The identification of blue triphenylmethane food dyes by thin-layer chromatography

Thin-layer chromatographic methods for the identification of the water-soluble synthetic dyes used as colorants in foods and drugs have been reported by authors using alumina (Mottier & Potterat, 1955), cellulose (Wollenweber, 1962), polyamide (Davidek & Davidkova, 1967), Sephadex (Parrish, 1968) and silica gel (Barrett & Ryan, 1963). Regulations governing usage of dyes vary widely from one country to another (Gill, 1962; Nieman, 1964) and reliable means of establishing unequivocal identification is essential.

Chromatography is used but the behaviour of chemically related dyes on chromatograms can be a source of error in identification among a group like the blue triphenylmethane dyes. The members of this group have a widely differing geographical acceptability particularly between North America and Europe (Gill, 1962; Nieman, 1964). Several authors have commented on the problem of their separation in particular of separating Brilliant Blue F.C.F., Blue V.R.S., Fast Green F.C.F., Green S, and Patent Blue V (Saenz Lascano Ruis, 1964; Ney, Bergner & others, 1965; Chapman & Oakland, 1968).

This paper describes a chromatographic method for differentiating between these dyes on ion exchange cellulose layers.

Thin layer chromatographic techniques were used. The plates were 10 × 5 cm diethylaminoethylcellulose pre-coated on plastic sheets by Machery-Nagel & Co. The plates were pre-treated by running in the solvent to half the length of the plate, dried and then running twice in deionized water and again dried. The spot loading was 1·0 μl of 0·01% w/v aqueous dye solution applied using microcapillaries 2·5 cm from base of plate and developed over a distance of 5 cm.

Two solvent series were used: (*a*) Molar solutions of ammonium halides; fluoride, chloride, bromide and iodide. (*b*) 0·2M solution of ammonium salts of organic acids; acetate, citrate, carbonate and benzoate. The $R_F \times 100$ values were expressed with reference to Patent Blue V and are the mean of five determinations.

The results (Table 1) indicate that an adequate separation of these blue triphenylmethane food dyes can be obtained in the listed solvents, the best result being obtained with M ammonium iodide and 0·2M ammonium benzoate solutions. Azo dyes which are the most commonly used food dyes have very low R_F values in this system

Table 1. R_F *values relative to Patent Blue V of the dyes in the solvent series a* (M) *and b* (0·05M) *on diethylaminoethylcellulose layers.*

Dye	C.I. (1956)	Ammonium fluoride	Ammonium chloride	Ammonium bromide	Ammonium iodide
Fast Green FCF	42053	7·0	8·0	5·0	10·0
Brilliant Blue FCF.. ..	42090	33·0	46·0	66·0	65·0
Green S	44090	48·0	72·0	86·0	87·0
Blue VRS	42045	61·0	78·0	75·0	76·0
Patent Blue V*	42051	54·0	72·0	75·0	90·0
		Ammonium acetate	Ammonium citrate	Ammonium carbonate	Ammonium benzoate
Fast Green FCF	42053	10·0	10·0	6·0	19·0
Brilliant Blue FCF.. ..	42090	10·0	41·0	44·0	65·0
Green S	44090	40·0	65·0	51·0	51·0
Blue VRS	42045	160·0	85·0	59·0	78·0
Patent Blue V*	42051	14·0	24·0	64·0	74·0

* R_F value.

and as a result it is easy to separate the blue dyes from them except for Fast Green FCF.

Pharmacognosy Department, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cathays Park, Cardiff, Glamorgan, U.K. T. D. TURNER

Elanco Division, Eli Lilly and Company Limited, Basingstoke, Hampshire, U.K. B. E. JONES

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Gas-liquid chromatographic estimation of paracetamol

Methods for the gas-liquid chromatographic estimation of paracetamol in plasma and urine have recently been described (Prescott, 1971). The drug could not be chromatographed directly in small amounts without significant absorption losses, and prior conversion to trimethylsilyl (TMS) derivatives was necessary. There are disadvantages however, in the use of silylating agents. The di-TMS derivatives of paracetamol formed with *N,O*-bis(trimethylsilyl)acetamide (BSA) is susceptible to hydrolysis, and silylation of other compounds in plasma extracts may give rise to unwanted peaks on the chromatograms. Although better results were obtained with *N*-trimethylsilylimidazole (TMSI), the sensitivity of the assay is limited by the broad solvent front of the slowly eluting TMSI. Furthermore, the flame ionization detector electrodes become contaminated by deposits of silica.

An improved method is now described in which both paracetamol and *N*-butyryl-*p*-aminophenol (the internal standard) are acetylated. The derivatives are stable, sensitivity is increased and the analysis can be completed in a much shorter time. Phenacetin does not interfere, and could be estimated in a sample at the same time as paracetamol.

Phosphate buffer (1·0 ml, M, pH 7·4) is added to plasma or urine (2·0 ml) containing up to 50 μg of paracetamol in a 15 ml glass-stoppered tube. Redistilled ethyl acetate (5·0 ml) containing *N*-butyryl-*p*-aminophenol (5 μg/ml) is then added and extraction effected by gentle mechanical shaking for 10 min. After centrifugation, the upper organic phase is transferred with Pasteur pipettes to 10 ml tapered stoppered centrifuge tubes and taken to dryness using a rotary vacuum evaporator. Pyridine (5 μl) and acetic anhydride (15 μl) are then added to the residue, the tubes stoppered and the contents mixed with a vortex mixer. The tubes are incubated on a water bath at 45° for 20 min and 1–3 μl aliquots are injected directly into the gas chromatograph. Samples containing paracetamol (50–500 μg/ml) are extracted with ethyl acetate containing *N*-butyryl-*p*-aminophenol (50 μg/ml), the residue is dissolved in pyridine (15 μl) and acetic anhydride (30 μl), and 1 μl aliquots are injected into the chromatograph. Appropriate dilutions are made of more concentrated samples, and total