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SEPARATION AND IDENTIFICATION OF FOOD COLOURS

I. IDENTIFICATION OF SYNTHETIC WATER SOLUBLE FOOD COLOURS USING THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A thin-layer chromatographic method is described for the separation and identification of forty-nine synthetic food colours which are used in food products or which have been used. R_F and R_X (with respect to Orange G) values are tabulated and a scheme for the rapid identification of the components of a mixture of dyes is proposed.

INTRODUCTION

Colouring matters in food

Processed foods are often coloured to retain the appearance of the original material and to provide a more appealing product. Foodstuffs may be coloured by (a) synthetic organic dyestuffs, (b) inorganic pigments and (c) natural colouring materials obtained from vegetable and animal sources. Synthetic organic dyestuffs are generally used. However, no two countries in the world have identical lists of permitted food colours because there are differences of opinion about the toxicity of the various food colours. Consequently it is possible that foodstuffs may be imported into a country which forbids the colouring matters present in the products. A method has been developed for the identification of synthetic food colours using thin-layer chromatography. The dyes covered by the method are those which are permitted in countries, who are members of the Codex Alimentarius Commission, or dyes which have been used in the past but are now considered too harmful for use in foodstuffs. These dyes are listed in Table I together with their colour index number and the countries in which they are permitted.

A number of thin-layer chromatographic separations of water soluble dyes used in food have been described but most of these deal with only a limited number of dyes; normally those permitted in one country only or a group of dyes of similar

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TABLE I

FOOD DYES PERMITTED IN VARIOUS COUNTRIES

Colour	*Colour index No. (1956)*	*Africa*	*America*		*Asia*					*Australia*		*Europe*												
		South Africa	*Canada*	*U.S.A.*	*India*	*Israel*	*Japan*	*Russia*	*Turkey*	*Australia*	*New Zealand*	*Austria*	*Czechoslovakia*	*Denmark*	*E.E.C. countries (six)*	*Finland*	*Norway*	*Poland*	*Portugal*	*Spain*	*Sweden*	*Switzerland*	*Great Britain*	*Yugoslavia*
Amaranth	16185	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Erythrosine	45430	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+	+	+
Ponceau 4R	16255	+			+	+	+			+	+	+	+	+	+	+	+	+		+	+	+	+	+
Citrus Red No. 2	12156		+	+																	+			
Orange I	14600																							
Quinoline Yellow	47005											+		+	+					+	+			+
Sunset Yellow FCF	15935	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Tartrazine	19140	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Fast Green FCF	42053	+	+	+			+									+	+				+	+		
Green S	44090										+			+	+								+	
Brilliant Blue FCF	42090	+	+	+					+	+														+
Indanthrene Blue	69800									+		+		+	+		+			+	+	+		+
Indigo Carmine	73015	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	
Patent Blue V	42051														+									
Carmoisine	14720	+			+	+			+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Scarlet GN	14815									+		+		+	+	+	+	+		+	+	+		+
Ponceau 6R	16290									+		+	+	+	+	+	+			+	+			
Fast Red E	16045				+								+	+		+	+			+	+	+	+	
Ponceau SX	14700											+		+										
Red FB	14780				+		+				+	+											+	
Red 6B	18055				+		+					+		+		+	+						+	

Red 10B	17200																				
Orange GGN	15980						+		+		+	+	+	+				+			
Orange RN	15970	+						+			+									+	
Orange G	16230	+									+									+	
Acid Yellow																					
(Fast Yellow AB)	13015						+		+		+	+	+	+	+		+	+	+		+
Chrysoin S	14270								+			+	+								
Oil Yellow GG (Sudan G)	11720	+												+				+		+	+
Oil Yellow XP	12740										+									+	
Yellow 2G	18965										+									+	
Guinea Green B	42085					+										+					
Light Green SF Yellowish	42095					+															
Blue VRS	42045				+	+		+	+		+		+								
Violet BNP	—							+			+									+	
Violet 6B																					
(Benzyl Violet 4B)	42640		+	+									+	+							
Brown FK	—						+	+			+									+	
Chocolate Brown FB	—						+	+			+									+	
Chocolate Brown HT	20285						+	+			+									+	
Black PN																					
(Brilliant Black BN)	28440				+		+		+	+	+	+	+	+	+			+	+	+	+
Black 7984	27755											+	+							+	
Ponceau 3R	16155															+					
Bordeaux B	16180	+																			
Eosine	45380					+															
Naphthol Yellow S	10316															+					
Violet 5BN	42650	+																			
Auramine	41000																				
Butter Yellow	11020																				
Chrysoidine	11270																				
Acid Magenta	42510																				
Methyl Violet	42535																				
Oil Orange E	12055																				
Oil Orange SS	12100																				
Oil Orange XO	12140																				
Oil Yellow AB	11380																				
Oil Yellow OB	11390																				
Rhodamine B	45170																				

