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ION-PAIR EXTRACTION AND ION-PAIR ADSORPTION THIN-LAYER CHROMATOGRAPHY FOR RAPID IDENTIFICATION OF IONIC FOOD DYES

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SUMMARY

An easy, rapid and accurate method for routine analysis of seventeen commonly used ionic food dyes by means of ion-pair extraction and ion-pair adsorption thin-layer chromatography on silica gel plates has been developed. Cetyltrimethylammonium bromide was selected as the counter ion for both isolation and separation. Its concentration in the sample before extraction with methylene chloride should be around 0.0125 *M*. A pH value of 2.5 for the extraction gives an acceptable recovery of most of the dyes. The thin-layer plates were impregnated with the counter ion which was also present in the eluent. Sharp spot application yields a very good separation, enabling satisfactory identification of the dyes.

INTRODUCTION

General features of the existing methods for analysis of dyes are that they are time-consuming, laborious and do not always yield a satisfactory separation. In most cases, identification is achieved by paper or thin-layer chromatography, although the number of high-performance liquid chromatographic (HPLC) methods is steadily increasing[1,2]. A thorough procedure for the systematic identification of 49 synthetic food dyes has been elaborated by Hoodless *et al.*[3].

Drevon and Laur[4] were the first to employ quaternary ammonium salts to isolate food dyes. Several workers have subsequently applied this method[5–8] but none has demonstrated the potential of the combination of ion-pair extraction and ion-pair chromatography. In the work presented here seventeen common food dyes, all containing sulphonate or carboxylate groups, are both isolated and chromatographed as their ion pairs with the cetyltrimethylammonium ion. The main advantage of this procedure is that it allows a rapid identification with a minimum of sample treatment and within a reasonable time, and is highly reliable.

Both permitted and non-permitted additives were considered. However, since no two countries in the world have identical lists of permitted colour additives, no distinction is made between the two categories.

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EXPERIMENTAL

Reagents

All compounds were used as purchased. Analytical grade chemicals were employed where available. The dye standards (Table I) were supplied by local distributors and were of "Food Grade" quality. They all contain sulphonate groups except erythrosine which has a carboxylate function. Cetyltrimethylammonium bromide (CTMA) was from UCB (Drogenbos, Belgium). Appropriate dilutions were made in distilled water. Dye solutions were prepared in buffer, except for erythrosine which was dissolved in buffer–methanol (1:1). Four standard solutions, each containing all

TABLE I

THE SULPHONATED DYES

Common name	*Colour index no.**	*EEC no.*	*Synonyms*
Yellow			
Tartrazine	19140	E102	FD & C Yellow No. 5; C.I. Food Yellow 4
Chrysoin S	14270	E103	C.I. Food Yellow 8; Resorcin Yellow
Quinoline yellow	47005	E104	C.I. Acid Food Yellow 3; D & C Yellow No. 10
Acid yellow	13015	E105	C.I. Food Yellow 2; Fast Yellow AB
Orange			
Sunset yellow FCF	15985	E110	FD & C Yellow No. 6; C.I. Food Yellow 3
Orange GGN	15980	E111	C.I. Food Orange 2
Red			
Azorubine	14720	E122	C.I. Food Red 3; Fast Red G; Carmoisine
Amaranth	16185	E123	FD & C Red No. 2; C.I. Food Red 9
Cochineal red A	16255	E124	C.I. Food Red 7; Ponceau 4R
Scarlet GN	14815	E125	C.I. Food Red 2
Ponceau 6R	16290	E126	C.I. Food Red 8, Scarlet 6R
Erythrosine**	45430	E127	FD & C Red No. 3; C.I. Food Red 14
Blue and black			
Patent blue V	42051	E131	C.I. Food Blue 3
Indigo carmine	73015	E132	FD & C Blue No. 2; C.I. Food Blue 1; Indigotine
Green S***	44090	E142	C.I. Food Green 4; Wool Green BS
Brilliant black BN	28440	E151	C.I. Food Black 1
Black 7984	27775	E152	

* *Rowe Colour Index*, 2nd ed., Bradford, Great Britain, 1956.

** Although not a sulphonated dye, this substance is included because of its widespread use among water-soluble food dyes.

