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# XAD-2 HPTLC METHOD OF IDENTIFICATION AND DETERMINATION OF SOME SYNTHETIC FOOD COLOURINGS

Key words: Food colourings, identification, determination, high pressure thin layer chromatography

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## ABSTRACT

*An HPTLC method for identification and determination of 12 synthetic dyes* (Amaranth, Allura red, Brilliant black BN, Brilliant blue E, Carmoisine, Erythrosine, Indigotine, Patent blue V, Ponceau 4R, Quinoline yellow, Sunset yellow, Tartazine) has been developed. Amberlite XAD-2 was used as a stationary phase for column chromatography. Elution was performed with various acidified

alcohols (*i*-propanol, *n*-propanol, *n*-butanol) and recoveries between 81.5 - 100.2% were obtained. The low limit of detection is in the range of 4 - 10 ng. The method was tested on commercial products as: carbonated soda, candies, chewing gum, identical natural flavours, etc.

## INTRODUCTION

Although a variety of synthetic dyes are used in the food industry all over the world, today there is a growing movement to restrict the use of synthetic colourants further or at least the amounts used. The permitted lists of food colourants vary from country to country, and in some of them concentrations of synthetic dyes are not limited by state legislation. Therefore a demand for quantitative methods for food colourants analysis exists.

The analyses of synthetic food dyes have been achieved by paper chromatography[1], high performance liquid chromatography[2,3], thin layer chromatography[4-6], visible absorption spectrophotometry[7] etc.

Thin layer chromatography (TLC) methods have been extensively used in the routine analysis of food dyes using various adsorbent layers such as different types of silica gel[4-6], cellulose[8], scolecite[9], etc.

The wool-dyeing method is generally proposed for extracting synthetic colourings from foods prior to identification by TLC. A method in which colourings are extracted with a liquid anion-exchange resin dissolved in butanol has also been proposed for food colour analysis[10]. The extracted dyes after that are purified and concentrated using polyamide as a stationary phase for column

chromatography[10]. This method, although suitable for quantitative measurements, is rather time consuming.

Accordingly, our aim was to establish a simple, fast and reliable method for the extraction, purification and at the same time concentration of the investigated synthetic dye. For this purpose we used the macromolecular nonpolar resin XAD-2 as a stationary phase for column chromatography. XAD-2 column chromatography extraction of food colourings from aqueous or readily soluble foods is as suitable and less time consuming as the extraction with a Sep - Pak sample preparation cartridge. Moreover, an advantage of the use of XAD-2 is the possibility of repeated use.

## EXPERIMENTAL

*Principal*

Colour additives were separated from the matrix by use of macromolecular resin XAD-2 as a stationary phase for column chromatography. After the elution the synthetic colourings were identified and quantificated by high pressure thin layer chromatography (HPTLC) on silica gel - Kieselgel $60F_{254}$.

*Reagents*

a. Methanol, *i*-propanol, *n*-propanol, ethanol (96%), all analytical grade.

b. Hydrochloric acid soln. - 25% (v/v) in $H_2O$.

c. Reference food colourings standards (Table 1) were obtained from the producers "Etol" IFF Celje, Republic of Slovenia and "Wurth", Wien, Austria.

TABLE 1

Current Status of Permitted Synthetic Food Colourings

| Synthetic colouring | EEC serial number | Colouring index number |
|---|---|---|
| Amaranth | E 123 | 16185 |
| Allura red | E 129 | 16039 |
| Brilliant black BN | E 151 | 28440 |
| Brilliant blue E | E 133 | 42090 |
| Carmoisine | E 122 | 14720 |
| Erythrosine | E 127 | 45430 |
| Indigotine | E 132 | 73015 |
| Patent blue V | E 131 | 42051 |
| Ponceau 4R | E124 | 16255 |
| Quinoline yellow | E 104 | 47005 |
| Sunset yellow | E 110 | 15985 |
| Tartazine | E 102 | 19140 |

