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Simultaneous Determination of Seven Synthetic Water-Soluble Food Colorants by Ion-Pair Reversed-Phase High-Performance Liquid Chromatography

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Abstract: A selective gradient ion-pair reversed-phase high-performance liquid chromatographic method for the simultaneous quantitative determination of seven synthetic water-soluble food colorants (Tartrazine E102, Quinoline Yellow E104, Sunset Yellow E110, Carmoisine E122, Ponceau 4R E124, Erythrosine E127, Carmine E132 and Brilliant Blue FCF E133) was developed. Analysis were performed on 125×4.6 mm i.d. Merck Lichrosher 100 RP C-18 (5 μm) column. The flow rate of the mobile phase was 1.0 mL min^{-1} and the injection volume was 50 μL. The mobile phase consisted of water:acetonitrile (50:50 v v^{-1}) Containing 0.35 M (1-Hexadecyl) Trimethylammonium Bromide (CTAB) and buffered to pH 7.5 (mobile phase A) and water acetonitrile (50:50 v v^{-1}) (mobile phase B). Successful separation and quantitative determination was obtained for all the colorants using an optimized gradient elution program. Detection limits were 1.59 (E142) and 22.1 (E124) ppm, with relative standard deviations in the range 0.37%. The method was applied to the determination of colorants in various types of drinks and food, such as carbonated fruit flavored drinks, concentrated fruit flavored drinks, jams and sugar confectionery.

Key words: Liquid chromatography, ion-pair, CTAB, diode-array detector, synthetic food-soluble colorants, food analysis, confectionary, fruit drinks

INTRODUCTION

Synthetic water-soluble food colorants are divided into five major colorant classes: the azo compounds (102, 110, 122, 123, 124, 128 and E129), the triarylmethane group (131, 133 and E142), the chinophthalon derivative of Quinoline Yellow (E104), xanthenes as Erythrosine (E127) and the indigo colorants (Indigo Carmine E132). The average daily intake of FD and C colorants over the age of 2 years ranged from 3.1 to 100 mg kg^{-1} per person. The use of synthetic colorants in foods is strictly controlled by legislation and harmonized across the European Union by formulating the directive 94/36/EC, 1994 on colors for use in foodstuffs. Consequently, accurate and reliable methods for the determination of synthetic colorants are required for the assurance of food safety. Many analytical techniques have been developed for the identification and determination of various synthetic food colorants, most of them require time-consuming pretreatment or cannot be applied to complex colorant mixtures. High-performance ion chromatography, reversed-phase liquid chromatography and ion-pair liquid chromatography are still the most preferred methods, as they provide unrivalled resolution, sensitivity and selectivity. Both isocratic and gradient systems are used and the latter are preferred for the separation of the more complex mixtures.

Prado and Godoy (2002) developed a method to determine eight synthetic food colorants using ODS-2 column and H_2O/Methanol (70:30) containing 0.08 m ammonium acetate for conditioning and H_2O/Methanol (70:30) mobile phase for the run. Kirschbaum *et al.* (2003) developed and evaluated an HPLC-DAD method for determination of 14 synthetic food colorants using R P18e column and mobile phase prepared from 0.1 m acetate buffer (pH 7.0) and acetonitrile. Chen *et al.* (1998) determined eight synthetic food colorants in drinks using an anion-exchange analytical column with very low hydrophobicity and gradient elution with mobile phase of hydrochloric acid-acetonitrile. Minioti *et al.* (2007) determined thirteen synthetic food colorants using C18 stationary phase and a mobile phase containing acetonitrile-methanol (20:80 v v^{-1}) mixture and a 1% (m v^{-1}) ammonium acetate buffer solution at pH 7.5. Falcon and Gandara (2005) determined five synthetic food colorants in soft drinks containing natural pigments using ODS column and gradient elution using methanol and ammonium acetate aqueous solution at pH 5. Ma *et al.* (2006) proposed a method for simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff using HPLC-DAD-electrospray mass spectrometry technique. The method is base on using C18 analytical column and a

