CHROM. 5247

Separation and identification of food colours

Part II. Identification of synthetic oil-soluble food colours using thin-layer chromatography

Oil-soluble food colours are also used as permitted food additives. A method of separation and identification of this type of food colouring is required to enable the enforcement of the permitted international food dye legislation. Ten dyes have been studied (see Table I), four of these are permitted for use in food in certain countries whereas the rest are dyes which are no longer permitted because they are considered too harmful for use in foodstuffs.

Thin-layer chromatography (TLC) was an obvious choice for the identification of these dyes. Cellulose[1], starch[2], silica gel[3], alumina[4], polyamide[5], and mixtures of these adsorbents[6] have all been used for the separation of oil-soluble dyes but with limited success. COPIUS-PEEREBOOM AND BEEKES[7] used silica gel plates impregnated with silver nitrate but this gave poor separation of the oil oranges. DAVÍDEK AND JANÍČEK[2] impregnated starch layers with liquid paraffin and RAMAMURTHY AND BHALERAO[8] used plates coated with calcium carbonate and starch dipped in a liquid paraffin–petroleum ether mixture.

Reversed-phase TLC appeared to be the most promising technique and a method has been devised for the separation of the ten dyes using cellulose coated plates impregnated with liquid paraffin and a single development solvent. Liquid paraffin was chosen as the stationary phase as it has been most widely used but other stationary phases have been suggested such as *n*-lauryl alcohol, oleic acid and diacetylethylene glycol monostearate[9].

Materials and methods

Apparatus. TLC apparatus for the preparation of layers 0.25 mm thick on 200 × 200 mm glass plates. Chromatographic development tanks. 5-μl pipettes, *e.g.* Microcap disposable pipettes. Large photographic dish.

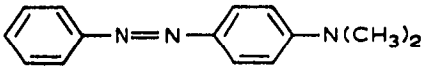
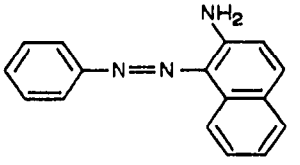
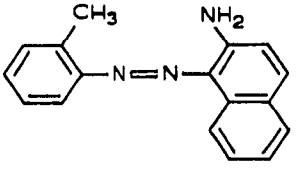
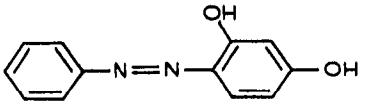
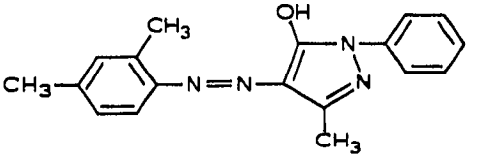
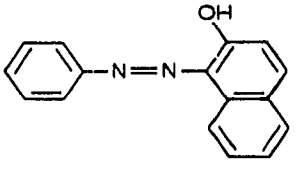
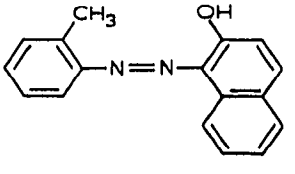
Reagents. Chromatographic development solvent, 2-methoxyethanol–methanol–water (55:15:30). Reference dye solutions, 0.1% w/v in ethanol. Cellulose powder, microcrystalline cellulose (available from Applied Science Laboratories, Inc.). Prepare plates as follows: Shake 20 g cellulose powder with 60 ml methanol for about 3 min and blend the mixture for 30 sec. Spread onto plates to produce a layer 0.25 mm thick and allow to air dry or dry in an oven at 80°. When the plates are dry immerse them in a 10% solution of liquid paraffin in petroleum ether (80–100°) for about 1 min and then air dry or dry in an oven at 80° in a horizontal position.

Procedure. Place a spot of 1–2 μl of the dye solution at least 20 mm from the edge and bottom of the plate. Also place 1–2 μl of each dye and of a mixture of all ten dyes. Dry the spots and develop the plate in the solvent for a distance of about 150 mm at room temperature. The dyes travel in the order shown in Fig. 1.

The identification of the sample dye is then confirmed by re-chromatographing on a plate where spots of the sample solution are overspotted with spots of the suspected dyes. The unknown dye is identified by giving a single spot with the correct standard while all the other standards give rise to double spots.