TABLE II

CODES FOR DYES CHROMATOGRAPHED IN SOLVENTS 1, 2, 3, AND 4

Code	*Possible identity of dye*	*Code*	*Possible identity of dye*	*Code*	*Possible identity of dye*
AAAA	Blue VRS	DADC	Orange RN	EBDE	Carmoisine
	Brilliant Blue	DBCA	Naphthol Yellow S	EBEB	Quinoline Yellow
	Light Green	DBCB	Orange GGN	EBEC	Quinoline Yellow
	Yellowish		Sunset Yellow	ECCA	Orange RN
	Patent Blue V	DBCC	Orange GGN	ECCB	Fast Red E
AAAB	Brilliant Blue		Sunset Yellow	ECCC	Fast Red E
	Fast Green	DCCA	Orange RN		Indigo Carmine
	Green S	DCCB	Orange GGN		Orange RN
	Light Green		Sunset Yellow		Red 10 B
	Yellowish	DCCC	Acid Yellow	ECCD	Carmoisine
	Patent Blue V		Orange GGN		Indigo Carmine
	Yellow 2G		Orange RN		Red 10 B
AAAC	Yellow 2G		Sunset Yellow	ECCE	Carmoisine
AABA	Scarlet GN	DCCD	Acid Yellow		Red 10 B
AACA	Scarlet GN		Orange GGN	ECDA	Orange RN
ABAA	Brilliant Blue		Sunset Yellow	ECDB	Fast Red E
	Light Green	DCDA	Orange RN	ECDC	Bordeaux B
	Yellowish	DCDC	Orange RN		Fast Red E
ABAB	Brilliant Blue	DCDD	Red 6B		Indigo Carmine
	Light Green	DCDE	Red 6B		Orange RN
	Yellowish	DCED	Red 6B		Ponceau 3R
ABAC	Acid Magenta	DCEE	Red 6B		Ponceau MX
ABAD	Acid Magenta	DDDD	Amaranth		Ponceau SX
ACAC	Acid Magenta		Red 6B		Red 10 B
ACAD	Acid Magenta	DDDE	Red 6B	ECDD	Carmoisine
ACCD	Tartrazine	DDED	Red 6B		Indigo Carmine
ADCD	Ponceau 6R	DDEE	Red 6B		Ponceau SX
	Tartrazine	DEDD	Red 6B		Red 6B
ADCE	Ponceau 6R	DEDE	Red 6B		Red 10 B
AECD	Ponceau 6R	DEED	Red 6B	ECDE	Carmoisine
AECE	Ponceau 6R	DEEE	Red 6B		Red 6B
BAAA	Guinea Green B	EAAA	Auramine		Red 10 B
	Violet 5BN		Methyl Violet	ECEC	Bordeaux B
	Violet BNP		Rhodamine B		Ponceau 3R
BBBB	Orange G		Violet 6B		Ponceau MX
BCBC	Ponceau 4R	EABA	Auramine		Ponceau SX
BCCC	Ponceau 4R		Methyl Violet	ECED	Ponceau SX
BCCD	Tartrazine		Rhodamine B		Red 6B
BDCD	Tartrazine	EACA	Auramine	ECEE	Red 6B
CAAA	Guinea Green B		Eosine	EDCC	Indigo Carmine
	Violet BNP		Erythrosine		Red 10 B
	Violet 5BN		Chrysoidine	EDCD	Indigo Carmine
	Violet 6B		Orange I		Red 10 B
CACB	Chrysoin S		Orange RN	EDCE	Red 10 B
CACC	Chrysoin S	EACB	Chrysoidine	EDDC	Indigo Carmine
CBCA	Naphthol Yellow S		Eosine		Red 10 B
CBCB	Orange GGN		Erythrosine	EDDD	Indigo Carmine
	Sunset Yellow		Orange I		Red 6B
CBCC	Orange GGN	EACC	Chrysoin S		Red 10 B
	Sunset Yellow		Orange RN	EDDE	Red 6B
CCBC	Ponceau 4R	EADA	Chrysoidine		Red 10 B
CCCB	Acid Yellow		Orange I	EDED	Black 7984
	Orange GGN		Orange RN		Black PN
	Sunset Yellow	EADB	Orange I		Red 6B
CCCC	Acid Yellow		Quinoline Yellow	EDEE	Black 7984
	Orange GGN	EADC	Orange RN		Black PN

