CHROM. 6163

Separation and identification of food dyes

III. Improved resolution of selected dye pairs

An earlier paper[1] described a thin-layer chromatographic (TLC) method for the separation and identification of forty-nine synthetic food colours. However, the separation achieved for dye pairs Orange GGN/Sunset Yellow FCF, Fast Green FCF/Green S, and Brilliant Blue/Light Green Yellowish was not satisfactory for identification of admixtures, and no separation was obtained for dye pairs Chocolate Brown HT/Chocolate Brown FB, Ponceau 3R/Ponceau MX and Violet 5BN/Violet BNP. Solvent systems for the improved separation for the first three dye pairs are now reported. A slight separation of Ponceau 3R and Ponceau MX has been obtained but not of the other two dye pairs.

Materials and methods

Chromatographic solvents

Mixtures were freshly prepared according to the following compositions:

(1) *n*-Butanol–water–glacial acetic acid (10:5:1)
(2) Isobutanol–ethanol–water–(0.88) ammonia (60:20:2:1)
(3) Ethyl acetate–methanol–(0.88) ammonia (10:3:3)
(4) *tert.*-Butanol–water–(0.88) ammonia (80:35:1)
(5) Isobutanol–water–glacial acetic acid (20:10:2)
(6) Isobutanol–ethanol–propionic acid (3:2:2)
(7) *n*-Butanol–water–glacial acetic acid–sodium tosylate (40:10:40:1)
(8) Dioxan–water–propionic acid (80:40:5)
(9) 10% w/v Aqueous chloral hydrate
(10) Dimethylsulphoxide–water–formic acid (20:10:1)
(11) Dimethylsulphoxide–*n*-butanol–water–glacial acetic acid (15:5:10:1)
(12) Pentane–acetonitrile–water–formic acid (60:20:15:5)
(13) *tert.*-Butanol–water saturated with phenol–propionic acid (50:38:12)
(14) Isopropanol–water–formic acid (40:10:2)
(15) Water saturated with phenol–*n*-butanol (30:70)
(16) Ethyl methyl ketone–ethanol–water–(0.88) ammonia (20:10:10:1)
(17) Isobutanol–ethanol–water–pyridine (3:2:1:1)
(18) Pyridine–methanol–water (2:1:1)
(19) Ethyl acetate–pyridine–water (7:3:1)
(20) *tert.*-Amyl alcohol–ethanol–water–(0.88) ammonia (10:1:1:1)
(21) *n*-Butanol–ethanol–water–(0.88) ammonia (2:2:1:1)
(22) Water
(23) 0.1% Aqueous sodium sulphate
(24) 4% Aqueous sodium sulphate.

Cellulose powder

Microcrystalline, available from Applied Science Laboratories.

Thin-layer plates

TLC plates were prepared as described previously with the exceptions noted below.

TABLE I

APPROXIMATE R_F VALUES OBTAINED

Plates: (a) Cellulose powder; (b) polyamide and Silica Gel G; (c) Alumina G; (d) alkaline Silica Gel G; (e) Sephadex. st = streak.

Colour	*Colour index No.*	*Solvent*								
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
		Plate								
		a	*a*	*a*	*d*	*a*	*b*	*a*	*b*	*a*
Fast Green FCF	42053	0.63	0.94	0.39	0.90	0.64	0.90	0.62	0.78	0.66
Green S	44090	0.63	0.84	0.35	0.90	0.64	0.90	0.62	0.78	0.66
Light Green Yellowish	42095	0.56	0.85	0.28	0.87	0.57	0.89	0.59	0.67	0.57
Brilliant Blue FCF	42090	0.56	0.92	0.40	0.87	0.57	0.89	0.59	0.67	0.57
Orange GGN	15980	0.42	0.56	0.30	0.81	0.44	0.24	0.28	0.30	0.42
Sunset Yellow FCF	15985	0.37	0.56	0.30	0.81	0.44	0.24	0.28	0.30	0.42
Orange G	16230	0.50	0.64	0.34	0.92	0.52	0.54	0.47	0.62	0.51
Ponceau MX	16150	0.47	0.77	0.22	0.90	0.46	0.07	0.31	0.15	0.46
Ponceau 3R	16155	0.47	0.77	0.22	0.82	0.46	0.07	0.31	0.15	0.46
Amaranth	16185	0.20	0.26	0.04	0.70	0.23	0.06	0.06	0.12	0.21
Violet BNP	—	0.77	0.81	0.80	0.95	0.78	0.89	0.68	0.88	0.81
Violet 5 BN	42650	0.77	0.81	0.80	0.95	0.78	0.89	0.68	0.88	0.81
Chocolate Brown FB	—	streak	0.17	0.00	0.78	st	st	st	st	0.72
Chocolate Brown HT	20285	streak	0.17	0.00	0.78	st	st	st	st	0.72

Alkaline Silica Gel G. Available from E. Merck. 20 g of Silica Gel G were stirred with 60 ml of a 2.5% sodium carbonate solution for 100 sec, the slurry spread, and the plates dried for 1 h at 105°.