Stock solution: 100 mg ref. std./100 ml $H_2O$. Working solution: a). Dilute 1 ml stock solution to 50 ml with water. b). Dilute 1 ml working solution (a) to 10 ml with ethanol (methanol, *i*-propanol, *n*-propanol).

d. TLC solvent system: *i*-propanol - *n*-propanol - *n*-butanol - $NH_4OH$ - $H_2O$ (4 + 2 + 2 + 0.5).

e. Amberlite XAD-2 resin (20 - 50 mesh), obtained from Rohm and Haas Co., Philadelphia PA. The resin was washed by stirring four times with four bed volumes of acetone, three times with three bed volumes of methanol and three

times with three bed volumes of distilled water. The XAD-2 resin remained in distilled water until transferred to the columns. The amount of resin used was 3 - 5 g.

*Apparatus*

a. Automatic high pressure sample applicator, Linomat IV (Camag).

b. Chromatogram scanner (Camag, Switzerland), capable of scanning the wavelength range from 400 - 700 nm when using tungsten lamp.

c. Chromatography horizontal chamber (Camag).

d. Columns, glass (200 x 10 mm) with flow regulator. The columns were plugged with fine glass wool and an aqueous slurry of the resin (3 - 5 g) was poured into the column. A small glass wool plug was placed on top of the resin.

e. HPTLC Kieselgel 60$F_{254}$ precoated plates (5628 E. Merck, Darmstadt)

f. Sintered glass filters

g. Syringe, glass 100 µl (Hamilton).

*Preparation of samples*

Sample size depends on concentrations of colourants present. The following are examples of preparations for commonly encountered samples:

a. Carbonated soda or similar drinks: Apply 2 ml to XAD-2 column, wash the column with 20 ml of distilled water, drain the liquid, then elute with 8 - 9 ml acidified ethanol. After the elution fill the volume up to 10 ml with ethanol. Elute at a flow rate of 0.5 ml/minute.

b. Candies or chewing gums: Place in beaker 1 or 2 units (depending on expected concentrations) with 15 - 20 ml water, heat the solution gently until sample becomes colourless. After cooling filter through sinter glass filter and dilute to 25 ml. Apply 5 ml to column.

c. Powder drink mix: Place 2 - 3 g product in beaker, add 30 ml water and mix by swirling until sample is dissolved. Filter solution, dilute to 50 ml. Apply 5 ml to column.

d. Natural identical flavours: Dilute 1 ml of the sample to 25 ml with distilled water. Apply 1 ml onto column.

e. Jams: Place 2 - 3 g in beaker, add 30 - 35 ml water, heat gently and swirl until dissolved and cool. Place liquid in the separatory funnel and mix with diethyl ether (twice with 30 ml of ether). Drain water layer through sinter filter and dilute filtrate to 50 ml. Apply 5 ml to column.

## RESULTS AND DISCUSSION

### *XAD-2 elution*

XAD-2 has been used as an adsorbent in the analysis of drugs of abuse[11], but we found that it is also good adsorbent for synthetic food colourings. It was necessary to establish the most suitable eluant, which would provide the best analytical recovery. For this purpose different solvents such as ethanol, methanol, *i*-propanol and *n*-propanol were applied. The obtained analytical recoveries are give in Table 2.

TABLE 2

Analytical Recoveries (R) Obtained for Some Synthetic Food Colourings Using Different Solvents as Eluants for XAD-2