TABLE II *(continued)*

Code	*Possible identity of dye*	*Code*	*Possible identity of dye*	*Code*	*Possible identity of dye*
	Ponceau 4R		Quinoline Yellow		Red 6B
	Red 2G	EAEB	Quinoline Yellow		Red FB
	Sunset Yellow	EAEC	Quinoline Yellow	EEDD	Red 6B
CCCD	Acid Yellow	EBCB	Fast Red E	EEDE	Red 6B
CCDC	Red 2G	EBCC	Fast Red E	EEED	Black 7984
DAAA	Rhodamine B	EBCD	Carmoisine		Black PN
	Violet 6B	EBCE	Carmoisine		Red 6B
DABA	Rhodamine B	EBDB	Fast Red E	EEEE	Black 7984
DACA	Orange RN		Quinoline Yellow		Black PN
DACB	Chrysoin S	EBDC	Fast Red E		Red 6B
DACC	Chrysoin S		Quinoline Yellow		Red FB
	Orange RN	EBDD	Carmoisine		Indanthrene Blue
DADA	Orange RN				

colour[1–13]. Cellulose and silica gel appeared to be the two most promising adsorbents for the separation of the water soluble dyes and so we have used only these two adsorbents with a variety of development solvents as listed in Table III.

A scheme for the quick identification of a colour or mixture of colours is proposed which is not dependent on the measurement of R_F values. This consists of running the dye or mixture of dyes in four solvents on thin-layer plates coated with cellulose with two standard dyes and then giving the dyes a code depending on where they travel to in relation to the two standard dyes. This code is compared with the list of codes given in Table II thereby giving an initial identification of the dyes. The identity of the food colour is then confirmed by running in solvents together with spots of the suspected food colours.

TABLE III

CHROMATOGRAPHIC SOLVENTS USED IN THE THIN-LAYER CHROMATOGRAPHIC SEPARATION OF THE DYES

Solvents 1–10 are used with cellulose plates; solvents 11–15 are used with silica gel plates.

Solvent No.	*Composition*	*Reference*
1	Trisodium citrate (2 g), water (85 ml), 0.88 ammonia (15 ml)	1
2	*tert.*-Butanol–propanoic acid–water (50:12:38)	1
3	Trisodium citrate (2 g), hexamine (5 g), water (50 ml), methanol (50 ml)	3
4	2-Methyl propan-1-ol–water–ethanol–0.88 ammonia (25:25:50:2)	2
5	Propan-1-ol–ethyl acetate–water (6:1:3)	1
6	Butan-1-ol–water–glacial acetic acid (20:12:10)	—
7	Hydrochloric acid, S.G. 1.18–water (23:77)	—
8	Butan-1-ol–water–pyridine–ethanol (4:4:2:2)	—
9	Ethyl methyl ketone–acetone–water–0.88 ammonia (70:30:30:0.5)	—
10	Butan-1-ol–water–ethanol–quinoline (4:4:3:2)	—
11	Propan-2-ol–0.88 ammonia (4:1)	2
12	Propan-2-ol–0.88 ammonia (85:15)	—
13	Methanol–chloroform–water–quinoline (4:2:2:2)	—
14	Methanol–chloroform–quinoline (4:4:2)	—
15	Propan-2-ol–chloroform–water–diethylamine (50:25:20:15)	—

MATERIALS AND METHODS

Apparatus

Thin-layer chromatographic apparatus for the preparation of thin layers 0.25 mm thick on 200 × 200 mm glass plates. Chromatographic development tanks. 5 μl pipettes *e.g.* Microcap disposable pipettes.

Reagents

Cellulose powder. Microcrystalline cellulose, available from Applied Science Laboratories Inc. Prepare plates as follows: Shake 20 g cellulose powder with 60 ml methanol for 3 min and blend at high speed for 30 sec. Spread onto plates and air-dry or dry in an oven at 80°.

Silica Gel G. Available from E. Merck. Prepare plates as follows: Shake 30 g Silica Gel G with 60 ml water for 1 to 2 min. Spread onto plates and, after the layer has set, activate the plates by heating to 105° for 1 h.

Reference dye solutions. 0.1 % in water.

Chromatographic solvents. See Table III. All solvent mixtures should be freshly prepared.

