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Identification and determination of red dyes in confectionery by ion-interaction high-performance liquid chromatography

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Abstract

In this paper an ion-interaction HPLC method for the separation and determination of red dyes used to decorate home-made sweets is presented. In particular E122, E123 and E124 dyes are considered, with special interest for E123 (amaranth), prohibited by the US Food and Drug Administration in 1976 and by Italian legislation in 1977 and then again permitted (with restrictions) in the European Community in 1994, (94/36 EEC Directive). A RP-ODS stationary phase is used and an ion-interaction reagent (octylammonium phosphate) is added to the hydro–organic eluent [water–acetonitrile (30:70, v/v), pH=6.4]. Spectrophotometric detection at 520 nm was employed and detection levels below 12 μg/l for the three dyes were achieved. The method is applied to two commercial products, respectively produced in France and in Italy, and the results are discussed in the light of the recent EEC Directive concerning food dyes.

Keywords: Dyes; Amaranth

1. Introduction

In 1960, an Amendment to the Food, Drug and Cosmetic Act permitted colours in use at that time in the USA to be used provisionally until their safety had been established. In the Federal Register of September 23, 1976 (85), the US Food and Drug Administration (FDA) addressed all the colours that were then provisionally listed for food, drug or cosmetic uses [1,2]. Since then many colours have been removed from the provisional list and prohibited, among them, amaranth [3]. In 1977 Italian legislation, DM 21.03.77, [4] banned the use of amaranth (E123) in most food and beverages: the dye could only be used in caviar and caviar succedanea.

On the other hand the dye was still allowed in other European countries. In 1994 the European Community, in order to unify in the member countries the legislation about food dyes, formulated the directive 94/36 [5]. This reports a positive list of the dyes permitted, two lists of foods in which these colours respectively can or cannot be used and, for some of the dyes, the maximum permitted amount. In concern of the time of application, the directive states that all the EC countries had to conform within December 31, 1995 legislations, regulations and administrative provisions to prohibit commercialization and use of all non-conforming products. Art. 9 also states that all the products already on the market or already labelled can be commercialized up to the exhaustion of the stock in hand.

As it particularly concerns red colours, the positive list reports E120, E122, E123 and E124. Among

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these, E120 (CI 75470) is the only natural colour, derived by coccinae. The others are synthetic colours: the structures, common names, European Community (EC), Colour Index (CI) and Food, Drug and Cosmetic (FD and C) denominations are reported in Table 1.

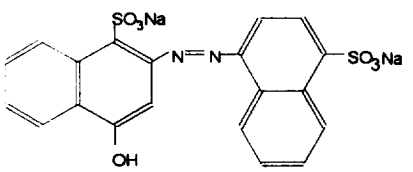
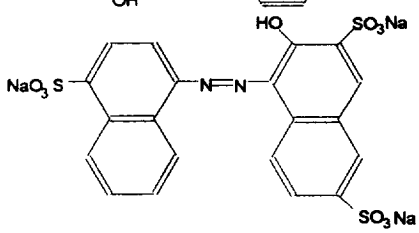
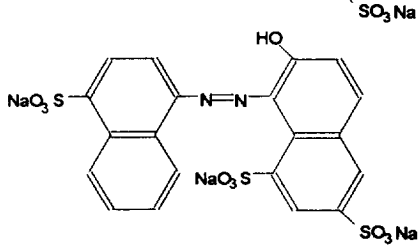
E123 (amaranth) is subjected also by the recent European directive to larger limitations than E122 and E124, both with concern to the kind of food (beverages, wines with alcoholic strength <15% and fish eggs) and concentration, which must be always lower than 30 mg/kg. Furthermore it cannot be directly sold to consumers.

For the analysis of food dyes, a large number of methods have been proposed, involving techniques such as paper and thin-layer chromatography [6–9], titrimetry [10], spectrophotometry [11], voltammetry [12], electrophoresis [13].

