allowed to separate and the upper layer only was then used as the mobile phase. The plate was eluted twice to a length of 15 cm with the same solvent (Fig. 2).

Influence of the relative humidity. Figs. 3a and b illustrate the influence of the relative humidity on the separation. At a relative humidity of 35% the separation of sorbic and benzoic acids is better, and as seen from the gradient plate they are well separated on silica gel, and are thus favoured by active plates. The esters of *p*-hydroxy-

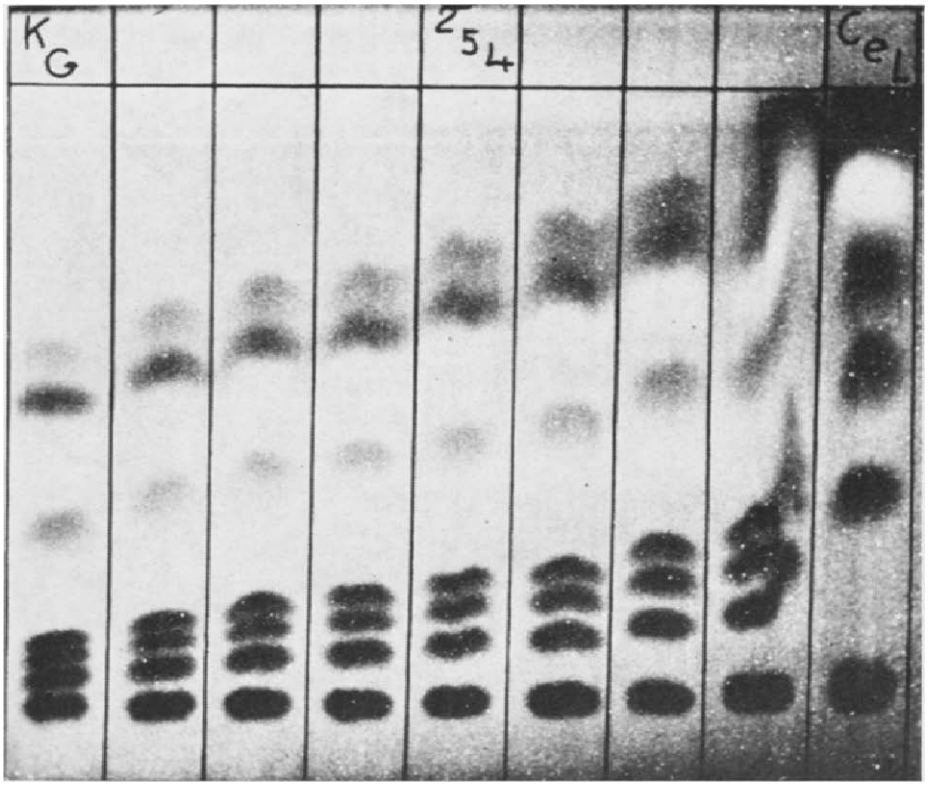

Fig. 1. Influence of the adsorbent. Left-hand side: Silica Gel GF_{254}; right-hand side: Cellulose MN 300 F_{254}.

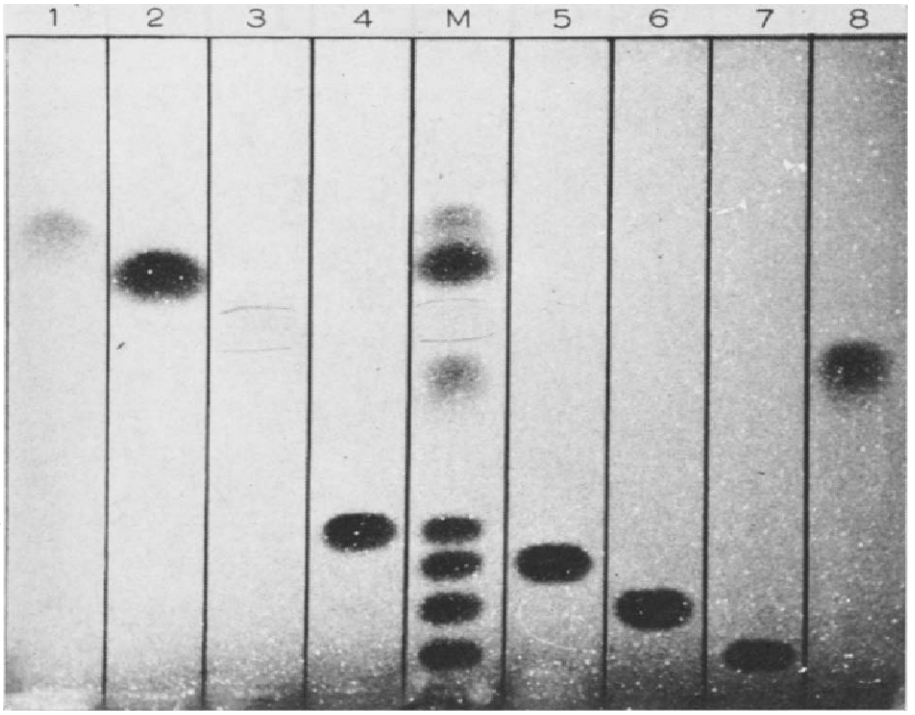

Fig. 2. Separation of nine preservatives. 1 = Benzoic acid, 2 = sorbic acid; 3 = salicylic acid; 4–8 = the propyl-, ethyl and methyl esters of *p*-hydroxybenzoic acid and dehydroacetic acid; M = mixture of preservatives. The bromoacetic acid spot is not visible in UV light.

benzoic acid are separated better at 60% relative humidity on cellulose layers. But we can see that the differences between the separations at 35% and 60% are not so great; thus a separation of the nine preservatives can be carried out on the proposed mixed layer of silica gel and cellulose under a wide range of normal conditions.