Procedure

Place two spots of 1–2 μl of the dye solution onto each of four cellulose plates at a distance of at least 20 mm from the edge and bottom of the plate. Also spot on the plates 1–2 μl of a solution of Orange G and a solution of Amaranth as reference spots and place a spot of a mixture of Orange G and Amaranth on top of one of the sample spots. Dry the spots by placing the plates in an oven at 105° for 5–10 min. Develop the cooled plates in solvents 1, 2, 3 and 4 for a length of run of about 150 mm at room temperature. Remove the plates from the tanks and allow them to air dry. When the plates are dry rule lines across so as to divide the plates into the following sections: code A: spots travelling above Orange G; code B: spots travelling with Orange G; code C: spots travelling below Orange G but above Amaranth; code D: spots travelling with Amaranth; code E: spots travelling below Amaranth.

Check whether the sample has affected the development characteristics of Orange G and Amaranth and if so make allowance for this when dividing the plate into sections. Observe which section the spots from the sample solution appear in for each plate and write down all possible composite codes for each spot by listing the code individual letters in the order—solvent 1, solvent 2, solvent 3, solvent 4. Compare the codes with the list given in Table II and hence obtain a preliminary identification of the dyes. When two or more spots are similar in colour, cross code the dyes so that all possible dyes are obtained from Table II. Also if a dye is visible in one solvent but not in another then this indicates that the dye is masked by another dye and so all codes for spots in that solvent must be used in constructing the composite codes. A further identification of the dyes may be obtained by calculating the R_F and R_X (with respect to Orange G) values and referring to the Tables IV–VII. This will eliminate some of the dyes obtained from Table II. All R_F and R_X values have been calculated by measuring to the leading edge of the spots.

The identification of the sample dye is then confirmed by chromatography on a plate with standard spots of the suspected colours using suitable solvents. Spots of

TABLE IV

R_F AND R_X (WITH RESPECT TO ORANGE G) VALUES FOR RED DYES

Colour	Colour index No.	*Approximate R_F values*									*Approximate R_X values*								
		Solvent No.									*Solvent No.*								
		1	*2*	*3*	*4*	*5*	*6*	*8*	*9*	*11*	*1*	*2*	*3*	*4*	*5*	*6*	*8*	*9*	*11*
Amaranth	16185	0.6	0.3	0.5	0.6	0.4	0.2	0.6	0.4	0.4	0.8	0.4	0.5	0.8	0.7	0.4	0.8	0.4	0.9
Bordeaux B	16180	0.2	0.6	0.4	0.6	0.5	0.6	0.7	1.0	0.4	0.3	0.9	0.4	0.8	0.9	1.0	1.0	1.0	0.9
Carmoisine	14720	0.3	0.7	0.6	0.5	0.7	0.6	0.8	0.9	0.4	0.4	1.1	0.6	0.7	1.1	1.0	1.1	0.9	0.9
Eosine	45380	0.2	1.0	0.7	0.8	1.0	1.0	0.9	1.0	0.6	0.3	1.5	0.7	1.1	1.6	1.7	1.3	1.0	1.4
Erythrosine	45430	0.1	1.0	0.7	0.9	1.0	1.0	0.9	1.0	0.7	0.2	1.5	0.7	1.2	1.6	1.7	1.3	1.0	1.6
Fast Red E	16045	0.4	0.7	0.6	0.7	0.6	0.5	0.8	1.0	0.4	0.6	1.0	0.6	1.0	1.0	0.9	1.1	1.0	0.9
Ponceau 3R	16155	0.2	0.6	0.4	0.6	0.5	0.5	0.8	0.9	0.3	0.3	0.9	0.4	0.8	0.7	0.9	1.1	0.9	0.7
Ponceau 4R	16255	0.7	0.5	0.9	0.6	0.4	0.3	0.7	0.6	0.2	1.0	0.6	1.0	0.8	0.7	0.5	1.0	0.6	0.4
Ponceau 6R	16290	0.8	0.2	0.8	0.4	0.3	0.1	0.6	0.2	0.1	1.1	0.2	0.8	0.5	0.4	0.2	0.8	0.2	0.2
Ponceau MX	16150	0.2	0.7	0.5	0.6	0.5	0.5	0.8	0.9	0.4	0.3	0.9	0.5	0.8	0.9	0.9	1.1	0.9	0.8
Ponceau SX	14700	0.4	0.7	0.5	0.6	0.5	0.5	0.8	0.9	0.4	0.6	0.9	0.5	0.8	0.9	0.9	1.1	0.9	0.8
Red 2G	18050	0.6	0.6	0.7	0.6	0.4	0.5	0.7	0.9	0.4	0.8	0.8	0.7	0.8	0.7	0.9	1.0	0.9	0.9
Red 6B	18055	0.4	0.3	0.4	0.5	0.4	0.2	0.6	0.5	0.4	0.6	0.4	0.4	0.7	0.7	0.4	0.8	0.5	0.9
Red 10B	17200	0.2	0.5	0.6	0.6	0.4	0.3	0.7	0.8	0.4	0.3	0.6	0.6	0.8	0.7	0.5	1.0	0.8	0.9
Red FB	14780	0.0	0.3	0.1	0.2	0.4	0.2	0.7	0.4	0.6	0.0	0.4	0.1	0.3	0.7	0.4	1.0	0.4	1.3
Rhodamine B	45170	0.5	1.0	0.9	1.0	1.0	1.0	0.9	1.0	0.8	0.7	1.5	1.0	1.3	1.6	1.7	1.4	1.0	1.8
Scarlet GN	14815	0.9	0.7	0.9	0.8	0.8	0.6	0.8	1.0	0.5	1.1	0.9	1.0	1.1	1.2	1.0	1.1	1.0	1.2