Synthetic azo dyes are highly ionic materials with several sulphonic acid groups per molecule. HPLC has been used for the analysis of highly polar components, but the use of normal-phase adsorption methods is complicated by high solute retention coupled with poor peak-shape and resolution. The application of reversed-phase chromatography is limited by the lack of sufficiently polar stationary phases while strong anion-exchange columns have been used successfully by a number of workers [14,15]. In recent years, micellar electrokinetic capillary chromatography [16] and capillary electrophoresis [17–19] have been developed as rapid techniques with high resolution. Another successful approach is the application of ion-pair chromatography [20,21].

This paper presents the development of a sensitive ion-interaction HPLC method for the separation and determination of the synthetic red dyes E122, E123 and E124 and its application to the analysis of coloured sugars used in confectionery (mompariglia). Two different products sold in Italian stores were

Table 1
Molecular structures, commercial common names, European Community (CE) number, colour index (CI) number and name, food and drug name of the three red food dyes studied

Molecular structure	Commercial name	CE number	CI number and name	F and D name
	Carmoisine	E122	14720 Acid red 14	Food red 3
	Amaranth	E123	16185 Acid red 27	Food red 9
	Ponceau 4R	E124	16255 Acid red 18	Food red 7

considered, one produced in Italy and the other imported from France.

With concern to the simultaneous separation of the red dyes E122, E123 and E124 only a ion-pair method is reported, which makes use of an isopropanol–water–cetrimide–acetic acid mobile phase and is characterized by detection limits around 10 mg/l [20]. The method here presented, based on a reversed-phase ion-interaction chromatography, permits very lower detection limits (around 10 μg/l) and makes use of a water–acetonitrile solution of octylamine orthophosphate at pH=6.4.

The principle of the technique involves the surface modification of a RP stationary phase when the lipophilic chain of the alkylammonium ion is adsorbed onto the ODS, giving rise to a positively charged layer. Through electrostatic forces the anion of the ion-interaction reagent is also bound. The formation of an electrical double-layer adsorbed onto the original RP phase [22,23] permits the separation of anionic and/or cationic [24,25] species.

2. Experimental

2.1. Apparatus

The analyses were carried out with a Merck–Hitachi (Tokyo, Japan) Lichrograph Model L-600, interfaced with a UV–Vis detector model L-4200 and an L-3720 conductivity detector with temperature control.

A Metrohm 654 pH meter equipped with a combined calomel electrode was employed for the pH measurement and a Hitachi (Tokyo, Japan) model 150-20 spectrophotometer for the absorbance measurements.

2.2. Reagents

Ultrapure water from Millipore Milli-Q (Bedford, MA, USA) was used for the preparation of all the solutions.

Octylamine was from Fluka (Buchs, Switzerland). Analytical grade chemical CH_3CN and orthophosphoric acid were purchased by Carlo Erba (Milan, Italy); carmoisine (E122), amaranth (E123) and Ponceau 4R (E124) were Aldrich (Milwakee, WI, USA) analytical-grade reagents.

2.3. Chromatographic conditions

A 5 μm ODS-2 Spherisorb Phase Separations column fully-endcapped 25.0 cm×0.46 cm with a carbon load of 12% (0.5 mmol/g) was used together with a 1.50 cm×0.46 cm, 5 μm, LiChrospher RP-18 guard pre-column.

The mobile phase consisted of a water–acetonitrile (70:30, v/v) 5.0 m*M* solution of octylamine, brought to pH 6.4 by orthophosphoric acid.

Column temperature was kept constant at 25°C and flow-rate was 1 ml/min.

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal was obtained; (a minimum of 1 h was necessary).

Between the use of different mobile phases and after use the stationary phase was washed by flowing water (1.0 ml/min for 15 min), a water–CH_3CN (50:50, v/v) mixture (0.5 ml/min for 30 min) and then CH_3CN (1.0 ml/min for 5 min).

2.4. Preparation of dye standard and sample solutions

1000 mg/l standard solutions of the dyes were prepared in ultrapure water in dark flasks. The solutions were kept at room temperature and then diluted with ultrapure water as necessary for the HPLC analysis.