mobile phase consisted of acetate buffer solution (containing 0.02 m ammonium acetate and 1% acetic acid) and methanol. Urquiza *et al.*, (2000) determined sulphonated food colorants by ion-interaction HPLC. The stationary phase was ODS and the mobile phase was acetonitrile-phosphate buffer (27:73 v v^{-1}) mixture at pH 6.7 and containing 2.4 mm butylamine as ion-interaction reagent. Angelino *et al.* (1998) developed a system suitable for tests for ion-interaction colorants using a mobile phase containing alkylammonium phosphate as an ion-interaction reagent. Gennaro *et al.* (1997) determined three synthetic food red colorants in confectionary by ion-interaction HPLC. The separation was performed using C18 column and the mobile phase was water-acetonitrile (30:70 v v^{-1}) buffered at pH 6.4 and containing octylammonium phosphate as an ion-interaction reagent. Fuh and Chia (2002) determined ten sulphonated azo dyes in food by ion-pair liquid chromatography using C18 column with gradient elution. The mobile phase was 3 mm triethylamine (solution A adjusted to pH 6.2) and methanol (solution B). Kiseleva *et al.* (2004) proposed a method for optimization of conditions for the HPLC separation and determination of ten synthetic dyes in food using C18 column and tetrabutylammonium dihydrogen phosphate as ion-pair reagent. The aim of this study was to develop a reversed-phase high-performance liquid chromatography method for the simultaneous determination of seven synthetic water-soluble colorants. The method was based on using C18 column for separation and a mobile phase Containing N-Ctyel-N,N,N-Trimethylammonium Bromide (CTAB) cationic surfactant as an ion-pair to achieve complete separation of the food colorants. The method was applied for the quantitative determination of colorants in various food products, such as carbonated fruit flavored drinks, concentrated fruit flavored drinks, jam, sweets and sugar confectionary.

MATERIALS AND METHODS

Reagents: All solutions were prepared with deionized water and all chemicals were of analytical reagent grade, unless otherwise stated.

(1-Hexadecyl) Trimethylammonium Bromide (CTAB) was from Alfa Aesar (England). The HPLC grade water and acetonitrile were supplied from Merck. The colorants Tartrazine (E102), Sunset Yellow FCF (E110), Amaranth (E123), Erythrosine (E127), Quinoline Yellow (E104), Carmoisine (E122), Ponceau 4R (E124), Brilliant Blue FCF (E133) were obtained from Fiorio Colori Industria Chemica (Italy). The common names, European Community numbers (E numbers), Color Index (CI numbers) and percent purities are reported in Table 1.

Table 1: Common names, E (European Community) and CI (Color Index) numbers, percent purity and λ_{max} of the standard synthetic food colorants studied

Common name	E number	CI number	%purity	λ_{max} (nm)
Tartrazine	E102	19140	87.6	427
Quinoline yellow	E104	47005	70.5	413
Sunset yellow FCF	E110	15985	86.2	481
Carmoisine	E122	14720	85.2	515
Ponceau 4R	E124	16255	82.7	507
Erythrosine	E127	45430	87.6	526
Brilliant blue FCF	E133	42090	90.3	624

Apparatus: Chromatographic analysis was carried out with a Waters separation module 2695 liquid chromatograph equipped with a gradient pump capable for mixing up four solvents, vacuum membrane degasser, an autosampler, a 100 μL loop injector and a Waters module 2996 photodiode array detector. The software was Waters Empower software.

A pH-meter (HANNA Instrument) equipped with a combined glass-calomel electrode was employed for pH measurements.

UV-2 UNICAM UV-Visible spectrophotometer was used for all spectrophotometric measurements. All spectrophotometric studies were carried out at room temperature (22±2°C) using quartz cell of 10 mm path length.

Chromatographic conditions: Analysis were performed on 125×4.6 mm i.d. Merck Lichrosher 100 RP C-18 (5 μm) column was used together with a C18 (25×4.6 mm i.d., 5 μm) guard column. The mobile phase consisted of water:acetonitrile (50:50 v v^{-1}) containing 0.35 M CTAB adjusted to pH 7.5 by drop wise addition of a sodium hydroxide solution 10% (m v^{-1}) (mobile phase A) and water:acetonitrile (50:50 v v^{-1}) (mobile phase B). The mobile phase was filtered by vacuum through a membrane filter with a pore diameter 0.45 μm. The flow-rate of eluent was always kept constant at 1.0mLmin^{-1} and the injection volume was set at 50 μL. All experiments were carried out at room temperature. The diode-array detector was programmed to monitor the colorants at the selected appropriate absorbance wavelength (Table 1).

