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Analytical Methods Section

The determination of food colours by HPLC with on-line dialysis for sample preparation

G. M. Greenway, N. Kometa & R. Macrae

School of Chemistry, University of Hull, Hull HU6 7RX, UK

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A simple method is reported for the determination of synthetic dyes in sugar rich foods. Extraction of the dyes is achieved with water at room temperature. The extract is then centrifuged or filtered prior to dialysis using an ASTED system. A portion of the dialysate is transferred directly to the HPLC column without further concentration, where adequate sensitivity is achieved for the determination of dyes in a range of foods. Samples studied include boiled sweets, fruit gums, lemon curd, jelly, blancmange and soft drinks.

INTRODUCTION

Synthetic colours, mainly azo dyes, have been used in a wide range of food products for many years. The sensory perception of colour is an important quality attribute and many processed products have been coloured either to replace natural colours destroyed during processing or to provide colour in products which would otherwise be colourless, as, for example, soft drinks. The current trend is, however, away from the use of such synthetic dyes despite the extensive toxicological screening which they have undergone. The lists of permitted synthetic dyes are progressively being reduced and a number of food processors are relying on the use of natural colours to impart the desired colour to their products. Unfortunately, many of the natural colours (e.g. anthocyanins, carotenoids and betalaines) do not have the same stability under processing conditions as their synthetic counterparts. There will always, therefore, be a tendency (or at least a temptation) for some food processors to include synthetic dyes in their products without the correct label designation.

There is, therefore, a well-defined need for precise and accurate methods for the determination of synthetic dyes in foods, particularly for the following reasons:

(i) to determine whether there are synthetic dyes present in foods and if so, whether they are correctly permitted;
(ii) to determine the levels of such dyes;
(iii) to confirm the absence of added dyes in foods where they are not declared;
(iv) to check on the stability of dyes during processing and storage (Damant *et al.*, 1989).

There are many well-documented methods for the chromatographic separation of synthetic dyes (Saag, 1988). These are either based on ion-exchange methods or now more commonly on ion-pair chromatography under reversed phase conditions. A detailed study of the factors affecting retention under these conditions has recently been published (Damant, 1990). The simplest mobile phase conditions are those based on ammonium acetate buffers. The problem in methods for the quantitative determination of synthetic dyes in foods does not, therefore, lie in their separation, but rather in the means for their quantitative isolation from the food matrix. Traditional methods, such as adsorption on to wool or polyamide powder (Lehmann, 1970) tend not to be quantitative and can lead to dye degradation. A milder means of extraction, either from the food itself (e.g. soft drinks) or from an aqueous extract of the food, would offer considerable advantages and this is the situation encountered with dialysis. This technique has been used as a means of sample preparation for vitamin analysis by HPLC (Nicholson *et al.*, 1984). However, only recently has a fully automated system been made commercially available, which allows considerable flexibility in terms of dialysis conditions, coupled with automated injection of the sample into the HPLC column (Green *et al.*, 1989). The power of the technique is further extended by allowing enrichment of the determinand in the dialysate on a small trace enrich-

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ment cartridge prior to elution to the analytical HPLC column. The combination of dialysis and trace enrichment then leads to a complete sample preparation systems for microconstituents of foods, which is marketed under the acronym ASTED (automated sample treatment through enrichment of dialysates).

MATERIALS AND METHODS

Reagents and standards

All reagents were of analytical grade. Solvents for chromatography were of HPLC grade.

Samples of synthetic dyes (Amaranth, Brown FK, Ponceau 4R and Sunset Yellow FCF) were kindly donated by Mr Stewart Reynolds (MAFF Laboratory, Norwich, UK). Stock solutions of the dyes, either singly or as a mixed standard (500 μg ml^{-1}) were prepared by dissolving the dye (50 mg) in 0·04 M ammonium acetate buffer (pH 7, 100 ml). These were diluted as necessary with the same acetate buffer to form working standards (0–30 μg ml^{-1}).

HPLC method

A Gilson pump (model 303) was used at a flow rate of 1·0 ml min^{-1}. The mobile phase consisted of 0·04 M ammonium acetate buffer (pH 7, 400 ml) and methanol (200 ml) made up to 1 litre with water. The column (150 × 4·6 mm i.d.) was packed with Spherisorb S5 ODS 2 (5 μm particle size) and was used at ambient temperature. Detection was achieved with an Applied Biosystems 757 absorbance detector set at 475 nm (0·005 AUFS).

