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Identification and Determination of Seven Synthetic Dyes in Foodstuffs and Soft Drinks on Monolithic C18 Column by High Performance Liquid Chromatography

NARUMOL VACHIRAPATAMA[1]*, JUTHAMAS MAHAJAROENSIRI[1] AND WONNOP VISESSANGUAN[2]

[1.] Department of Chemistry, Faculty of Science and Technology, Thammasat University, Rangsit, Pathumthani, 12121, Thailand
[2.] BIOTEC-Central Research Unit, National Center for Genetic Engineering and Biotechnology, 113 Thailand's Science Park, Phaholyothin Rd, Klong 1, Klong Luang, Pathumthani 12120, Thailand

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ABSTRACT

Seven synthetic dyes (amaranth, ponceau 4R, sudan red 1, tartrazine, sunset yellow FCF, fast green FCF and brilliant blue FCF), commonly used as colorant in foodstuffs and soft drinks, were investigated on monolithic C18 column by high performance liquid chromatography. The compositions of mobile phase containing methanol-water in acetate buffer at different pH were optimized at the maximum detection wavelength of each synthetic dye. Relative standard deviations of retention time, peak area and peak height for seven synthetic dyes were 0.18-0.83, 0.44-3.49 and 0.49-3.56%, respectively. The detection limit (at a signal-to-noise ratio of 3) for amaranth, ponceau 4R, sudan red 1, tartrazine, sunset yellow FCF, fast green FCF and brilliant blue FCF were 3.38, 2.89, 2.62, 1.92, 3.38, 1.23 and 1.56 μg/L, respectively. The optimal methods were applied to identify and determine the synthetic dyes in seven foodstuffs and four soft drinks. The results showed that some samples contained synthetic dye at a higher concentration than the maximum legal limit in foods and soft drinks of Thailand.

Key words: synthetic dyes, foodstuffs, soft drinks, high performance liquid chromatography

INTRODUCTION

Natural or synthetic food colorants are often added to foodstuffs and soft drinks in order to maintain the natural color during process or storage and to create the desired colored appearance. However, synthetic dyes have more advantages than natural dyes such as low price and high stability. At present, synthetic dye is widely used to make food more attractive and appetizing. Due to its toxicity, especially when consumed in excess, synthetic dyes is strictly controlled by laws, regulations and acceptable daily intake (ADI) values for food safety (Table 1).

The chromophore groups in synthetic dyes (Figure 1) can be analyzed with several methods such as visible spectrophotometry[(1)], thin layer chromatography[(2)], high performance liquid chromatography (HPLC)[(3-5)], capillary electrophoresis (CE)[(6-9)] and ion chromatography (IC)[(10)]. Among the methods mentioned above, HPLC provided the highest sensitivity and the separation of synthetic dyes were performed on a reversed phase C18 column[(3-5)]. While most HPLC and CE studies showed the separation of red or the mixture of red and yellow synthetic dyes, only a few papers[(10,11)] showed the separation of blue, green, red and yellow of synthetic dyes which was useful for the determination of dyes in real samples. In addition, carcinogenic dyes of Sudan and amaranth are found in foods such as chili spice[(12)] and chili tomato with cheese sauce samples[(13)]. No paper has shown the separation of synthetic dyes on monolithic column to date. Therefore, the purpose of this study was to develop HPLC method on monolithic RP 18 for the separation of synthetic dyes over the range of blue, green, yellow and red colors in a relatively short analysis time and under high sensitivity. The representative dyes for this study were amaranth, ponceau 4R, sudan red 1, tartrazine, sunset yellow FCF, fast green FCF and brilliant blue FCF. The proposed method was applied to identify and determine the synthetic dyes in foods and soft drinks.