TABLE V

R_F AND R_X (WITH RESPECT TO ORANGE G) VALUES FOR YELLOW AND ORANGE DYES

Colour	Colour index No.	*Approximate R_F values*									*Approximate R_X values*								
		Solvent No.									*Solvent No.*								
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*11*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*11*
Auramine	41000	0.3	1.0	0.9	1.0	0.9	1.0	not visible	0.8	0.8	0.4	1.4	1.0	1.6	1.4	1.9	not visible	1.3	1.8
Acid Yellow	13015	0.7	0.6	0.9	0.6	0.6	0.5	0.7	0.7	0.4	0.8	0.9	1.0	0.9	0.9	0.9	1.1	1.0	1.0
Chrysoidine	11270	0.1	0.8	0.7	0.9	0.8	0.9	0.1	0.9	0.8	0.2	1.2	0.7	1.6	1.2	1.7	0.2	1.4	1.8
Chrysoin S	14270	0.5	0.8	0.8	0.6	0.9	0.7	0.4	0.8	0.5	0.6	1.2	0.9	0.9	1.3	1.4	0.7	1.2	1.0
Naphthol Yellow S	10316	0.6	0.7	0.8	0.7	0.7	0.6	not visible	0.7	0.5	0.7	1.0	0.9	1.1	1.1	1.1	not visible	1.1	1.0
Orange G	16230	0.8	0.7	0.9	0.6	0.7	0.5	0.7	0.7	0.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Orange GGN	15980	0.6	0.7	0.8	0.6	0.6	0.5	0.2	0.7	0.4	0.8	1.0	0.9	1.0	1.0	1.0	0.3	1.0	1.0
Orange I	14600	0.4	0.8	0.8	0.7	0.9	0.7	0.1	0.8	0.5	0.6	1.2	0.9	1.1	1.3	1.4	0.2	1.2	1.2
Orange RN	15970	0.4, 0.5	0.9	0.7	0.6, 0.8	0.6, 0.9	0.5, 0.8	0.1	0.7, 0.8	0.7	0.5, 0.7	1.2	0.8	0.9, 1.3	0.8, 1.4	0.9, 1.5	0.2	1.0, 1.2	1.6
Quinoline Yellow	47005	0.1, 0.3	0.7	0.4	0.6	0.7	0.6	0.1	0.6, 0.7	0.7	0.1, 0.4	1.0	0.5	1.0	1.1	1.2	0.1	0.9, 1.0	1.6
Sunset Yellow	15985	0.6	0.7	0.8	0.6	0.6	0.5	0.2	0.7	0.4	0.7	0.9	0.9	1.0	0.9	1.0	0.4	1.0	1.0
Tartrazine	19140	0.8	0.4	0.8	0.4	0.5	0.3	0.4	0.5	0.3	1.0	0.6	0.8	0.6	0.7	0.6	0.6	0.7	0.6
Yellow 2G	18965	0.9	0.8	1.0	0.6	0.8	0.6	0.9	0.7	0.4	1.1	1.2	1.1	1.0	1.2	1.2	1.5	1.0	1.0