*** Has a bluish shade at certain concentrations.

dyes of the same colour group (yellow, orange, red, blue + black) at a concentration of 0.025 % in water–methanol (1:1), were used as references for thin-layer chromatography (TLC). Buffers of different pH values were prepared by mixing known amounts of two stock solutions and appropriate dilution. The pH values were measured with a glass electrode and carefully adjusted where necessary. Elution solvents were freshly prepared before each chromatographic run. The thin-layer plates were pre-coated silica gel plates (Merck No. 5721, 20 × 20 cm, layer thickness 0.25 mm).

Isolation of the dyes

As the isolation from the samples was to be undertaken by batch extraction, the influence of several parameters on the efficiency of the procedure had first to be established.

pH value. A 0.01 % solution of each dye was prepared in buffers of different pH value (2, 2.5, 3, 3.5, 4, 5, 6). One millilitre of each dye solution, 1 ml of a 0.01 *M* CTMA solution and 2 ml methylene chloride were mixed in a glass-stoppered tube on a Vortex for 30 sec. After separation of the phases, the extraction efficiency was evaluated visually.

Counter-ion concentration. It is known from the theory of ion-pair extraction that the distribution ratio can be varied within certain limits by changing the nature and the concentration of the counter ion. As the nature of the counter ion selected was based upon empirical observations, the influence of the concentration remained to be examined. Therefore, 1 ml of the 0.01 % dye solutions in buffer (pH 2.5), 1 ml of aqueous CTMA solutions, having concentrations ranging from 0.1 *M* to 0.001 *M* and 2 ml methylene chloride were treated as described above. The dye concentration in both layers was evaluated visually.

Dye concentration. One millilitre of each of a series of dye solutions in buffer (pH 2.5) having concentrations ranging from 0.002 % to 0.1 %, 1 ml 0.025 *M* CTMA solution and 2 ml methylene chloride were treated as described above. The results were evaluated visually.

Sample extraction

Surface coloured sweets were shaken with buffer (pH 2.5) until the coloured substance had dissolved. The phases were immediately separated to avoid dissolution of the sugar and other substances which complicate the separation of the immiscible liquid layers in the next stage of the analysis.

Other samples were used as such after adjustment to pH 2.5 (*e.g.*, beverages) or macerated in methanol–water (1:1) slightly alkalinized with ammonium hydroxide (*e.g.*, preserved fruits, instant desserts, caviar). After filtration and evaporation to dryness, the residue was taken up in buffer (pH 2.5) solution.

Jellies, containing large amounts of gelatine, were treated as follows. A quantity of jelly was dissolved in the minimum volume of water. Two volumes of methanol containing 0.1 % ammonium hydroxide were added and the mixture cooled in a refrigerator at 4°C for 2 h to precipitate the proteins. After centrifugation the supernatant was evaporated, the residue taken up in 20 ml of water–methanol (1:4), alkalinized with 0.1 % ammonium hydroxide, and the entire procedure repeated. The final dry residue was dissolved in buffer (pH 2.5).

One millilitre of a coloured solution or of an extract, 1 ml of a 0.025 *M*

TABLE II

EXTRACTION EFFICIENCY OF A CONSTANT AMOUNT OF DYE (0.01%) AS A FUNCTION OF CONCENTRATION OF COUNTER IONS AT pH 2.5

Code: −, no dye extracted; =, both layers coloured; +, complete extraction.

*Concentration of counter ion (M)**	*E102*	*E103*	*E104*	*E105*	*E110*	*E111*	*E122*	*E123*	*E124***	*E125*	*E126*	*E127****	*E131*	*E132*	*E142*	*E151*	*E152*
0.1	−	−	−	−	−	−	−	−	−	−	−	(+)	−	−	−	−	−
0.05	+	+	=	+	+	+	−	−	−	−	+	(+)	+	−	+	=	+
0.025	=	+	=	=	+	+	+	+	+	+	+	(+)	+	=	+	+	+
0.01	−	=	=	=	=	=	+	=	=	+	=	(+)	+	=	+	+	+
0.005	−	=	=	−	=	=	+	=	=	+	−	(+)	+	−	=	=	−
0.0025	−	−	−	−	−	−	=	−	−	=	−	(+)	+	−	=	−	−
0.001	−	−	−	−	−	−	=	−	−	=	−	(+)	=	−	=	−	−

* Of the solution added to the dye solution (actual dilution 1:2).