Alumina G. Available from E. Merck. 25 g of Alumina G were stirred with 50 ml of water, the slurry spread, and the plates dried for 1 h at 105°.

Polyamide powder–Silica Gel G (1:4). Separately available from E. Merck. 20 g of a homogeneous mixture (ball milled during 24 h) was slurried with 60 ml of methanol, spread, and the plates dried at 80° for 1 h.

Sephadex G-25 (superfine). Available from Pharmacia. Slurries of the gel were made using the following media: (a) distilled water, or (b) 0.1% aqueous sodium sulphate solution, or (c) 4% aqueous sodium sulphate solution.

20 g of Sephadex G-25 were mixed with 100 ml of liquid and stood overnight. A 0.9-mm layer of gel was spread on the plates. Plates were run at an angle of 20° by downward displacement for 12 h prior to sample application and chromatography in the usual manner[2].

Results

The list of separations is given in Table I. The most useful results are listed in the first four columns. R_F values have been calculated, as before, by measuring to the leading edge of a spot.

Discussion

It was reported earlier[3] that most problems arise from the possibility of red and yellow dyes being present together in a mixture. This aspect has secured attention in the present study and some improved separations have been achieved. The separation

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
b	*a*	*b*	*a*	*b*	*a*	*d*	*c*	*c*	*d*	*a*	*c*	*e*	*e*	*e*
0.97	0.96	0.21	0.42	0.34	0.63	0.40	0.78	0.90	0.80	0.64	0.72			
0.97	0.96	0.21	0.42	0.34	0.63	0.40	0.78	0.90	0.80	0.64	0.72			
0.97	0.94	0.26	0.39	0.30	0.57	0.38	0.74	0.88	0.78	0.61	0.68			
0.97	0.94	0.26	0.39	0.30	0.57	0.38	0.74	0.88	0.78	0.61	0.68			
0.89	0.89	0.15	0.28	0.18	0.68	0.42	0.62	0.70	0.45	0.44	0.50			
0.89	0.89	0.15	0.28	0.18	0.68	0.42	0.62	0.70	0.45	0.44	0.50			
0.97	0.90	0.21	0.42	0.25	0.72	0.51	0.65	0.74	0.50	0.52	0.56			
0.91	0.89	0.17	0.39	0.23	0.48	0.40	0.74	0.82	0.39	0.42	0.48	0.40	0.16	0.08
0.91	0.89	0.17	0.39	0.23	0.48	0.40	0.74	0.82	0.39	0.42	0.48	0.40	0.16	0.08
0.56	0.50	0.08	0.21	0.04	0.28	0.16	0.52	0.61	0.15	0.20	0.21			
0.95	0.90	0.33	0.53	0.53	0.68	0.67	0.82	0.94	0.90	0.76	0.78	0.48	0.40	0.31
0.95	0.90	0.33	0.53	0.53	0.68	0.67	0.82	0.94	0.90	0.76	0.78	0.48	0.40	0.31
0.97	0.88	st	0.23	st	0.35	0.14	st	st	st	0.40	0.44	0.50	0.44	0.34
0.97	0.88	st	0.23	st	0.35	0.14	st	st	st	0.40	0.44	0.50	0.44	0.34

of Orange GGN and Sunset Yellow was only achieved on cellulose powder using solvent 1. Orange G could be separated from either of the above colours using a variety of stationary phases and solvents. Fast Green and Green S, and Light Green Yellowish and Brilliant Blue may be resolved on cellulose powder using solvent systems 2 and 3, respectively. None of the stationary phase and solvent systems examined allowed resolution of Ponceau MX and Ponceau 3R although on alkaline Silica Gel G using solvent system 4 these two dyes had slightly different R_F values. Amaranth is readily separated from the two Ponceau dyes using a variety of solvent mixtures. No separation was achieved for the dye pairs Chocolate Brown HT/ Chocolate Brown FB, and Violet BNP and Violet 5BN. Further studies on the Ponceau dyes are in progress.

One of us (N.Y.N.) wishes to thank the British Council for financial support for postgraduate studies. The authors thank the Government Chemist and Mr. A. W. HUBBARD and Dr. J. THOMSON of the Laboratory of the Government Chemist for their helpful association with this work.

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Received May 19th, 1972