ASTED conditions

The standard ASTED system (Gilson Medical Electronics, Villiers-le Bel, France) was used without modification. This consisted of a model 231 automatic sampling injector and two dilutors (Model 401). The dialyser (100 μl) was constructed of poly(methyl methacrylate) and fitted with a 15 kD molecular weight cut-off membrane. The dialyser recipient solvent was 0·04 M ammonium acetate (pH 7) and water was used as the priming solvent for dilutor 0. File 181 was configured as shown in Table 1. The method File 151 was prepared as shown in Table 2.

Table 1. File 181 configuration

Prompt	Value	Program variable
Rack code	50	AA0
Process number	2	AA1
Donor volume	100	AA2
Recipient volume	175	AA3
Concurrent	0	AA4

Table 2. File 151 format

Program	Value	Function variable
A0	130	Sample volume (μl)
C2	0	Sample height (mm)
A1	0	Reagent number
A17	5	Air gap volume (μl)
B0	0	Pulse mode
A13	200	Load volume (μl)
A15	3	Dispense speed 0
B1	500	Dialyser volume (μl)
B2	0	Aspirate speed 1
B3	5	Dispense speed 1
C11	200	Wait after injection (s)
A16	2000	Donor purge volume (μl)
B4	2000	Recipient purge volume (μl)
B5	500	Regeneration volume (μl)

Sample preparation

Soft drinks

Samples were degassed by purging with helium for 5 min and then used directly for analysis.

Boiled sweets and fruit gums

A number of sweets (usually a maximum of four) were weighed into a small beaker and dissolved in 0·04 M ammonium acetate buffer (15 ml) with the aid of an ultrasound bath. The solution was allowed to stand for 15 min and the supernatant was decanted into a volumetric flask (20 ml). Any residue was washed with ammonium acetate buffer, and the washings were used to dilute the extract to volume.

Jelly and blancmange

A portion of the sample (*c.* 10 g) was weighed into a small beaker and thoroughly mixed with 0·04 M ammonium acetate (10 ml). This was dispersed with the aid of an ultrasound bath for 20 min. The sample was then filtered into a 15 ml volumetric flask and made up to volume with further washings.

Assay procedure

The system was set-up for Process 2, using the ASTED User's Guide (Gilson, 1988). The HPLC column was equilibrated with mobile phase (*c.* 30 ml). The integration file was set up, followed by selection and running of the Configure file 181 and the Reset file 150. The samples were added to the sample rack (code 50) as described in the Users' Guide. The method file 151 was then selected and run after responding to tube number and number of samples.

RESULTS AND DISCUSSION

The original intention in this study was to exploit the full capabilities of the ASTED system for the quanti-

Table 3. Performance data for aqueous dye standards

	Linear range[a] (μg ml^{-1})	Precisions (% RSD)		Detection limit (2 σ) (μg ml^{-1})
		5 μg ml^{-1} (n = 10)	60 μg ml^{-1} (n = 10)	
Amaranth	0–150	1·53	0·54	0·64
Brown FK	0–150	1·57	0·81	1·58
Ponceau 4R	0–150	1·44	0·36	0·50
Sunset Yellow	0–200	1·83	0·53	0·50

[a] Correlation coefficients better than 0·9995.

tation of selected azo dyes in foods. That is to say, after suitable extraction from the food matrix, the aqueous dye solution would be subjected automatically to dialysis, and the dye, which transferred across the membrane, would then be concentrated from the recipient stream with a trace enrichment cartridge (TEC) (reversed phase), i.e. Process 1. In order for trace enrichment to be possible, each component must have a significant breakthrough volume (i.e. the volume of dye solution which can be passed through the TEC before dye appears in the effluent). These values were obtained for Amaranth (0·4 ml), Brown FK (major component 1·7 ml), Ponceau 4R (0·7 ml) and Sunset Yellow (1·7 ml). Only Brown FK and Sunset Yellow showed any significant degree of retention and so the system was configured for Process 2, in which a sample of the dialysate (recipient stream) is transferred directly to the analytical column without enrichment. Under these conditions the system still showed more than adequate sensitivity for the determination of colours in foods.