MATERIALS AND METHODS

I. *Apparatus*

The chromatographic system consisted of a Waters (Milford, MA, USA) Model 600E gradient pump, a Rheo-

* Author for correspondence. Tel and Fax: +66-2-5644483; E-mail: nvachira@yahoo.com

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Table 1. Amount of synthetic dyes permitted by Ministry of Public Health (Thailand), Issue 281 (A.D. 2547), acceptable daily intake (ADI) values for food safety and countries prohibiting these dyes

Synthetic dye	color	Maximum allowance limit (mg/kg of food)	ADI (mg/kg of body weight)	Use prohibited
Amaranth	Red	0	0-0.5	Spain, USA, Austria, Norway, Russia, France, Thailand
Sudan red 1	Red	0	0	All countries
Ponceau 4 R	Red	50	0-4.0	USA, Norway
Brilliant blue FCF	Blue	50	0-12.5	Belgium, France, Germany, Switzerland, Sweden, Austria, Norway
Fast green FCF	Green	100	0-25	USA, Austria
Tartrazine	Yellow	200	0-7.5	Norway, Austria
Sunset yellow FCF	yellow	200	0-2.5	Norway

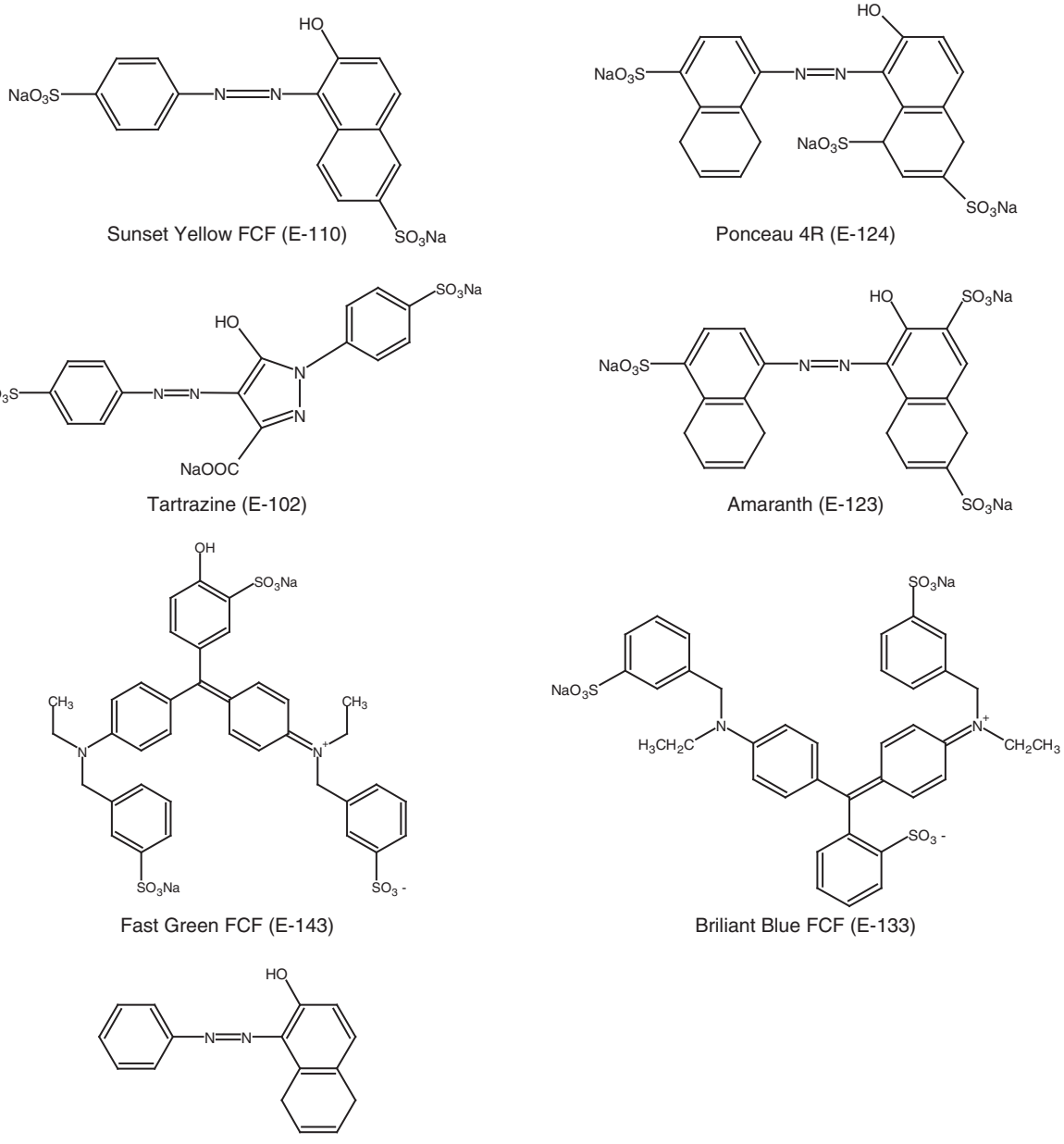

Figure 1. Structure formula of synthetic dyes.