TABLE VI

R_F AND R_X (WITH RESPECT TO ORANGE G) VALUES FOR BROWN AND VIOLET DYES

Colour	*Colour index No.*	*Approximate R_F values*								*Approximate R_X values*							
		Solvent No.								*Solvent No.*							
		1	*2*	*3*	*4*	*5*	*11*	*14*	*15*	*1*	*2*	*3*	*4*	*5*	*11*	*14*	*15*
Brown FK	—	streak	streak	streak	0.6	streak	0.7, 0.8	streak	0.4, 0.5	streak	streak	streak	0.9	streak	1.1, 1.3	streak	0.6, 0.7
Chocolate Brown FB	—	streak	streak	streak	streak	streak	0.0	0.0	small streak	streak	streak	streak	streak	streak	0.0	0.0	small streak
Chocolate Brown HT	20285	streak	streak	streak	streak	streak	0.0	0.0	long streak	streak	streak	streak	streak	streak	0.0	0.0	long streak
Acid Magenta	42685	1.0	0.4, 0.6	0.9	0.6	0.5, 0.6	0.2	not visible	not visible	1.4	0.7, 1.0	1.1	0.8	0.8, 0.9	0.3	not visible	not visible
Methyl Violet	42535	streak	1.0	0.8, 0.9	1.0	1.0	0.8, 0.9	0.8	0.7	streak	1.5	1.0, 1.1	1.4	1.6	1.3, 1.4	1.9	1.1
Violet BNP	—	0.7	0.8	0.9	0.9	0.9	0.5	0.4	0.5	1.0	1.3	1.1	1.3	1.4	0.7	1.1	0.7
Violet 5BN	42650	0.7	0.8	0.9	0.9	0.9	0.5	0.4	0.5	1.0	1.3	1.1	1.3	1.4	0.7	1.1	0.7
Violet 6B	42640	0.6	0.8	1.0	1.0	0.9	0.5, 0.6, 0.7	0.4, 0.5	0.6	0.8	1.3	1.2	1.4	1.4	0.8, 0.9, 1.0	1.1, 1.2	0.9

ABLE VII

$_F$ AND R_X (WITH RESPECT TO ORANGE G) VALUES FOR GREEN, BLUE AND BLACK DYES

olour	Colour index No.	*Approximate R_F values*									*Approximate R_X values*								
		Solvent No.									*Solvent No.*								
		1	2	3	4	5	11	12	13	10	1	2	3	4	5	11	12	13	10
ast Green FCF	42053	0.9	0.8	1.0	0.8	0.8	0.1	0.1	0.8	0.8	1.2	1.1	1.1	1.0	1.1	0.5	0.2	1.0	1.0
reen S	44090	0.9	0.8	0.9	0.8	0.8	0.1	0.1	0.7	0.8	1.2	1.1	1.0	1.0	1.2	0.4	0.2	0.9	1.0
uinea Green B	42085	0.7	0.9	1.0	1.0	0.9	0.3	0.3	0.8	0.9	0.9	1.2	1.1	1.3	1.3	1.1	1.4	1.0	1.2
ight Green Yellowish	42095	0.9	0.8	1.0	0.9	0.8	0.3	0.1	0.8	0.8	1.1	1.1	1.1	1.2	1.1	0.9	0.5	1.0	1.0
lue VRS	42045	0.9	0.9	1.0	0.9	0.9	0.3	0.2	0.8	0.9	1.1	1.2	1.1	1.2	1.3	1.1	1.0	1.0	1.2
rilliant Blue FCF	42090	0.9	0.8	1.0	0.9	0.8	0.3	0.2	0.8	0.8	1.1	1.1	1.1	1.2	1.1	1.0	0.7	1.0	1.0
ndanthrene Blue[a]	69800	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ndigo Carmine	73015	0.2, 0.3	0.4	0.5	0.6	0.5	0.3	0.2	0.8	0.7	0.3, 0.4	0.6	0.6	0.8	0.7	1.0	1.0	1.0	0.9
atent Blue V	42051	0.9	0.9	1.0	0.9	0.9	0.1	0.0	0.8	0.9	1.2	1.2	1.1	1.1	1.3	0.2	0.0	1.0	1.2
lack 7984	27755	0.2	0.3	0.2	0.4	0.4	0.1	0.0	0.7	0.5	0.2	0.4	0.2	0.6	0.5	0.4	0.0	0.9	0.7
lack PN	28440	0.4	0.3	0.2	0.4	0.4	0.1	0.0	0.7	0.7	0.4	0.4	0.2	0.6	0.5	0.4	0.0	0.9	0.9

[a] Indanthrene Blue is insoluble in water and most organic solvents.

the sample solution are also 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.

If the sample contains several dyes, more than one solvent may be necessary for complete confirmation of the dyes.