** Experiments carried out at pH 3.

*** Due to the instability of the dye in an acidic medium, the aqueous layer always becomes discoloured after centrifugation, erroneously indicating complete recovery.

TABLE III

EXTRACTION EFFICIENCY OF VARIOUS AMOUNTS OF DYE AT CONSTANT pH (2.5) AND COUNTER-ION CONCENTRATION (0.025 *M* CTMA)

Code as in Table II.

Dye concentration (%)*	*E102*	*E103*	*E104*	*E105*	*E110*	*E111*	*E122*	*E123*	*E124***	*E125*	*E126*	*E127****	*E131*	*E132*	*E142*	*E151*	*E152*
0.1	=	=	=	=	=	=	=	=	=	+	=	(+)	+	=	+	+	=
0.08	=	=	=	=	=	=	=	=	=	+	=	(+)	+	=	+	+	=
0.06	=	=	=	=	=	=	+	+	=	+	+	(+)	+	=	+	+	+
0.04	=	=	=	=	=	=	+	+	=	+	+	(+)	+	=	+	+	+
0.02	=	=	=	=	+	+	+	+	=	+	+	(+)	+	=	+	+	+
0.01	=	+	=	+	+	+	+	+	+	+	+	(+)	+	=	+	+	+
0.008	=	+	+	+	+	+	+	+	+	+	+	(+)	+	=	+	+	+
0.006	=	+	+	+	+	+	+	+	+	+	+	(+)	+	=	+	+	+
0.004	=	+	+	+	+	+	+	+	+	+	+	(+)	+	=	+	+	+
0.002	=	+	+	+	+	+	+	+	+	+	+	(+)	+	=	+	+	+

* Of the sample.

****** See footnotes to Table II.

cetyltrimethylammonium bromide solution and 2 ml of methylene chloride were mixed in a glass-stoppered tube on a Vortex for 30 sec. In most cases, centrifugation was applied to speed up phase separation. Concentration of the organic layer, where indispensable, was carried out in a stream of nitrogen.

Thin-layer chromatography

Preliminary work had shown that adsorption chromatography of sulphonated dyes on impregnated layers was highly promising on silica gel plates. Therefore, attention was concentrated on the potential of silica gel as the sorbent. It soon became obvious that the counter ion should be present both in the eluent system and in the sorbent layer. This was achieved either by dipping the pre-coated plates in a dilute solution of the counter ion (0.1 *M* in methanol) followed by air drying or by developing them twice with a 0.1 *M* solution of the counter ion in methanol with careful air drying after each run. Aliquots (1–5 μl) of the extracts were spotted on thin-layer plates 2.0 cm from the bottom and 1.0 cm apart using a capillary pipette or a syringe. Reference standard solutions were similarly applied (2 μl), each spot corresponding to 0.5 μg of the individual dye. The mobile phases were: A, methanol–acetone (9:1) + 1 % glacial acetic acid, 0.1 *M* counter ion; B, methanol–acetone (1:1), 0.1 *M* counter ion. After solvent development in a saturated chamber to a distance of 15 cm, the plate was removed, air dried and examined both visually and under UV light of 366 nm.

RESULTS AND DISCUSSION

Isolation of the dyes

The highest extraction efficiency for most of the 0.01 % dye solutions was achieved with the buffer of pH 2.5 (50 ml 0.1 *M* potassium hydrogen phthalate + 38.8 ml 0.1 *M* HCl, diluted to 100 ml), although for some the yield was apparently higher with the buffer of pH 3 (50 ml 0.1 *M* potassium hydrogen phthalate + 22.3 ml 0.1 *M* HCl, diluted to 100 ml). For each of the dyes a certain amount of the ion pair formed was extracted into the organic layer. That ion-pair extraction had occurred was demonstrated by the fact that when the counter-ion solution was substituted by water no colour could be observed in the methylene chloride layer. The influence of counter-ion concentration is summarized in Table II. It can be seen that, with few exceptions, the concentration of the counter-ion solution should lie between 0.01 and 0.05 *M*. Higher concentrations of 0.1 *M* prevent extraction. Although no clear explanation of this behaviour can be given, Knox and Laird[9] suggested that, above a certain concentration, adsorption or micelle formation may be relevant. The poor extraction efficiency with less concentrated CTMA solutions results from poor ion-pair formation. The results in Table III show that the extraction efficiency is little or not affected by the dye concentration in most instances, in agreement with the theory of ion-pair extraction which assumes that any excess of counter ion should be present for ion-pair formation.