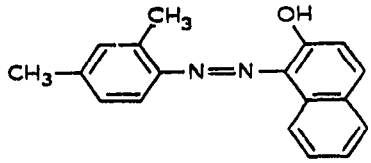
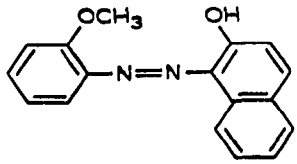
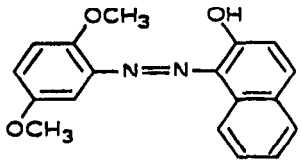
TABLE I

DYES STUDIED

Dye	*Colour index number*	*Structure*
Butter Yellow	11020	
Yellow AB	11380	
Yellow OB	11390	
Oil Yellow GG Ceres Orange GN	11920	
Oil Yellow XP	12740	
Oil Orange E Sudan I	12055	
Oil Orange SS	12100	

(Continued on p. 334)

TABLE I (*continued*)

Oil Orange XO Sudan II	12140	
Sudan Red G Ceres Red G	12150	
Citrus Red No. 2	12156	

Discussion

Alumina, Kieselguhr G and Silica Gel G were tried in place of cellulose for coating the plates all being impregnated with liquid paraffin. Although alumina and kieselguhr gave a similar separation to cellulose the colours tended to fade very readily and silica gel did not give as good a separation. The various factors which might affect the separation of the dyes have been studied.

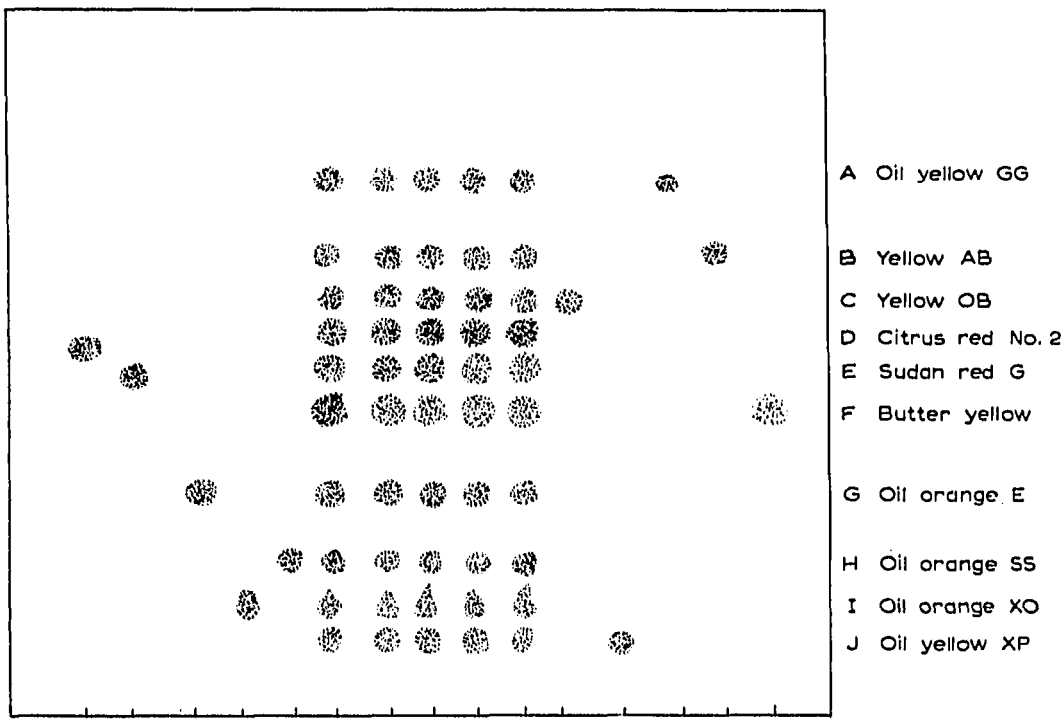


Fig. 1. Typical separation of the oil-soluble dyes.

