Journal of Chromatography, 84 (1973) 195–199

CHROM. 6818

Note

Separation and identification of food dyes

V. Examination of Ponceau 6R dyes: extraction of dyes from confectionery products (cakes, cake mixtures and pastries)

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(Received March 21st, 1973)

The first paper in this series[1] described a thin-layer chromatographic (TLC) method for the separation and identification of 49 water-soluble synthetic food dyes. It was noted that most problems arise when red and yellow dyes are present in a mixture. Improved resolutions of certain dye pairs were later developed[2]. The use of permitted dyes is constantly under review and with Great Britain's entry into the Common Market the question of the revision of such lists, in order to ensure uniform action, suggests that further studies of dyes is pertinent. In the present U.K. list of permitted dyes there occurs the red colour Ponceau 4R, the trisodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-6,8-disulphonic acid (C.I. 16255). In some countries a similar dye, Ponceau 6R is used[1] and confusion may arise from the fact that two different dyes are commonly referred to as Ponceau 6R[3]. U.K. samples of Ponceau 6R were confirmed to be the disodium salt of 1-naphthylazo-1-naphthyl-6,7-disulphonic acid (C.I. 16250), also known as Acid Red 44, and German samples the tersodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-3,6,8-trisulphonic acid (C.I. 16290), also known as Monazo dye, Acid Red 41 and Food Red 8. The samples of both materials were examined by normal chemical and spectroscopic methods in order to confirm their identities and were shown to be free from major impurities by thin-layer chromatography (TLC). As neither compound is on the permitted list, it is assumed that they were not used in U.K. foods. None the less, the possibility that in the process of preparing foods the Ponceau 6Rs could be reduced to give toxic materials has been examined.

EXPERIMENTAL

TLC plates

The TLC plates were prepared as described previously[2] from microcrystalline cellulose powder (Applied Science Lab., State College, Pa., U.S.A.) and from silica gel G (E. Merck, Darmstadt, G.F.R.).

Solvents

The following solvent systems were used:

(1) *n*-Butanol–water–glacial acetic acid (20 : 10 : 2)
(2) Trisodium citrate–water–0.88 ammonia (2 : 85 : 15)
(3) Isobutanol–ethanol–water–0.88 ammonia (60 : 20 : 20 : 1)
(4) *tert.*-Butanol–1% aqueous ammonia solution (100 : 44)
(5) Ethyl methyl ketone–acetone–water (7 : 3 : 3)
(6) Trisodium citrate–hexamine–water–methanol (2 : 5 : 50 : 50)
(7) *tert.*-Butanol–propionic acid–water (50 : 12 : 38).

TABLE I

APPROXIMATE R_F VALUES OBTAINED FOR DYES

Solvent No.	*Plate type*	*Dye C.I. 16250*	*Dye C.I. 16290*
1	Cellulose powder	0.46	0.00
2	Cellulose powder	0.36	0.77
3	Cellulose powder	0.57	0.08
4	Cellulose powder	0.87	0.77
5	Silica gel G	0.71	0.41
6	Cellulose powder	0.58	0.75
7	Silica gel G	0.95	0.93

RESULTS AND DISCUSSION

Chromatography

The list of separations is given in Table I. The R_F values were calculated, as before, by measuring to the leading edge of a spot. The structures of the dyes were confirmed by chemical and UV, IR, NMR and mass spectroscopic procedures (C.I. 16250, $\lambda_{max.}$ 514 nm, $\varepsilon_{max.}$ 14,000; C.I. 16290, $\lambda_{max.}$ 519 nm, $\varepsilon_{max.}$ 16,000).

Drastic reduction of dyes

Samples of each dye were heated with tin(II) chloride in hydrochloric acid, excess of sodium hydroxide was added and the solutions were extracted with diethyl ether. The extract was washed with water and concentrated. The extracts were spotted on to appropriate TLC plates alongside authentic samples of the expected reduction products and plates were then developed with solvent 5 and viewed under UV light. The R_F values of the various materials are given in Table II.

The reduction products were further confirmed as α-naphtylamine or naphthionic acid by using chemical and UV (Unicam SP1800), IR (Unicam SP200G), NMR (Perkin-Elmer R10) and mass spectroscopic (A.E.I. MS12) procedures.

TABLE II

APPROXIMATE R_F VALUES FOR REDUCED PRODUCTS FROM DYES

Plate	*Compound*	R_F
Cellulose powder	Reduced C.I. 16250	0.97
Cellulose powder	α-Naphthylamine	0 97
Silica gel F	Reduced C.I. 16290	0.71
Silica gel F	Naphthionic acid	0.71

Reduction of dyes in foodstuffs

Drastic reducing conditions similar to those used above would not normally be encountered at any stage of food preparation. However, as the reduction product of the U.K. Ponceau 6R (dye C.I. 16250), α-naphthylamine, is known to be carcinogenic, the following experiments were carried out in order to determine whether α-naphthylamine is likely to be produced during the use of the dye in foodstuffs. The common reducing substances in foodstuffs are ascorbic acid, sulphur dioxide and various sugars. Mixtures were calculated to simulate proportions likely to occur in practice as follows: 0.05 g of dye with 0.025 g of ascorbic acid or 0.01 g of anhydrous sodium sulphite or 5 g of sucrose made up in each in 10 ml distilled water. The mixtures were boiled for 30 min, cooled and the products were spotted on to TLC plates and developed in solvent No. 5. The R_F values of the various materials obtained are given in Table III.