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Table 2. The optimal composition of the mobile phase for seven synthetic dyes

Synthetic dye	Methanol (%, v/v)	[acetic acid] (mM)	Eluent pH
tartrazine	12	10	6.5
amaranth	15	10	6.5
ponceau 4R, sunset yellow FCF	25	10	6.5
fast green FCF, brilliant blue FCF	45	10	5.5
Sudan red 1	85	10	6

dyne (Cotani, CA, USA) model 7125 stainless steel injector (5 µL loop), a UV-VIS detector (Jasco, Tokyo, Japan) operated at 429, 484, 488, 509, 521, 623 and 633 nm for tartrazine, sunset yellow FCF, sudan red 1, ponceau 4R, amaranth, fast green FCF and brilliant blue FCF. A Chromolith RP-18 endcapped column (4.6 mm i.d., 100 mm, Merck) was used as the analytical column and was fitted with a Chromolith RP-18 (4.6 mm i.d., 5 mm, Merck) guard column. The optimal compositions of the mobile phase are shown in Table 2. The flow rate of the mobile phase was 1 mL/min and the column temperature was kept at 30°C by using a column heater (model TCM, Waters). Each analysis was performed in three replicates. Ultrasonic 1510 (Branson, Danbury, USA) was used to degas sample solution.

II. *Reagents and Solutions*

The standard synthetic dyes were amaranth (E-123), ponceau 4R(E-124), sudan red1, sunset yellow FCF (E-110) and fast green FCF (E-143) from Sigma-Aldrich (Steinheim,Germany), tartrazine (E102) from Acros organics(New Jersey, USA), and brilliant blue FCF (E-133) from Proquimac (Vacarisses, Spain). Stock solutions of synthetic dyes were prepared at a concentration of 1000 mg/L. All dyes were dissolved in deionized water, except Sudan red 1 that was dissolved in 50% (v/v) methanol. HPLC methanol and sodium hydroxide were obtained from Merck (Darmstadt, Germany) and acetic acid was from BDH (Poole, UK).

III. *Sample Preparation for Foodstuffs and Soft Drinks*

Triplicate 1-5 g of foodstuffs was added in 20 mL of water and diluted to the final volume of 25 mL in volumetric flask after the synthetic dyes were completely dissolved. In case of soft drinks, samples were degassed in an ultrasonic bath and diluted as required. Due to the precipitation appear in some dissolved sample solution, filtration was required before injection into HPLC. However, filtration made the synthetic dyes absorbed on nylon filter. To prevent the decrease intensity of dyes, the filter was required until saturated dyes were absorbed completely. The filtered solution was used afterward.

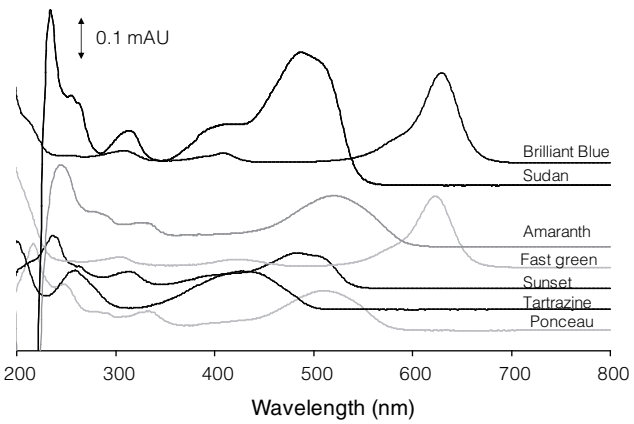

Figure 2. Absorption spectra of seven synthetic dyes dissolved in methanol-water (50%, v/v) at pH 5.

