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Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array detection

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Abstract

Forty synthetic food colors were determined in drinks and candies by reversed-phase high-performance liquid chromatography with photodiode array detection. The following food colors were analyzed within 19 min using a short analytical column (50 mm × 4.6 mm i.d., 1.8 μm) at 50 °C with gradient elution: Ponceau 6R, Tartrazine, Fast yellow AB, Amaranth, Indigotine, Naphthol yellow S, Chrysoine, Ponceau 4R, Sunset yellow FCF, Red 10B, Orange G, Acid violet 7, Brilliant black PN, Allura red AC, Yellow 2G, Red 2G, Uranine, Fast red E, Green S, Ponceau 2R, Azorubine, Orange I, Quinoline yellow, Martius yellow, Ponceau SX, Ponceau 3R, Fast green FCF, Eosine, Brilliant blue FCF, Orange II, Orange RN, Acid blue 1, Erythrosine, Amido black 10B, Acid red 52, Patent blue V, Acid green 9, Phloxine B, Benzyl violet 4B, and Rose bengal. The recoveries of these compounds added to soft drinks and candies at 5 μg/g ranged from 76.6 to 115.0%, and relative standard deviations (R.S.D.s) were within 6.0%. The limits of detection and the limits of quantitation were 0.03 and 0.1 μg/g, respectively.

Keywords: Synthetic food colors; Drinks and candies; HPLC; Photodiode array detection; Short column

1. Introduction

Food colors are used for maintenance and improvement of color appearance in foods. In recent years, natural food colors have been increasingly used for the consumer preference. However, they are relatively unstable and the costs are higher than synthetic food colors. Therefore, synthetic food colors are still used instead of natural colors in many foods such as drinks, candies, and sweets.

The lists of permitted food colors vary from country to country. In Japan, there are some violation cases of food products containing non-permitted food colors such as Azorubine, Quinoline yellow, and Patent blue V [1]. These food colors are permitted and frequently used in EU countries, however, they are not permitted in other countries including Japan and USA.

All ingredients including food colors are required to be listed on the food labels. Some people are sensitive to particular food colors, so the colors in the food products must be the same as those on their labels. Thus, determination of synthetic food colors is required to ensure the food safety.

A large number of analytical methods for food colors have been proposed, such as thin layer chromatography (TLC) [2,3], voltammetry [4], polarography [5], spectrophotometry [6,7], capillary electrophoresis (CE) [8–10], ion chromatography [11], and high-performance liquid chromatography (HPLC) [12–17]. Voltammetry, polarography, and spectrophotometry are simple and rapid methods, however, they are not suitable for determining multiple food color mixtures in the food matrix. Capillary electrophoresis gives high resolution and short analysis time, but tailing or fronting peaks of some food colors are observed in the electropherograms. In HPLC methods, the followings were reported: determination of 38 food colors within 40 min [12], 14 food colors within 50 min [14], 10 food colors within 25 min [15], and 13 food colors within 29 min [17].

In this study, we analyzed 40 synthetic food colors within 19 min: 12 colors permitted in Japan (Tartrazine, Amaranth, Indigotine, Ponceau 4R, Sunset yellow FCF, Allura red AC, Fast green FCF, Brilliant blue FCF, Erythrosine, Acid red 52, Phloxine B, and Rose bengal), 6 colors permitted in EU and their analytical standards were commercially available in Japan

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(Brilliant black PN, Red 2G, Green S, Azorubine, Quinoline yellow, and Patent blue V), and 5 colors occasionally detected as illegal use at quarantine stations and public health centers in Japan (Red 10B, Fast red E, Orange II, Orange RN, and Amido black 10B) [1,18].

This paper describes an analytical method of 40 synthetic food colors in drinks and candies by HPLC equipped with a photodiode array detector using a short analytical column.

2. Experimental

2.1. Chemicals and reagents

Ponceau 6R (CI 16290), Chrysoine (CI 14270), Brilliant black PN (CI 28440), Yellow 2G (CI 18965), Fast red E (CI 16045), Green S (CI 44090), Azorubine (CI 14720), Martius yellow (CI 10315), Ponceau SX (CI 14700), Ponceau 3R (CI 16155), Eosine (CI 45380), Acid blue 1 (CI 42045), Amido black 10B (CI 20470), and Acid green 9 (CI 42100) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Tartrazine (CI 19140), Amaranth (CI 16185), Indigotine (CI 73015), Ponceau 4R (CI 16255), Sunset yellow FCF (CI 15985), Allura red AC (CI 16035), Fast green FCF (CI 42053), Brilliant blue FCF (CI 42090), Erythrosine (CI 45430), Acid red 52 (CI 45100), Phloxine B (CI 45410), and Rose bengal (CI 45440) were purchased from Society of Japanese Pharmacopoeia (Tokyo, Japan). Naphthol yellow S (CI 10316), Red 10 B (CI 17200), Orange G (CI 16230), Uranine (CI 45350), Orange I (CI 14600), Orange II (CI 15510), and Benzyl violet 4B (CI 42640) were supplied by Wako Pure Chemical (Osaka, Japan). Acid violet 7 (CI 18055), Red 2G (CI 18050), Quinoline yellow (CI 47005), and Orange RN (CI 15970) were obtained from MP Biomedicals (Irvine, CA, USA). Ponceau 2R (CI 16150) was purchased from Waldeck (Münster, Germany). Fast yellow AB (CI 13015) was from Sigma–Aldrich (St. Louis, MO, USA), and Patent blue V (CI 42051) was from Fluka (Buchs, Switzerland). Sodium salts of the standards were used except Martius yellow and Acid blue 1. These food color standards were used as received. The purity was over 85% in the standards which had purity data on the labels or certificates of analysis. Other food color standards met or exceeded company standards of purity.