| Dye | conc. (mg/l) | Ethanol, R% | Methanol, R% | *n*-propanol, R% | *i*-propanol, R% |
|---|---|---|---|---|---|
| Allura red | 20.0 | 99.4 | 98.4 | 99.4 | 96.9 |
| | 40.0 | 99.7 | 99.8 | 100.0 | 97.6 |
| | 60.0 | 100.2 | 100.0 | 99.9 | 98.2 |
| Amaranth | 10.0 | 99.4 | 98.0 | 87.0 | 90.4 |
| | 20.0 | 100.0 | 97.3 | 88.5 | 91.7 |
| | 30.0 | 99.9 | 99.6 | 90.4 | 92.4 |
| Brilliant black BN | 20.0 | 99.7 | 99.4 | 99.4 | 96.0 |
| | 40.0 | 100.0 | 98.7 | 100.5 | 97.4 |
| | 60.0 | 100.1 | 99.8 | 99.9 | 98.6 |
| Brilliant blue | 20.0 | 90.2 | 94.4 | 82.6 | 83.1 |
| | 40.0 | 91.2 | 93.8 | 84.7 | 83.6 |
| | 60.0 | 93.4 | 94.9 | 85.0 | 84.7 |
| Carmoisine | 20.0 | 89.2 | 90.0 | 87.4 | 86.2 |
| | 40.0 | 88.3 | 88.5 | 86.2 | 84.5 |
| | 60.0 | 90.4 | 87.2 | 85.0 | 87.7 |
| Erythrosine | 25.0 | 94.4 | 92.2 | 98.7 | 95.9 |
| | 50.0 | 95.2 | 93.2 | 99.8 | 97.9 |
| | 75.0 | 97.8 | 96.4 | 99.6 | 98.7 |
| Indigitine | 20.0 | 80.4 | 82.6 | 80.6 | 84.0 |
| | 40.0 | 82.5 | 83.3 | 81.5 | 84.0 |
| | 60.0 | 80.8 | 85.7 | 82.0 | 86.6 |
| Patent blue V | 10.0 | 98.2 | 97.4 | 96.0 | 86.4 |
| | 20.0 | 99.0 | 97.0 | 96.1 | 87.7 |
| | 30.0 | 99.4 | 97.6 | 96.7 | 88.0 |
| Ponceau 4R | 20.0 | 98.6 | 98.7 | 99.8 | 93.0 |
| | 40.0 | 99.9 | 99.8 | 100.1 | 93.4 |
| | 60.0 | 100.1 | 100.0 | 99.9 | 94.1 |
| Quinilone yellow | 20.0 | 97.0 | 96.4 | 96.0 | 98.5 |
| | 40.0 | 97.6 | 97.8 | 97.3 | 99.9 |
| | 60.0 | 96.7 | 98.0 | 99.0 | 99.9 |
| Sunset yellow | 20.0 | 99.4 | 98.1 | 98.6 | 97.8 |
| | 40.0 | 100.0 | 99.0 | 99.2 | 97.5 |
| | 60.0 | 100.0 | 100.2 | 100.0 | 98.4 |
| Tartazine | 20.0 | 99.4 | 98.3 | 88.9 | 91.2 |
| | 40.0 | 100.1 | 97.7 | 88.5 | 90.1 |
| | 60.0 | 100.2 | 98.0 | 89.6 | 92.0 |

As can be seen from Table 2, analytical recoveries are between 80.4 - 100.5%. They are less in the case of Indigotine and Carmoisine, but quite satisfactory for the rest of the dyes. Although all the solvents provide good recoveries, because of its lower toxicity ethanol was always the solvent of choice.

It is important to note that all the elutions were made with acidified eluants. Therefore different volumes of 25% HCl were used, depending on the type of colouring analyzed, and they were: for Patent blue V, Indigotine and Quinoline yellow 0.05 ml, for Brilliant blue and Sunset yellow 0.1 ml, for Ponceau 4R and Allura red 0.2 ml, for Tartazine 0.25 ml, Amaranth 0.3 ml, Carmoisine 0.4 ml, and for Erythrosine and Brilliant black 0.5 ml. In the case of Patent blue V, Indigotine and Brilliant blue, in order to avoid changes of colour from blue to green blue it was necessary after the elution to neutralize the solutions with 25% $NH_4OH$.

By use of small volumes of eluants we avoided evaporation and therefore possible loss of colouring. A small amount of resin (3 g) was quite suitable when mixtures of 2 - 3 colourings were used, but in the case of more then 3 colourings, the amount of resin used was 5 g.

*UV densitometry*

The use of an automatic high pressure applicator made the application of the samples onto plates much easier. A volume of 10 ml was quite satisfactory for application on the plate, but in the case of lesser concentrations it could be increased up to 30 ml. More then 30 ml are not recommended because of the interferences which may occur from the complex matrices (jams, candies).