RESULTS AND DISCUSSION

I. *Absorption Spectra of Synthetic Dyes*

Absorption spectra of each standard synthetic dye dissolved in methanol-water (50% v/v) measured at pH 5 are shown in Figure 2. The maximum absorbance of each dyes occurred in both UV and visible wavelength. Due to UV cut off of methanol and acetate buffer interfered the absorbance of dye in UV range, detection wavelengths for HPLC were selected from the maximum absorbance in visible range at 429, 484, 488, 509, 521, 623 and 633 nm for tartrazine, sunset yellow FCF, sudan red 1, ponceau 4R, amaranth, fast green FCF and brilliant blue FCF, respectively.

II. *Separation Parameters*

The amount of methanol in the mobile phase strongly affected retention time. The seven synthetic dyes used in this study exhibited different hydrophobic interaction to stationary phase. Therefore, the dyes were eluted from the Chromolith RP-18 using different percentages of methanol and small molecule containing high polarity was initially eluted. The optimal values are summarized in Table 2. Other factors of the mobile phase were inves-

tigated as follows. First, the concentration of acetate buffer over the range 10-15 mM provided similar retention time and peak height. Thus, 10 mM acetate buffer was selected. Second, the eluent pH had an effect on peak height and peak area of synthetic dyes and pH 5.5 to 7 was studied. The optimal eluent pH was selected from the highest peak height observed. The best pH was obtained from fast green and brilliant blue at pH 5.5, sudan at pH 6 and, tartrazine, amaranth, ponceau and sunset yellow at pH 6.5 (Table 2). Chromatogram of standard synthetic dyes under the optimal composition of mobile phase is illustrated in Figure 3. Because brilliant blue and fast green contain isomers, these dyes showed three peaks and two peaks, respectively (Figure 3). Thus, the total peak area was used for the determination of synthetic dye in sample. However, detection wavelength of tartrazine, amaranth, ponceau 4R and sunset yellow FCF was 429, 521, 509 and 484, respectively. Methanol for elution of each dye from monolithic RP-18 column was 12, 15, 25, 25% v/v, respectively. Therefore, the simultaneous separation of these dyes at single wavelength within 429-521 nm and using the gradient elution from 15-25 % methanol in 10 mM acetate buffer at pH 6.5 was possible. For example, the chromatogram in Figure 4 shows simultaneous separation of tartrazine (T), amaranth (A), ponceau 4R (P) and sunset yellow FCF (S) at detection wavelength of 484 nm. Although polarity of tartrazine was higher than that of amaranth, retention of amaranth was less than that tartrazine because tartrazine used less % methanol in mobile phase than amaranth (12% and 15% of methanol for tartrazine and amaranth, respectively) (Figure 3). Comparison of the elution order of tartrazine and amaranth in the same composition of mobile phase is showed in Figure 4. The result showed the retention of tartrazine was less than amaranth because hydrophobic interaction between amaranth and C18 stationary phase was higher than tartrazine. From Figure 4, the capacity factors for tartrazine, amaranth, ponceau and sunset yellow were 0.33, 0.71, 3.29 and 4.56, respectively.

III. *Analytical Performance Parameters*

The detection limits (determined at signal-to-noise ratio of 3) obtained from the optimal composition of mobile phase at wavelength providing the maximum absorbance of each dye in visible for amaranth, ponceau 4R, sudan red 1, tartrazine, sunset yellow FCF, fast green FCF and brilliant blue FCF were in range from 1.92- 3.38 µg/L, respectively. The external calibration exhibited good linearity over the range of 25 µg/L - 20 mg/L and R^2 ranged from 0.9998 to 1. The % relative standard deviations (n = 5) for migration time, peak area and peak height were 0.1820-0.8290, 0.4480-3.4894 and 0.4901-3.5603, respectively. The performance characteristics of HPLC method are summarized in Table 3.

IV. *Determination of Synthetic Dyes in Samples*

The developed HPLC method was applied to analysis of synthetic dyes in seven foodstuffs and four soft drinks.