The system was characterised with a series of aqueous standards of the four dyes studied, Amaranth, Brown FK, Ponceau 4R and Sunset Yellow, with the results shown in Table 3. Even at low levels of dye (5 μg ml^{-1}) precision was found to be better than ±2% (RSD) and detection limits were around 0·5 μg ml^{-1}. The somewhat higher value for Brown FK is due to the fact that this is a multicomponent dye and only the major component (detectable at 475 nm) was used in these calculations. The detection limit for those dyes that can be retained on the TEC, such as Sunset Yellow, can be greatly reduced. The degree of retention of the dyes on the cartridge, and indeed the range of such dyes, could be increased by the addition of a suitable ion-pairing reagent into the recipient stream. However, this was found to be unnecessary in the present study.

The simplest and most effective way to extract water-soluble dyes from products with a high sugar or polysaccharide content is to use aqueous extraction assisted with ultrasound at room temperature. Under these conditions, products such as sweets and jellies lead to viscous solutions, while starch-based products such as blancmange lead to a suspension with the dye in solution in the supernatant. Simple filtration then leads to solution which can be applied directly to the ASTED system. In fact, even fine particulate material in suspension does not cause a problem, provided that the sample probe does not become blocked.

Precision for the method was checked on a sample of orange fruit gums, and for 10 replicate injections an RSD of 4·3% was found for Ponceau 4R (3 μg ml^{-1}) and 1·7% for Sunset Yellow (10 μg ml^{-1}). Between-batch precision was further tested on the same sample with 10 replicates per day for 20 days and an RSD of 4·3% was obtained. These levels of precision would appear to be more than adequate for routine determinations of food colours.

The extraction procedure adopted for the various food samples was designed to be simple and as rapid as possible. The single stage extraction at room temperature produced a mean recovery of *c.* 90%, which could be increased if necessary by a second washing of the residue after filtration or centrifugation. The recovery data (shown in Table 4) are only of relevance to that portion of the dye which remains free in the food system. Any dye which is protein bound, for example, will not be extracted under these conditions and a high recovery of added dye should not be taken as an indication that bound material will also be extracted. Using this simple extraction technique the aqueous extracts contain very high levels of sugars (mainly sucrose and components of glucose syrups in the present examples). These extracts could not be submitted directly to HPLC analyses as the sugars would cause shifts in retention times and would also lead to distorted peaks. Any high molecular weight polysaccharide material would additionally result in deterioration of the column. The extracts after dialysis produced chromatograms of identical quality to aqueous standards and no deterioration in column performance was observed throughout the course of the study.

The levels of dyes in most artificially coloured foods are such that a portion of the dialysate (100 μl) can be used directly for HPLC, while still providing adequate sensitivity and precision. Although this approach does not utilise the full potential of the ASTED system it does mean that all the common dyes can be determined under the same conditions. With the TECs currently available, only selected dyes could be retained, and hence concentrated.

Table 4. Determination of colours in commercial foods samples

Food sample	Colours identified	Level (ppm)	Recovery (%)
Blancmange dessert (banana)	Ponceau 4R	12·6	88·9
	Sunset Yellow FCF	70·1	90·4
Blancmange dessert (vanilla)	Ponceau 4R	4·45	92·2
	Sunset Yellow FCF	26·7	91·2
Blancmange dessert (traditional)	Ponceau 4R	14·1	92·0
Lemon curd	Sunset Yellow FCF	50·5	—
Boiled sweets (yellow)	Ponceau 4R	2·63	85·3
Boiled sweets (red)	Ponceau 4R	10·5	87·2
Jelly (orange)	Sunset Yellow FCF	56·4	95·1
Fruit gums (orange)	Ponceau 4R	7·45	84·3
	Sunset Yellow FCF	27·3	86·3
Fruit gums (red)	Ponceau 4R	12·8	86·7
Fruit gums (yellow)	Ponceau 4R	3·11	88·8
'Irn Bru' drink	Ponceau 4R	2·92	88·1
Orange flavour	Sunset Yellow FCF	5·47	90·0
Blackcurrent drink	Sunset Yellow FCF	29·7	—
'Bali Hi' drink	Sunset Yellow FCF	6·71	93·1

CONCLUSION

Simple room temperature aqueous extraction of sugar-rich foods allows the isolation of water-soluble dyes in a form which can be processed directly by dialysis. Samples of the dialysate contain adequate amounts of the dyes for HPLC analysis without further treatment. The method possesses a good level of precision and large numbers of samples can be processed using the autosampler, once the initial aqueous extraction has been achieved.

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