The commercial products studied consist of little balls of sugar variously coloured. Two products were analyzed, respectively produced in France and in Italy. The French sample declares, among dyes of other colours, the presence of E123 and E124. The Italian one reports the presence of only E124.

The little red balls were separated from the others and 1.00 g was stirred in 10.0 ml of CH_3OH until complete decoloration; the red extract so obtained was diluted 1:10 (v/v) in ultrapure water and filtered 0.45 μm before injection in the HPLC system.

3. Results

3.1. E122, E123 and E124 standard separation

Ion-interaction chromatography is very suitable for the analysis of the dyes, if conditions are chosen in which the ionized analytes are able to form ion-pairs with octylamine while the interference by sugars is reduced to a minimum extent.

UV–Vis detection at the maximum absorbance wavelength of the amaranth (520 nm) was employed. In the condition optimization process, the use of different mobile phases was compared. Octylamine concentrations ranging between 0.00 m*M* and 8.00 m*M*, acetonitrile percentages between 0% and 40% and three pH values (equal to 3.0, 6.4 and 8.0) were tested.

Experiments performed in reversed-phase mode (without addition of octylamine phosphate in the mobile phase) did not obtain the separation of the dyes (which were very poorly retained) and demonstrated evidence of ion-interaction mechanisms. In turn, the addition of organic modifier was necessary, in order to obtain the separation in reasonably low total analysis time.

The optimized composition of the mobile phase corresponds to a 5.00 m*M* solution of octylamine in acetonitrile–water (30:70), brought at pH=6.4 with orthophosphoric acid. Fig. 1 reports a typical chromatogram recorded under these conditions for a mixture containing 0.10 mg/l of E123, 0.10 mg/l of E124 and 0.15 mg/l of E122.

Calibration plots were built reporting peak area (relatives units as given by the integrator) as a function of standard concentration ranging between 0.05 and 0.20 mg/l. The plots could be fitted by straight lines with correlation coefficient r^2 always greater than 0.995. Through the data of sensitivity (peak area for 1.0 mg/l) and for a signal-to-noise ratio=3, detection limits always below 12.0 μg/l were evaluated for the three dyes.

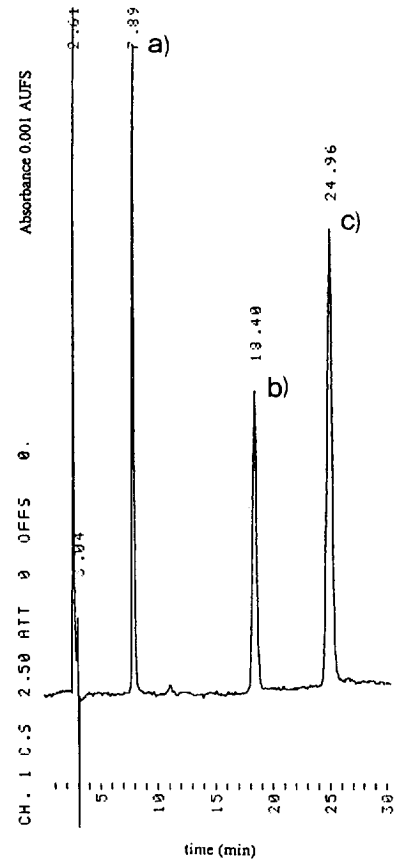


Fig. 1. Chromatogram recorded for a mixture of (a) E123 (0.10 mg/l), (b) E124 (0.10 mg/l) and (c) E122 (0.15 mg/l) standard solutions. Chromatographic conditions: stationary phase: 5 μm ODS-2 Spherisorb Phase Separations, fully-endcapped. Mobile phase: water–acetonitrile (70:30) 5.00 m*M* solution of octylamine, at pH 6.4 for orthophosphoric acid. Spectrophotometric detection: 520 nm. Flow-rate: 1.0 ml/min, 100 μl injected.