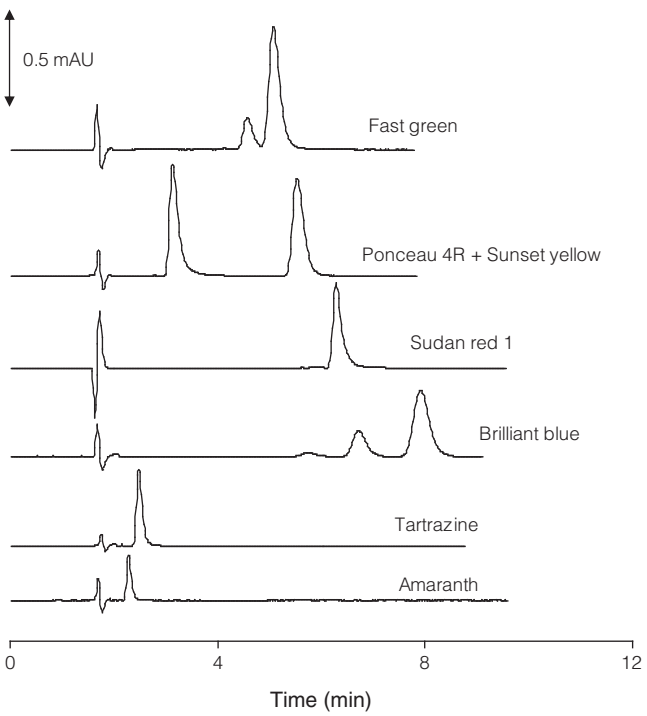

Figure 3. Chromatogram of seven standard synthetic dyes under optimal condition of each dyes (Table 2). Concentration: 2 mg/L fast green, a mixture of 5 mg/L ponceau 4R and 5 mg/L sunset yellow, 2 mg/L sudan red 1, 2 mg/L brilliant blue, 2 mg/L tartrazine and amaranth 1 mg/L.

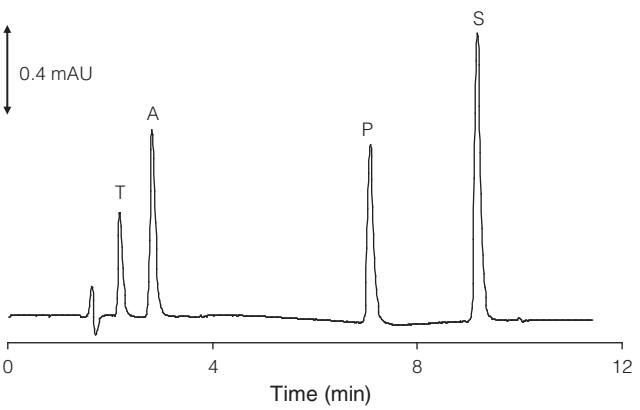

Figure 4. Chromatogram of a mixture of standard tartrazine (T), amaranth (A), ponceau 4R (P) and sunset yellow FCF (S) with a concentration of 0.5 µg/mL. Mobile phase was gradient program; A=15 mM acetate buffer pH 6.5; B=100% methanol; 0-1 min: isocratic elution: 85% A: 15% B; 1-2 min: linear gradient from 85% A: 15% B to 80% A: 20% B; 2-3 min: linear gradient from 80% A: 20% B to 75% A: 25% B; 3-10 min: isocratic elution at 75% A:25% B; 10-18 min back to initial condition at 85% A: 15% B; analytical column: 100 x 4.6 mm I.D, Chromolith RP-18 endcapped column; flow rate: 1 mL/min; 30 °C; injection volume: 5 µL; detection at 484 nm.

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Table 3. Performance characteristics for the determination of synthetic dyes by HPLC

Synthetic dye	Linearity correlation	Detection limit (μg/L)	Quantitation limit (μg/L)	% RSD for intraday (n = 5)		% RSD for interday (n = 5)	
				Retention time	Peak area	Retention time	Peak area
Tartrazine	1	1.92	6.41	0.25	0.45	0.23	2.08
Amaranth	1	3.38	11.26	0.30	2.62	0.49	3.08
Ponceau 4R	1	2.89	9.64	0.20	2.20	0.10	6.38
Sunset Yellow FCF	0.9999	3.38	11.26	0.18	1.44	0.48	2.52
Fast green FCF	1	1.23	4.09	0.62	1.47	0.39	3.08
Brilliant blue FCF	0.9999	1.56	5.20	0.26	3.49	0.22	2.91
Sudan red 1	0.9998	2.61	8.73	0.83	2.23	0.16	2.65

• Linear range for each dye was 25 ng/mL-20 μg/mL
• Linear equation for tartrazine: y=18938x+291.4; amaranth: y=16062x-544.51; ponceau 4R: y=15214x-196.44; sunset yellow FCF: y=19974x-694.81; Fast green FCF: y=61201x-525.81; brilliant blue FCF: 58111x-760.92 and sudan red 1: y=30897x-282.05.