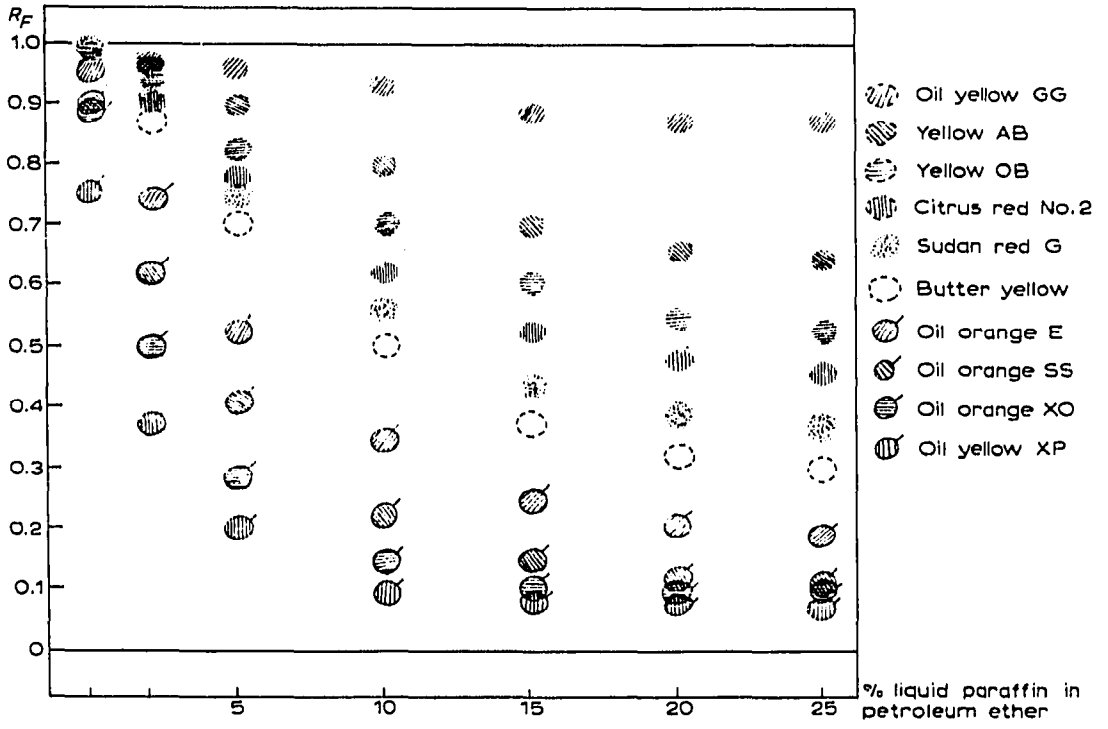


Fig. 2. Effect of liquid paraffin on the separation of the dyes.

Concentration of liquid paraffin solution for impregnating plates. Separation of the dyes is not affected when 5–15% liquid paraffin in petroleum ether is used. However the concentration of liquid paraffin affects the R_F values of the dyes as shown in Fig. 2 and also the time required for the developing solvent to run a particular distance up the plates. The lower the concentration of liquid paraffin in the dipping solution the quicker the developing solvent moved up the plate (see Table II).
At higher concentrations of liquid paraffin the spots begin to blur and streak. The optimum concentration of liquid paraffin is 8–10%.

Drying time after dipping plates. DAVÍDEK AND JANÍČEK[2] reported that the R_F

TABLE II

THE EFFECT OF THE CONCENTRATION OF LIQUID PARAFFIN IN THE DIPPING SOLUTION ON THE DISTANCE MOVED BY THE DEVELOPING SOLVENT IN A GIVEN TIME

Percentage liquid paraffin in petroleum ether	*Distance travelled by solvent front in 3 h (cm)*
1	14.0
2	15.2
3	14.5
4	13.9
5	13.7
7.5	12.0
10	11.9
15	9.9
25	8.7