3.2. Analysis of the food dyes

The samples of the dyes, prepared as described in Section 2.4, were then analyzed under the optimized conditions. Fig. 2 reports the comparison between the chromatogram obtained for the French (Fig. 2A) and the Italian (Fig. 2B) sample. As declared on the labels, the French sample resulted to contain E123 and E124, while the Italian one contains only E124. Both of them do not contain E122.

The chromatogram also suggests that the method is interference free by the other components present in the product, such as sugar, glucose, tragacanth and vegetable fats. The standard addition method was employed to quantify E123 in the French sample. An

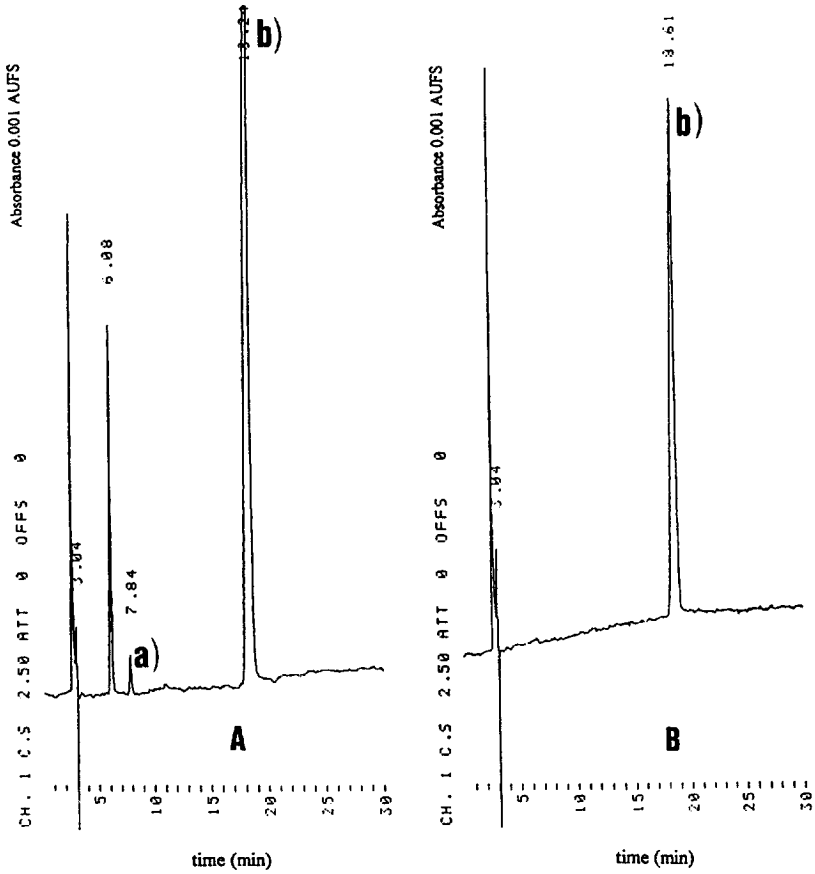


Fig. 2. (A) Chromatogram recorded for the French product: a=E123, b=E124. (B) Chromatogram recorded for the Italian product: b=E124. Conditions as in Fig. 1.

average (three samples) content of E123 of 1.04±0.06 mg/kg was found.

The results obtained can be discussed at the light of two main points of view, respectively bound to legal implications and to health safety.

As regards the legal aspects, according to the EEC 94/36, E123 could be contained only in beverages, wines and fish eggs. The French product which contains E123 can still be commercialized up to the exhaustion of the stock in hand.

No consideration can be made about the content, just because the product is not allowed to contain E123.

Even if the laws are respected, health safety must be taken into consideration, namely, the fact that a commercial product contains E123, which is a suspected possible toxin.

4. Note added

Since completing this article, the authors have observed that the ion-interaction chromatography systems described can be readily implemented using other 5 μm C_{18} reversed-phase packings, rather than currently available batches of the material used for the present work.

Acknowledgments

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