Preparation of colorant standards: Individual standard stock solutions containing each colorant were prepared by dissolving 100 mg unpurified colorant in 100 mL distilled water. The solutions were kept in dark flasks. The working standard solutions of each color were prepared by appropriate dilution of stock solutions with water to give concentrations between 0.10 and 100 ppm, taking into consideration the purity of the colorants. The mixed standard solutions containing all colorants at concentrations between 0.10 and 10 ppm were also prepared by mixing and dilution of appropriate aliquots

from standard stock solution of each substance. All solutions were stored at 4°C in the dark and were stable at least for 2 months.

Preparation of sample solutions: All the tested samples were obtained from the local market (included carbonated fruit flavored drinks, concentrated fruit flavored drinks, jams and sugar confectionary). A 10 gm sample of drink was diluted with water in a volumetric flask of 50 mL. The samples were degassed, if necessary. Concentrated fruit flavored drinks were prepared by diluting 2 gm sample with water in a volumetric flask of 50 mL. The solid samples were homogenized. A portion of 10 g of jam, or confectionary product was accurately weighted and dissolved in 50 mL of water. The sample solution was placed in ultrasonic bath for 15 min for the complete extraction of the colorants. These solutions were filtered through a folded paper filter and the filtrate was collected in volumetric flask of 50 mL. All solutions were injected after filtering through 0.45 μm disposable syringe filters.

RESULTS AND DISCUSSION

Prior to HPLC analysis the visible spectrum of 20 ppm of each standard colorant was obtained in order to establish its maximum absorbance wavelength (Table 1).

Effect of CTAB concentration on the retention time of the colorants: Each colorant was chromatographed individually at the recommended chromatographic conditions using mobile phase A consisted of water:acetonitrile (50:50 v v^{-1}) containing 0.35 M CTAB and mobile phase B consisted of water:acetonitrile (50:50 v v^{-1}). The injection volume of each colorant was 20 μL of 20 ppm and the absorbance was measured at the corresponding peak at λ_{max} (Table 1). The obtained results are presented in Table 2. It can be seen from the results that as the percentage of mobile phase A in the eluent decreases (CTAB concentration decreases) and the percentage of mobile phase B increases, the retention time (t_R) of the peaks of all the colorants increase. Resolution problems can be solved by eluting the mixtures containing the 7 colorants at low percentage of mobile phase A in order to delay the elution of the colorants which have short retention times (104, 133, 110 and E102), then the percentage of mobile phase A increases gradually in order to elute the colorants which have long retention times (122, 127 and E124).

Table 2: Effect of CTAB concentration in the eluent on retention time of each colorant

Mobile phase			Retention time (min)						
A (%)	B (%)	CTAB (M)	E104	E133	E110	E102	E122	E127	E124
30	70	0.14	6.40	10.00	13.23	16.22	35.5	N.T.*	N.T.*
60	40	0.21	2.70	3.30	7.00	8.05	30.24	39.37	N. T.*
80	20	0.28	2.00	2.32	2.64	3.47	24.40	27.50	34.17
100	0	0.35	1.73	1.88	1.98	2.70	15.07	19.74	23.73

N.T.: The peak has not been detected during an elution time of 60 min

Optimization of the separation: Many experiments were performed to obtain an efficient separation for all the colorants with no interference. The selection of the percentage of mobile phases A and B in the eluent were based on the results presented in Table 2. The colorants 104, 133, 102 and E110 were eluted at 30% A: 70% B, the colorants 122, 127 and E124 were eluted at 80% A: 20% B. Under these conditions separation of the seven colorants in mixtures can be achieved with no interference (Fig. 1).

The gradient elution program used was as follows. For the initial 18 min, an isocratic elution at 30% A: 70% B was carried out, between 18 and 20 min, a linear gradient from 30% A: 70% B to 80% A: 20% B was carried out. Finally, a 2 min equilibrium phase of the column was taking place to recover initial conditions of 30% A: 70% B for the next run. The elution of the colorants was observed in a chromatogram that was obtained by scanning the wavelength range from 400 to 700 nm, continuously (Table 3). Using the above described optimized gradient program, the retention times (t_R), Resolution coefficient (R_s) for two successive peaks and the width (w) of each peak at the optimum conditions were calculated and presented in Table 4.