DISCUSSION

In constructing the table of codes for the dyes, slight variations in the development characteristics of the dyes have been taken into account so that some dyes occur under a number of different codes. Brown FK, Chocolate Brown FB and Chocolate Brown HT have not been included in this table as they streak in the solvents used. If the standard dyes, Orange G and Amaranth, run very differently when overspotted on the sample from when they are spotted separately on the plate then the spots in the sample should be coded twice, once using the standards in the sample to divide up the plate and once using the standard spotted separately to divide up the plate. By this means all possible dyes will be obtained, but a number of these will be rejected on the basis of colour and R_F value. However, do not discount dyes which could give rise to the colour of the spot, *e.g.* an orange coloured spot may be a red and yellow dye superimposed.

As most problems arise from the possibility of a red and yellow dye being together in the mixture, the separation of the reds, oranges and yellows are set out in Table VIII. All R_F and R_X values have been calculated by measuring to the leading edge of a spot as this was found to be more reliable for spots which tail. When confirming the identity of a dye by running it with standard dyes it is useful to observe

TABLE VIII

SEPARATION OF REDS, ORANGES AND YELLOWS IN SOLVENTS 1, 2, 3 AND 4
Dyes in italics are completely separated from the others.

$R_F = 1.0$

Solvent 1	*Solvent 2*	*Solvent 3*	*Solvent 4*
Yellow 2G	Eosine Erythrosine Rhodamine B	*Yellow 2G*	Rhodamine B Auramine
Scarlet GN	*Auramine*	*Rhodamine B*	Chrysoidine Orange RN
Tartrazine	*Orange RN*	*Auramine*	*Ethyrhrosine*
Ponceau 6R	Chrysoin S Chrysoidine Orange I	*Orange G*	Eosine Scarlet GN Naphthol Yellow S Orange I
Orange G	*Yellow 2G*	Scarlet GN Acid Yellow Chrysoin S Naphthol Yellow S	Orange G Yellow 2G
Ponceau 4R	Scarlet GN Naphthol Yellow S	Eosine Erythrosine Ponceau 4R Ponceau 6R Orange GGN Orange I Orange RN Sunset Yellow Tartrazine	Fast Red E Acid Yellow Chrysoin S Orange GGN Quinoline Yellow Sunset Yellow
Acid Yellow Red 2G Orange GGN Sunset Yellow Naphthol Yellow S	Orange G Carmoisine Quinoline Yellow	Amaranth Carmoisine Fast Red E Ponceau SX Red 2G Red 10 B Chrysoidine	Bordeaux B Ponceau 3R Ponceau 4R Ponceau MX Ponceau SX Red 2G Red 10 B Orange RN
Orange RN Amaranth Chrysoin S	Fast Red E Orange GGN Sunset Yellow	Bordeaux B Ponceau 3R Ponceau MX Quinoline Yellow	Amaranth Carmoisine Tartrazine
Orange RN Orange I Rhodamine B Red 6B Ponceau SX Fast Red E	Bordeaux B Ponceau 3R Ponceau MX Ponceau SX Acid Yellow Orange RN	*Red 6B*	Ponceau 6R Red 6B
Carmoisine Bordeaux B Auramine Quinoline Yellow	Ponceau 4R Red 2G	*Red FB*	*Red FB*
Eosine Ponceau 3R Ponceau MX Red 10 B Erythrosine	Red 10 B Tartrazine		
Chrysoidine Quinoline Yellow	Amaranth Ponceau 6R Red 6B Red FB		
Red FB			

$R_F = 0$

the plate under UV light of 254 nm and 350 nm as some of the dyes fluoresce.

The following mixtures of dyes could not be separated in any of the solvents tried: Chocolate Brown HT and Chocolate Brown FB, Ponceau 3R and Ponceau MX, Violet 5BN and Violet BNP.

Chocolate Brown HT can be tentatively distinguished from Chocolate Brown FB by running in solvent 15 on silica gel. Chocolate Brown FB produces a small streak from the spotting line whereas Chocolate Brown HT produces two spots and a streak from the spotting line. The two spots travel higher than the streak from Chocolate Brown FB.

ALDRED[6] has reported that Violet 5BN and Violet BNP can be separated on silica gel using a mixture of 2-methyl propan-1-ol, ethanol and water as developing solvent. When this system was tried we did not obtain a separation of the samples of Violet 5BN and Violet BNP which we were using. Some of these dyes may be broken down during extraction from the foodstuffs, or in the foodstuff itself, and the decomposition products may affect the separation of the dyes. Work is in progress on these aspects to see how they will affect the identification scheme and further publications of the results of this work will follow.