A solvent system consisting of *i*-propanol - *n*-propanol - *n*-butanol - $H_2O$ - 25% $NH_4OH$ (4 + 2 + 2 + 0.5 + 1) provided good separation of eight colorings : Tartazine, Ponceau 4R, Amaranth, Allura red, Erythrosine, Brilliant black BN, Brilliant blue and Patent blue V. In the case of Indigotine, Sunset yellow, Quinoline yellow and Carmoisine it was necessary, because of their close Rf values, to repeat the development (2-3 times) with the same system. Therefore these colourings are treated separately. The hRf values of the investigated colours are given in Table 3.

Each colouring was scanned at its maximum wavelength which were : for Patent blue V 638 nm, Indigotine 615 nm, Brilliant blue 630 nm, Carmoisine 505 nm, Amaranth 516 nm, Erythrosine 532 nm, Brilliant black BN 568 nm, Ponceau 4R 507 nm, Allura red 499 - 502 nm, Sunset yellow 470 - 473 nm, Tartazine 427 nm and Quinoline yellow 414 nm. No difference in spectral data between acid alcoholic solutions and water neutral solutions scanned on the plate occurred.

The reliability of the method was checked by the method of standard addition, when a known concentration of colourings was added to the noncoloured carbonated soda within the range of 10 - 50 mg/l. A linear plot of absorbance against concentration was obtained between 10 - 50 ng. The limit of detection was between 4 ng for Brilliant blue to 10 ng for Sunset yellow, Quinoline yellow and Indigotine.

The between-days precision was evaluated by analysis at three known concentrations (10.0, 20.0 and 30.0 mg/l for Amaranth and Patent Blue V; 20.0, 40.0 and 60.0 mg/l for Allura red, Brilliant black BN, Brilliant blue E, Carmoisine, Indigotine, Ponceau 4R, Quinoline yellow, Sunset yellow and Tartazine; and 25.0

TABLE 3

The hRf Values of Some Synthetic Colourings Obtained on Silica gel $60F_{254}$ (Solvent System: *i*-propanol - *n*-propanol - *n*-butanol - $H_2O$ - 25% $NH_4OH$ (4+2+2+0.5+1)

| Dye | hRf value (Rf · 100) |
|---|---|
| Allura red | 52 |
| Amaranth | 43 |
| Brilliant black BN | 36 |
| Brilliant blue | 31 |
| Carmosine | 50 |
| Erythrosine | 71 |
| Indigotine | 47 |
| Patent blue V | 48 |
| Ponceau 4R | 33 |
| Quinoline yellow | 49 |
| Sunset yellow | 50 |
| Tartazine | 39 |

50.0 and 75.0 mg/l for Erythrosine) on six days. The coefficient of variation ranged from: 3.0% to 5.6% for Amaranth, 1.9 to 4.1% for Allura red, 2.3% to 4.4% for Brilliant black BN, 2.8% to 7.4% for Brilliant blue E, 4.2% to 6.4% Carmoisine, 2.4 to 4.2% for Erythrosine, 5.4% to 7.4% for Indigotine, 2.98% to 5.6% for Patent blue V, 2.0% to 3.4% for Ponceau 4R, 2.8% to 4.2% for Quinoline yellow, 2.6% to 4.1% for Sunset yellow and 3.0% to 5.2% for Tartazine.

Using this method the most frequently encountered commodities in the Republic of Macedonia were checked and it was found that the most frequently used colourings for carbonated soda were Tartazine (3.5 - 10.5 mg/l) and Sunset yellow ( 5 - 15.4 mg/l), for candies and chewing gums Ponceau 4RS (5 - 8.7 mg/kg) and for natural identical flavours Tartazine (0.4 - 3.6 g/kg), Brilliant black BN (0.51 - 0.72 g/kg), Ponceau 4R (0.2 - 0.33 g/kg), Carmoisine (0.3 - 2.6 g/kg), Sunset yellow (0.2 - 0.3 g/kg) and Patent blue V (0.18 - 0.27 g/kg).

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