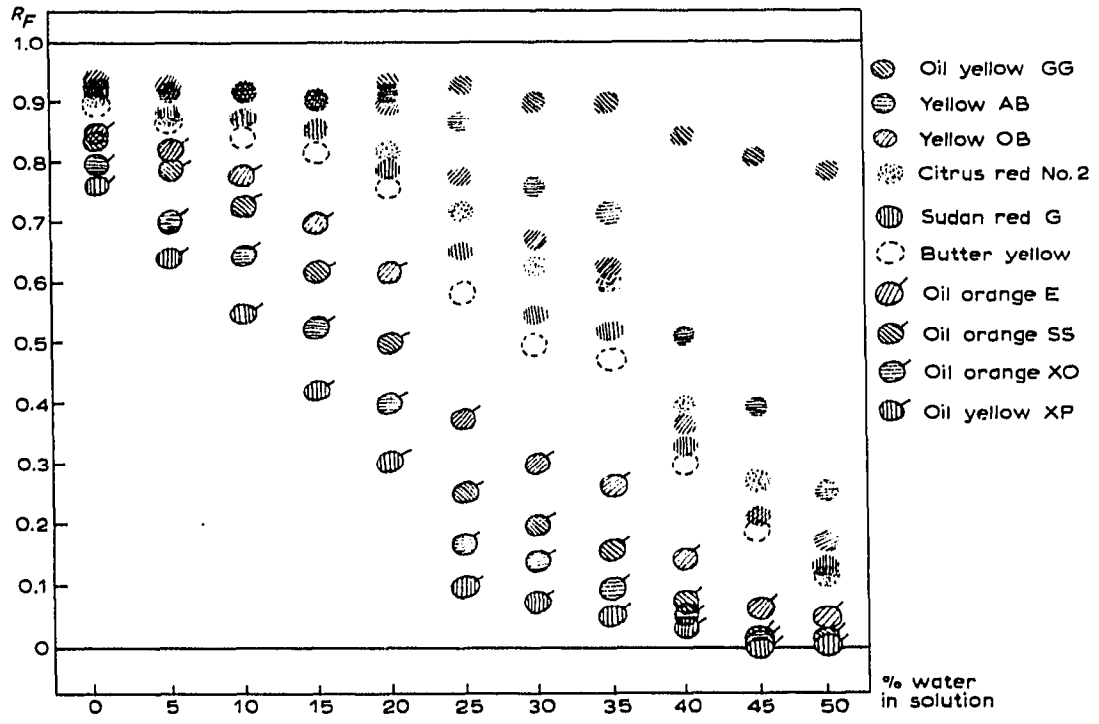


Fig. 3. Effect of water content of the developing solvent.

values of most oil-soluble dyes varied with the drying time after the preparation of the plates. We have found that the time the plates are left to dry has very little effect.

Time of immersion in the liquid paraffin–petroleum ether mixture. There was no significant change in R_F values when the immersion time was increased from a few seconds to over 5 min in a 10% solution of liquid paraffin in petroleum ether.

Water content of the development solvent. The amount of water in the solvent system is important since it not only effects the R_F values but also the separation of the dyes (see Fig. 3). The optimum amount of water in a 50:50 mixture of methanol and 2-methoxyethanol lies between 25 and 33%. As the percentage water is increased the development time increases.

Alcohol content of the development solvent. Different alcohols were investigated, *i.e.* methanol, ethanol and propan-2-ol. With propan-2-ol streaking of the spots occurred and a limited separation was obtained whereas methanol gave compact spots and a good separation of the dyes. Ethanol was intermediate.

A series of plates were run in solvents where the ratio of methanol to 2-methoxyethanol in the solvent was varied keeping the water content constant at 25%. These showed that a high proportion of 2-methoxyethanol was required for the best separation and that the optimum ratio was 4 parts of 2-methoxyethanol to 1 part methanol.

Effect of acid and alkali in the development solvent. With 1% ammonia solution (s.g. 0.88) in the development solution the R_F value of Oil Yellow XP increases and merges with Oil Orange E and the R_F value of Butter Yellow increases and merges with Citrus Red No. 2.

When 10% concentrated hydrochloric acid is added to the development solvent Oil Yellow GG travels with the solvent front, Yellow AB travels just below the solvent front and Yellow OB is not visible. Confirmation of the presence of Butter Yellow may be obtained by exposing the developed plate to hydrochloric acid vapour, when the spot changes from yellow to red.

Although each variable was investigated separately with all the others constant, there are probably a number of different combinations which will give a separation of the dyes, *e.g.* high R_F values produced by a low percentage of liquid paraffin in the dipping solution may be reduced by a high percentage of water in the developing solvent. The conditions laid down in the method are those which were found to give a good separation of the dyes.

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