Thin-layer chromatography

For the chromatographic separation several counter ions with different lipophilic character and of various sizes were examined. The best results with regard to

separation efficiency were obtained with CTMA, so that this was selected for general use. The poor discriminating ability of many other ions was reflected in a moderate mobility of the ion pair or in a very pronounced tailing of the spots.

The two modes of plate impregnation did not yield identical results. Although both generally gave satisfactory results, the quality of the pre-developed plates was sometimes difficult to reproduce, considerable run-to-run differences in separation efficiency being encountered. An acceptable reproducibility was achieved when the plates were immersed for exactly 1 min in a fresh 0.1 *M* solution of the counter ion in methanol, followed by careful air drying in hot air.

A typical chromatogram required 35–45 min which is a relatively short time compared with the elution time of one to several hours with conventional butanol–water mixtures. Usually, well defined circular spots were obtained, an advantage of the present method compared to many others, where peak tailing considerably decreases separation efficiency and identification possibilities.

Identification in foodstuffs was achieved by comparison of the R_F values and colours of the unknowns with those of the constituents of the reference mixtures. UV detection at 366 nm was shown to be useful in corroborating the identity of azorubine (red fluorescence) or erythrosine (pale orange fluorescence) on chromatograms of a somewhat poorer quality.

A satisfactory separation of all dyes within each colour group was not possible with only one eluent system. Table IV shows the R_F values in the two selected mobile phases. The orange dyes may be developed in any of the solvent systems, but they were not separated. This means that when a spot with a given R_F value is detected one

TABLE IV

R_F VALUES OF SOME SULPHONATED DYES SEPARATED BY ION-PAIR THIN-LAYER CHROMATOGRAPHY ON IMPREGNATED SILICA GEL LAYERS

Solvents: A, methanol–acetone (9:1) + 1% glacial acetic acid, 0.1 *M* CTMA; B, methanol–acetone (1:1), 0.1 *M* CTMA.

Dye	*A*	*B*
Tartrazine	0.14	0.07
Chrysoin S	0.48	0.33
Quinoline yellow	0.33	0.28
Acid yellow	0.37	0.31
Sunset yellow FCF	0.32	0.25
Orange GGN	0.32	0.25
Azorubine	0.28	0.25
Amaranth	0.26	0.19
Cochineal red A	0.07	0.03
Scarlet GN	0.46	0.40
Ponceau 6R	0.17	0.07
Erythrosine	0.34	0.20
Patent blue V	0.36	0.34
Indigo carmine	0.38	0.29
Green S	0.44	0.39
Brillant black BN	0.12	0.09
Black 7984	0.16	0.12

should proceed to a further differentiation by other chromatographic procedures such as high-performance thin-layer chromatography (HPTLC)[10] or on conventional cellulose plates with water–concentrated HCl–*n*-butanol (62:28:10) as the eluent[11]. It was also shown that chromatographic spot resolution is especially dependent on the sharpness of the spots applied.

Low extraction efficiency may be overcome by adjusting the pH and/or counter-ion concentration. A practical example is quinoline yellow which over the whole dye concentration range was completely recovered at pH 4 upon addition of a 0.005 *M* CTMA solution. The determination of optimum conditions for quantitative extraction of each dye was beyond the scope of this paper, which was directed to rapid identification. Further research on the former is being undertaken.

Samples heavily loaded with interfering materials should be subjected to a pre-extraction with methylene chloride. After removal of the organic layer, the counter-ion solution is added and another portion of methylene chloride which will now extract the dye–CTMA ion pair. The appropriateness of the method was fully demonstrated with the various kinds of sample, all the dyes being correctly identified.

CONCLUSION

The method for ion-pair extraction and ion-pair adsorption TLC presented here requires only limited sample treatment and is very easy to apply. TLC development is much faster than conventional methods with butanol- and/or water-containing eluents. The high separation efficiency, due to the sharpness of the spots, offers a highly reliable means of identification.

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