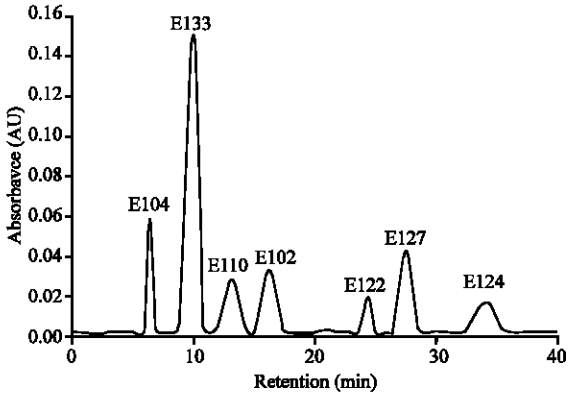

Fig. 1: Chromatogram of a mixed standard solution using the optimized gradient program of Table 3. The concentrations of all colorants were 20 mg L^{-1}. Identification of the peaks and their corresponding t_R are given in Table 4

Table 3: Optimized gradient program for separation of 7 colorants at a flow rate of 1.0 mL min^{-1}

Time (min)	A (%)	B (%)		Eluted colorants
0.0	30	70	0	
18	30	70	1	E104, E133, E110, E102
40	80	20	1	E122, E127, E124

Table 4: Chromatographic data of the colorants with the optimized gradient program of Table 3

Colorant	t_R (min)	R_s	w (min)	LOD (mg L^{-1})	Linear range (mg L^{-1})	R. S. D (%)*
E104	6.40		0.96	0.02	0.3-65	1.3
E133	10.00	2.37	2.04	0.01	0.15-45	1.8
E110	13.20	1.43	2.43	0.01	0.4-70	1.6
E102	16.22	1.26	2.37	0.03	0.3-73	1.1
E122	24.40	4.32	1.42	0.03	0.4-82	1.3
E127	27.50	1.74	2.15	0.04	0.5-85	1.2
E124	34.17	2.55	3.07	0.06	0.8-93	1.6

*Relative standard deviations for 20 mg L^{-1} of each colorant for 5 measurements

Table 5: Determination of colorants in food products collected from the local market

Sample	Colorants found	Concentration mgL^{-1}	R. S. D (%)[1]
Carbonated soft drink strawberry	E110	65.2	1.2
	E122	8.3	1.3
Carbonated soft drink orange	E110	24.1	1.7
	E102	24.5	1.5
Concentrated fruit drink raspberry	E124	2348	1.8
	E133	54.3	1.6
Concentrated fruit drink orange	E110	500	1.4
	E102	500	1.6
Jam strawberry[2]	E124	68.6	1.5
Coated candy[2]	E102	12.3	1.3
	E110	18.9	1.4
	E127	32.3	1.4
	E133	3.6	1.3
	E124	8.7	1.3

[1]Relative standard deviations for 5 measurements, [2]concentration in mg kg^{-1}

Validation of the method: The concentrations of the range of linearity were in the ranges given in Table 4. The calibration curves were constructed by plotting peak areas against concentrations. The Limit of Detection (LOD) of each colorant was calculated and corresponds to concentration equivalent to a signal-to-noise ratio of three. The Relative Standard Deviations (RSD) of 5 independent replicate of each colorant were calculated at 20 mg L^{-1} standard solutions.

Application to real samples: The developed method was applied for quantitative determination of food colorants in different foodstuffs collected from the local market. The samples were prepared and analyzed as described in the section of materials and method and the obtained results are summarized in Table 5.

CONCLUSION

An efficient and accurate analytical method for the simultaneous determination of seven food colorants in a single run by liquid chromatography-diode array detection was developed and optimized. The proposed method includes a simple pretreatment procedure for the extraction of colorants from food and offers a combination of sensitivity and selectivity, simplicity and relatively short time of analysis. This method permits the detection of colorants at very low concentrations (μg kg^{-1} range). The applicability was verified by the determination of colorants present in various water-soluble foodstuffs.

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