TABLE III

APPROXIMATE R_F VALUES OF PRODUCTS OBTAINED UNDER MILD REDUCING CONDITIONS

Substance	*Cellulose powder*	*Silica gel G*
Dye C.I. 16250	0.84	0.71
After boiling with ascorbic acid	0.78	0.52
After boiling with sodium sulphite	0.84 and 0.80	0.64
After boiling with sucrose	0.84	0.71
α-Naphthylamine	0.97	0.93

Although ascorbic acid and sodium sulphite had some action on one Ponceau 6R (C.I. 16250), the products produced did not contain α-naphthylamine. No evidence was found of the presence of α-naphthylamine in any of the above mildly reducing solutions. It seems unlikely that Ponceau 6R will break down to α-naphthylamine in ordinary food preparation, but this would not preclude its formation by biological degradation prior to or after ingestion.

Extraction of dyes from confectionery products (cakes, cake mixtures or pastries)

Cakes and pastries were prepared so as to contain measured amounts of the two dyestuffs. The separation of the dyes from the prepared foodstuffs was carried

out according to the instructions given in the following procedure, which was adapted from that described by Lehmann *et al.*[4]. The major changes are in the solvent mixtures used and the use of silica gel–cellulose mixture in place of polyamide as absorbent.

Chromatographic columns. A 250 × 15 mm column with a ground-glass stopcock and two 50 × 15 mm columns with ground-glass stopcocks are required.

Reagents. All reagents must be of analytical grade purity. The following are required: acetone; absolute ethanol; methanol; light petroleum (boiling range 40–60°); 90% formic acid; 0.5 and 0.1 *N* hydrochloric acid; Celite 545; cellulose microgranular/CT (without additives) (H. Reeve Angel, Clifton, N.J., U.S.A.); silica gel/CT (without additives) (H. Reeve Angel); acetone–ammonia solution (40 ml of acetone, 9 ml of water, 1 ml of ammonia of sp. gr. 0.88) (this solution should be freshly prepared); polyoxyethylene sorbitan mono-oleate solution (1% in water); and tetramethylammonium hydroxide solution (25%, w/w, in water).

Procedure. Weigh 5 g of the sample into a glass evaporating basin and place it in a drying oven at 100° for 30 min. Add sufficient light petroleum to cover the dried sample (about 30 ml) and stir the mixture. Allow the solid to settle and decant off the light petroleum. Repeat this procedure two more times and then allow the residual light petroleum to evaporate. Grind the sample gently so as not to form too fine a powder, add 4 g of Celite to the sample and mix.

Place a plug of glass-wool in the end of a chromatographic tube (250 mm × 15 mm) and transfer the powdered sample to the tube. Pour 30 ml of acetone on to the top of the column and when the solvent has percolated through the whole length of the column apply a slight air pressure so as to aid uniform packing. Discard the eluate.

Carefully pour 50 ml of a mixture of methanol, water and tetramethylammonium hydroxide solution (40 : 9 : 1) through the column. Adjust the pH of the eluate to approximately 6 by the addition of dilute hydrochloric acid. Add 5 ml of 1% polyoxyethylene sorbitan mono-oleate solution and reduce the volume of the mixture to about one half on a steam-bath with the aid of a current of air blown over the surface of the liquid. Add an equal volume of water to the solution and allow it to cool.

Place a plug of glass-wool in a 15 × 500 mm chromatographic tube and add a suspension of equal amounts of cellulose powder and silica gel in water to the tube so as to give a column *ca.* 20 mm high. Rinse the walls of the tube with a small volume of acetone in order to aid the settling of the column packing and then place sand on top of the packed column so as to form a layer about 6 mm deep.

Pour the solution of extracted dye through the column and wash the column three times with 5 ml portions of acetone, five times with 5 ml portions of a mixture of chloroform, absolute ethanol, water and formic acid (100 : 90 : 10 : 1), three times with 5-ml portions of acetone and finally three times with 10-ml portions of water. Elute the dyes with the minimum volume of acetone–ammonia solution, rejecting the eluate until the dyes are eluted. Remove the ammonia by blowing a current of air over the surface of the liquid and then reduce the volume to about half on a steam-bath. Add an equal volume of water and adjust the pH to approximately 6 with hydrochloric acid. Pour the solution through a column of cellulose powder–silica gel in a second 15 × 500 mm chromatographic tube prepared as above and wash the column with the same volumes of solvents in the sequence as described above for the first column. Elute the dyes with the minimum volume of acetone–ammonia solution. Remove the ammonia by blowing a current of air over the surface of the liquid and then evaporate

the solution almost to dryness on a steam-bath. Dissolve the residue in a few drops of 0.1 *N* hydrochloric acid and use this solution for TLC.

Results

The recovery of dyes from the confectionery was compared with the same amount of dyestuff passed directly through the column by spectrophotometric means. The recovery of either dye was 98% from jam and 95% from cake. No breakdown products of the dyestuffs were observed on the TLC plate.

For materials that are mainly water-soluble, such as jams and jellies, the initial step of extraction with light petroleum is not necessary. In many instances, the third adsorption on to a chromatographic column is an unnecessary refinement.

ACKNOWLEDGEMENTS

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 other members of his staff for their helpful association with this work.

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