Table 4. Concentration of synthetic dyes in food and soft drink samples

Sample	Synthetic dye found	Concentration (mg/kg)
HB candy (red)	Ponceau 4R	258.7
	Tartrazine	127.9
C candy (orange)	Sunset yellow	46.6
	Tartrazine	45.6
C candy (blue)	Brilliant blue	36.4
C candy (green)	Brilliant blue	13.0
	Tartrazine	20.3
Chocolate candy (green)	Brilliant blue	31.1
	Tartrazine	74.3
Chilly-salt seasoning (orange-red)	Sunset yellow	44.2
	Ponceau 4R	13.5
Baked green pea (green)	Brilliant blue	2.6
	Tartrazine	40.9
Iced black tea (orange)	Sunset yellow	682.1
Soft drink (canned packaging, orange)	Sunset yellow	51.5
Soft drink (bottle packaging, orange)	Sunset yellow	37.3
Soft drink (canned packaging, green)	Fast green	7.5
	Tartrazine	84.5

The optimized compositions of the mobile phase for the determination of dyes in samples were in Table 2, analytical column: 100 × 4.6 mm I.D., Chromolith RP-18 endcaped column; flow rate: 1 mL/min; 30°C; injection volume: 5μL.

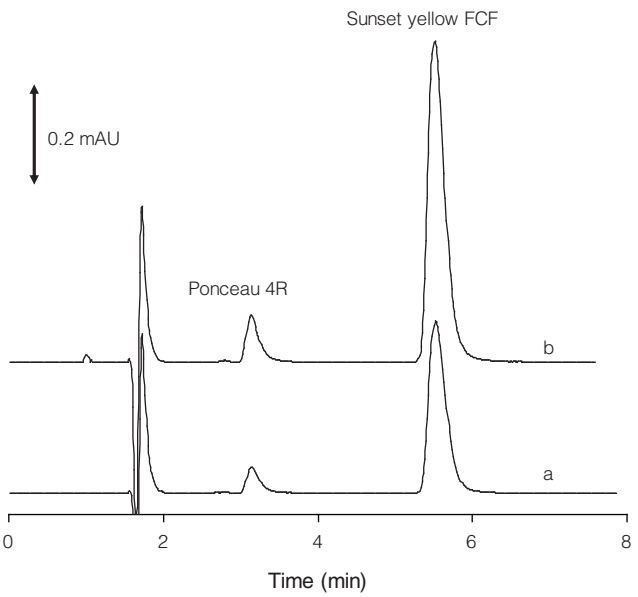

Figure 5. Chromatogram of (a) chili-salt seasoning sample and (b) chili-salt seasoning sample spiked with 1 mg/L of ponceau 4R and 1 mg/L of sunset yellow FCF. Mobile phase was methanol-water (25:75, v/v) containing 10 mM acetate buffer at pH 6.5; analytical column: 100 x 4.6 mm I.D, Chromolith RP-18 endcapped column; flow rate: 1 mL/min; 30°C; injection volume: 5 μL; detection at 484 nm.

Five synthetic dyes of ponceau 4R, sunset yellow FCF, tartrazine, fast green and brilliant blue FCF were found in the samples. Chromatograms of chili-salt seasoning and the spiking of the sample with standard Ponceau 4R and sunset yellow FCF to confirm the peak identity are shown in Figure 5. Table 4 shows the concentrations of synthetic dyes containing in the samples. One or two synthetic dyes were found in samples. In addition, HB candy and iced black tea had concentration of synthetic dyes higher than the maximum legal limit in food and soft drink of Thailand. However, the seven synthetic dyes used in this study are not allowed in some countries due to their effect on human health (see detail in Table 1). For examples, fast green FCF has been found to have tumorigenic effects in animal and mutagenic effects in both animals and humans. Ponceau 4R is considered carcinogenic by the U.S. including Norway and Finland. The % recovery for tartrazine, ponceau 4R, sunset yellow FCF, fast green FCF and brilliant blue FCF in samples were 97.59, 103.33, 103.11, 101.22 and 103.28, respec-

tively. The advantages of using monolithic column C18 compared to regular C18 were smaller peak width (0.32 min for monolithic C18 and about 1 min for regular C18), shorter analysis time (9 min for monolithic C18 and 19-25 min for regular C18[3,4]) and higher sensitivity. The detection limit of dyes for 5 µL injection loop on monolithic C18 column was in range 1.2-3.4 µg/L, whilst for 20 µL injection loop on regular C18 column the detection limit was in the range 0.3-1 mg/L[4].