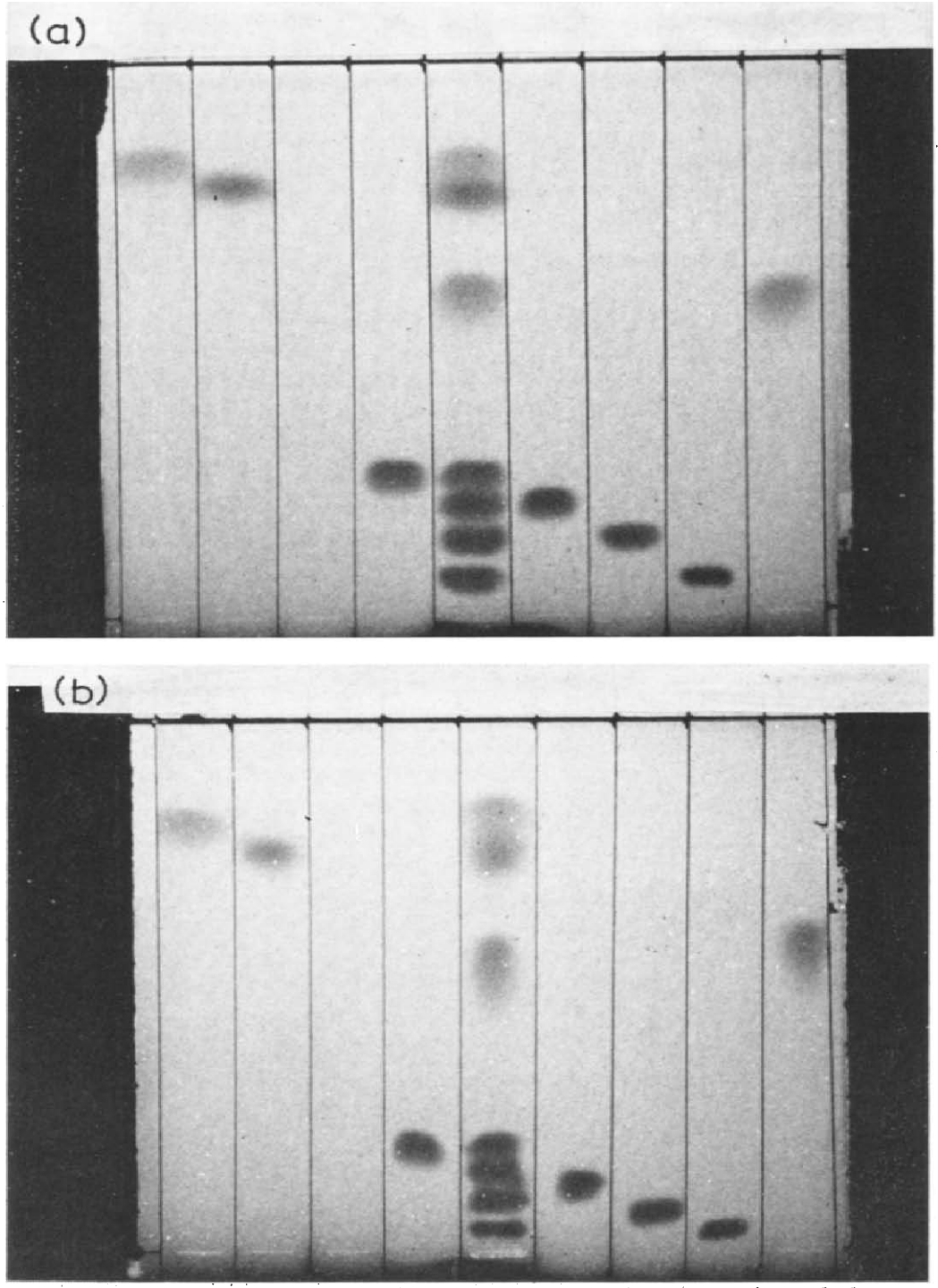

Fig. 3. Influence of the relative humidity on the separation of the preservatives. (a) Relative humidity, 35%; (b) relative humidity, 60%.