Violet 5BN is permitted only in South Africa and Violet BNP is permitted only in Denmark, New Zealand and the United Kingdom. Consequently the need to separate these two dyes should not arise very often. However, they can be distinguished by their IR spectra. No work has been carried out on extraction of these dyes from foodstuffs and it is realised that co-extractives may affect the running characteristics of various dyes but by overspotting the sample with the suspected dyes in the final confirmation any irregularities should not affect the identification of the dyestuff.

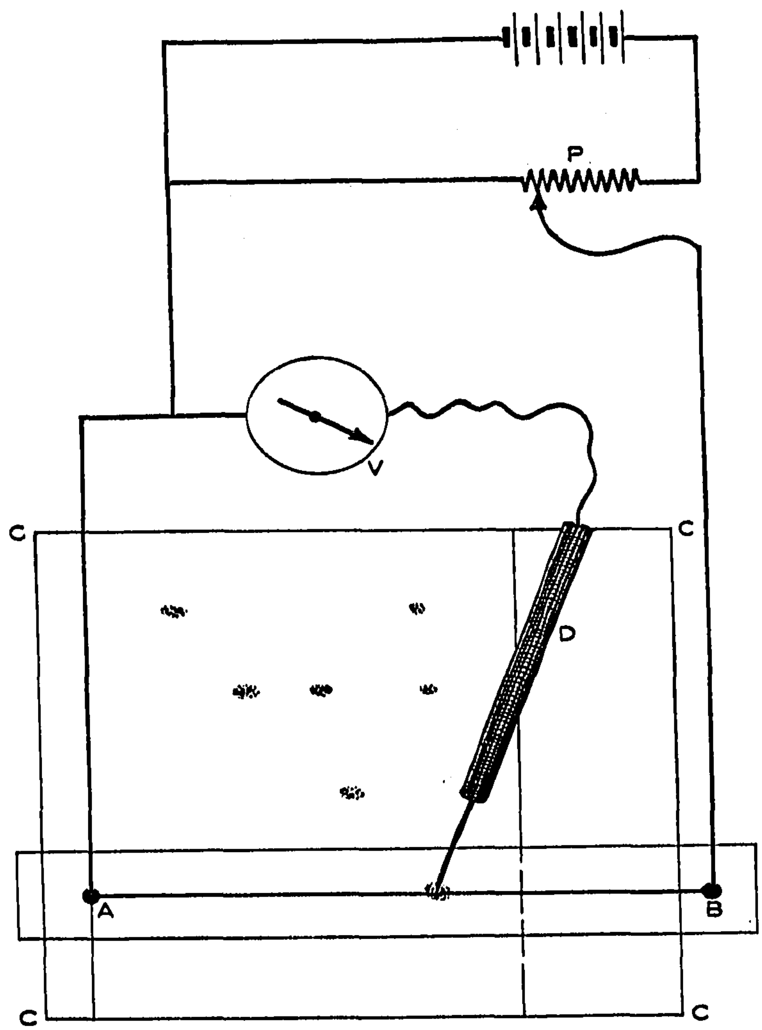

Fig. 1. A simple device for the measurement of R_F values. A — B, resistance wire mounted on perspex; C, a developed thin-layer plate; P, 1 kΩ potentiometer; V, 2.5 V. f.s.d. voltmeter; D, contact probe.

THIN-LAYER CHROMATOGRAPHIC TECHNIQUES

R_F measurement

To relieve the tedium of measuring a large number of R_F values a simple electrical device was constructed. The device consists of a perspex template which slides over the thin-layer plate. The template has a length of resistance wire stretched between two terminals and a sliding contact for making contact with the resistance wire. The resistance wire is made part of a simple potentiometer circuit as shown in Fig. 1. The template is placed over the thin-layer plate and adjusted so that terminal "A" is over the spotting line. The sliding contact is moved to the solvent front or the standard spot, if R_X values are required, and the potentiometer "P" adjusted so that the voltmeter reads 1.0 units. The sliding contact is then moved over the spot whose R_F or R_X value is required and the voltmeter reading noted. The template is then moved along keeping it in contact with the bottom edge of the plate until it is over the next spot.

Documentation of chromatograms

Copies of the thin-layer chromatograms were made by a simple blue print type procedure. The spots on the plates are scribed round with a needle and then placed coated surface down on a piece of "Blackline" paper. (Blackline paper for ammonia development, ZY5M, obtainable from Mason Ltd., Colchester.) The back of the plate is illuminated by means of photoflood bulbs for approximately 40 sec. The plate is removed and the paper is suspended in a tank containing a few millilitres of 0.88 ammonia solution for about 1 min. The print obtained consists of black spots ringed with a white line.

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