CONCLUSIONS

The separation and determination of seven synthetic dyes were successful on monolithic RP-18 column by using methanol-water in acetate buffer as a mobile phase. This method is simple and requires minimal clean-up for sample preparation. Detection limits were satisfactory for all real samples. The results showed that this HPLC method was useful for the identification and determination of mixed synthetic dyes in food and soft drink samples. However, some samples have to investigate more than one HPLC experiment because they composed of mixed synthetic dyes which absorbance of each dye showed in different wavelength.

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REFERENCES

1. Altinoz, S. and Toptan, S. 2002. Determination of Tartrazine and Ponceau-4R in various food samples by Vierordt's mathod and ratio spectra first-order derivative UV spectrophotometry. J. Food Compos. Anal. 15: 667-683.
2. Oka, H., Ikai, Y., Kawamura, N., Yamada, M., Inoue, H., Ohno, T., Inagaki, K., Kuno, A. and Yamamoto, N. 1987. Simple method for the analysis of food dyes on reversed-phase thin-layer plates. J. Chromatogr. A 411: 437-444.
3. Gennaro, M. C., Gioannini, E., Angelino, S., Aigotti, R. and Giacosa, D. 1997. Identification and determination of red dyes in confectionery by ion-interaction high performance liquid chromatography. J. Chromatogr. A 767: 87-92.
4. Garcia-Falcon, M. S. and Simal-Gandara, J. 2005. Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal clean-up. Food Control 16: 293-297.
5. Zhang, Y. P., Zhang, Y. J., Gong, W. J., Gopalan, A. I. and Lee, K. P. 2005. Rapid separation of Sudan dyes by reverse-phase high performance liquid chromatography through statistically designed experiments. J. Chromatogr. A 1098: 183-187.
6. Jaworska, M., Szulinska, Z., Wilk, M. and Anuszewska, E. 2005. Separation of synthetic food colourants in the mixed micellar system: Application to pharmaceutical analysis. J. Chromatogr. A 1081: 42-47.
7. Perez-Urquiza, M. and Beltran, J. L. 2000. Determination of dyes in foodstuffs by capillary zone electrophoresis. J. Chromatogr. A 898: 271-275.
8. Huang, H. Y., Shih, Y. C. and Chen, Y. C. 2002. Determining eight colorants in milk beverages by capillary electrophoresis. J. Chromatogr. A 959: 317-325.
9. Giovine, L. D. and Bocca, A. P. 2003. Determination of synthetic dyes in ice-cream by capillary electrophoresis. Food Control 14: 131-135.
10. Chen, Q. C., Mou, S. F., Hou, X. P., Riviello, J. M. and Ni, Z. M. 1998. Determination of eight synthetic food colorants in drinks by high performance ion chromatography. J. Chromatogr. A 827: 73-81.
11. Huang, H. Y., Chiu, C. W., Sue, S. L. and Cheng, C. F. 1995. Analysis of food colorants by capillary electrophoresis with large-volume sample stacking. J. Chromatogr. A 995: 29-36.
12. Ma, M., Luo, X., Chen, B., Su, S. and Yao, S. 2006. Simultaneous determination of water-soluble and fat-soluble synthetic colorants in foodstuff by high performance liquid chromatography-diode array detection-electrospray mass spectrometry. J. Chromatogr. A 1103: 170-176.
13. Calbiani, F., Careri, M., Elviri, L., Mangia, A. and Zagnoni, I. 2004. Accurate mass measurements for the confirmation of Sudan azo-dyes in hot chilli products by capillary liquid chromatography-electrospray tandem quadrupole orthogonal-acceleration time of flight mass spectrometry. J. Chromatogr. A 1058: 127-135.