Detection. For the detection of benzoic acid a solution of 4.5 ml H_2O_2 (30 %), 4.5 ml water and 1 ml saturated $MnSO_4$ was used, followed by a 0.3% aqueous solution of $FeSO_4$; for the detection of sorbic acid a solution of 5 ml 0.5% $K_2Cr_2O_7$ and 5 ml 0.3 *N* H_2SO_4, followed by a saturated solution of thiobarbituric acid; for the detection of salicylic acid a 0.1% aqueous solution of $FeCl_3$; and for the detection of dehydroacetic acid a 3% aqueous solution of $TiCl_3$ or a 0.1% aqueous solution of $FeCl_3$. For the detection of bromoacetic acid a mixture of three volumes of Phenol Red (24 mg Phenol Red in 2.4 ml 0.1 *N* NaOH made up to 100 ml with acetone) and one volume of a CH_3COONa solution (6 g CH_3COONa, 3 ml CH_3COOH and water made up to 100 ml) is sprayed on the chromatogram, followed by a spray of a Chloramine T solution (25 mg Chloramine T in 15 ml of water–acetone, 1:1).

Fig. 4 shows the results obtained with the above detection reagents using the same detection procedure as recommended earlier.

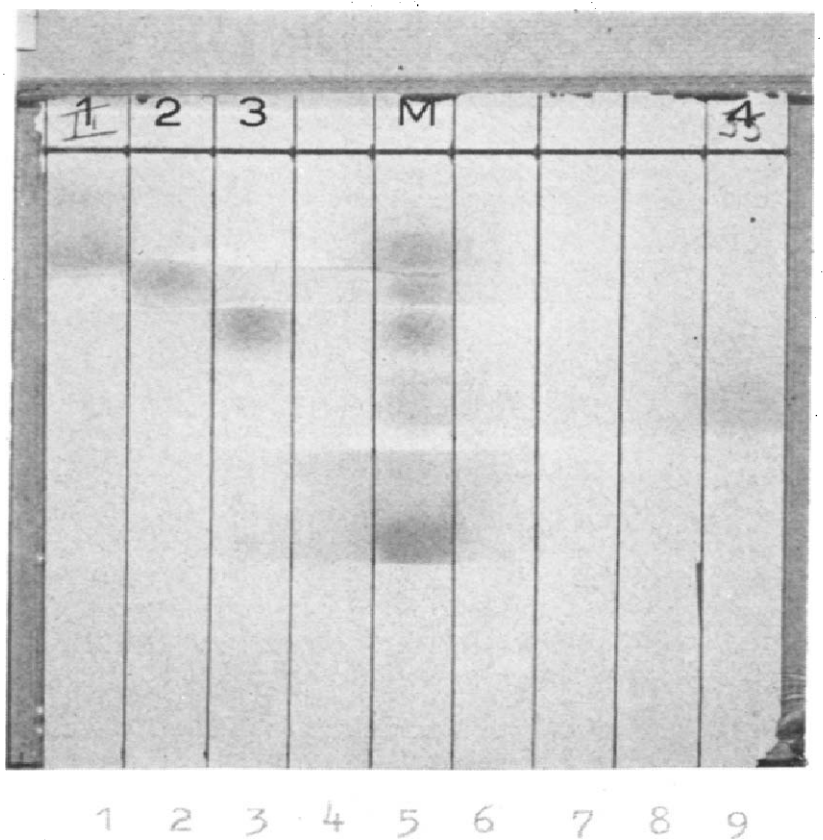

Fig. 4. Plate sprayed with the proposed detection reagents. The original colour picture was taken with a Polaroid camera. 1 = Benzoic acid; 2 = sorbic acid; 3 = salicylic acid; 4 = dehydroacetic acid; M = mixture of preservatives. The lowest spot is that of bromoacetic acid.

For the detection of the esters and the free *p*-hydroxybenzoic acid a 10% NaOH solution, a 2% alcoholic solution of aminoantipyrine and an 8% aqueous solution of $K_3Fe(CN)_6$ were used. The esters were hydrolysed by spraying with the NaOH solution. The plate was heated for 5 min at 80° and sprayed again with distilled water and heated for another 5 min. Spraying with the aminoantipyrine and $K_3Fe(CN)_6$ solutions gave red to red-brown spots.

TABLE I

hR_F VALUES AND DETECTION LIMITS WITH THE PROPOSED METHOD

Preservative	*Approximate hR_F value*	*Quantity visible in UV light (254 nm) (μg)*	*Quantity detectable by the proposed detection reagents (μg)*
Benzoic acid	70	10	25
Sorbic acid	63.5	0.6	0.25
Salicylic acid	56	1.2	15
Dehydroacetic acid	50	5	25
Bromoacetic acid	30	not visible	5
Propyl *p*-hydroxybenzoate	24.5	0.25	1
Ethyl *p*-hydroxybenzoate	20	0.25	1
Methyl *p*-hydroxybenzoate	13	0.25	1
p-Hydroxybenzoic acid	6	0.25	0.25

Special attention should be given to the remark made above, namely that only small amounts of the reagent should be sprayed on the plate, exposing only the part under scrutiny. If this is insufficient to give an unambiguous identification, the plate should be heated and re-sprayed, small amounts of reagent again being employed.

The detection limits of the proposed method in UV light and after spraying with the reagents described above are given in Table I.

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