Ethanol and methanol (HPLC grade), polyamide (particle size 150–425 μm), acetic acid, ammonia solution, and ammonium acetate were obtained from Wako Pure Chemical. Syringe filter (PVDF, 0.45 μm, 13 mm) was purchased from Whatman (Clifton, NJ, USA).

Water was purified using Milli-Q water purification system (Millipore, Bedford, MA, USA).

All of the stock solutions (1000 μg/ml) except for Martius yellow were prepared in water. Martius yellow was dissolved in methanol. Working solutions (20 μg/ml) were prepared freshly by mixing the stock solutions and diluting with water.

Forty food colors were divided into 2 groups, groups A and B for working solutions.

Working solution A contains following food colors (group A): Ponceau 6R, Tartrazine, Amaranth, Indigotine, Chrysoine, Ponceau 4R, Sunset yellow FCF, Orange G, Acid violet 7, Allura red AC, Red 2G, Uranine, Green S, Azorubine, Quinoline yellow, Ponceau SX, Fast green FCF, Brilliant blue FCF, Orange II, Acid blue 1, Erythrosine, Acid red 52, Acid green 9, Phloxine B, and Rose bengal.

Working solution B contains following food colors (group B): Fast yellow AB, Naphthol yellow S, Red 10B, Brilliant black PN, Yellow 2G, Fast red E, Ponceau 2R, Orange I, Martius yellow, Ponceau 3R, Eosine, Orange RN, Amido black 10B, Patent blue V, and Benzyl violet 4B.

2.2. Liquid chromatography and separation conditions

The analyses were performed with a Shimadzu (Kyoto, Japan) LC-10 system equipped with LC-10AT pumps and a diode array detector SPD-M10AVP. Separations were done using a ZORBAX Eclipse XDB-C18 Rapid Resolution HT (50 mm × 4.6 mm i.d., 1.8 μm, Agilent Technologies, CA, USA). Solvent A was 0.1 mol/l of ammonium acetate aqueous solution (pH 6.7) and solvent B was methanol–acetonitrile (50:50, v/v). The flow rate was set at 1.5 ml/min and the injection volume was 5 μl. In gradient-elution analysis, the initial mobile phase was 3% of solvent B, increased linearly to 60% in 18 min, and held at 60% for 2 min. A return to the initial conditions was carried out in 10 min. The column was kept at 50 °C in the column oven.

The column eluate was monitored at 450, 490, 520, and 620 nm for the yellow, orange, red, and blue colors, respectively. The absorption spectra of the food colors were recorded between 200 and 700 nm. Peak identification was done by comparing the retention times and absorption spectra of the samples with food color standards.

2.3. Sample preparation

Sample preparation was performed as follows based on the modified Japanese official analytical method [19].

For drinks and syrups, a 10 g amount of the sample was weighed accurately in a beaker. If the sample was carbonated, it was degassed by ultrasonication for 5 min. In case of alcoholic beverages, ethanol in the sample was evaporated on a hot plate and the evaporated volume of water was added. Ten milliliters of water was added to the sample and mixed. The content was adjusted to approximately pH 3 with 6% acetic acid.

For candies, gelatin candies, and marshmallows, a 10 g amount of the crushed sample was weighed accurately in a beaker. Adding 50 ml of water, the sample was dissolved heating on a hot plate. The content was adjusted to approximately pH 3 with 6% acetic acid.

Polyamide column was prepared by making slurry of polyamide and packing it on to a column with a 10 mm i.d. The column was packed up to a height of ca. 5 cm.

The sample solution was applied to the column conditioned with 20 ml of 1% acetic acid, and then the column was washed with 20 ml of 1% acetic acid followed by 20 ml of water. The column was eluted with 15 ml of 1% ammonia solution/ethanol (1:1, v/v), and then the eluate was filtrated through a 0.45 μm syringe filter. The filtrate was neutralized with 6% acetic acid

and evaporated to dryness on a rotary evaporator at 40 °C. The residue was dissolved in 2 ml of methanol/water (1:1, v/v). For Quinoline yellow, Acid green 9, and Benzyl violet 4B, sum of the two major peak areas were used for quantitation.

2.4. Precision and accuracy

Method validation samples were prepared by fortifying 10 g of food samples (soft drinks and candies) with 2.5 ml of 20 μg/ml working solutions. Each fortification level was 5 μg/g to the sample. Recoveries were calculated comparing the peak areas of the fortifying samples with those of standard solutions.

Intra-day and inter-day reproducibility of the method were assessed by performing replicate analyses. Intra-day precision was determined by analyzing six fortified samples on the same day. Inter-day repeatability was determined by analyzing the fortified sample on six different days.

3. Results and discussion

3.1. Separation of mixed food colors by liquid chromatography

Simultaneous analysis of 40 different food colors by HPLC is difficult. As using ion-pair reagents, which increase the retention time and improve separation of charged analytes, is tedious and time-consuming for column equilibration [13,15], gradient elution without ion-pair reagent was adopted.

Ammonium acetate aqueous solution was used for solvent A. Ammonium acetate is often used as a mobile phase additive of LC/MS [20]. Addition of ammonium acetate promotes ionization of analytes and enhances the sensitivity. For the further confirmation analysis by LC/MS, we used ammonium acetate solution which can also be used for LC/MS.

For solvent B, the common reversed-phase solvents such as methanol, acetonitrile, and methanol–acetonitrile were compared. Methanol–acetonitrile (50:50, v/v) gave the best separation of the peaks.

The elution order of synthetic food colors mainly determined by their polarities, depending on the number of polar functional groups such as hydroxyl groups or sulfonate groups. Azo groups generally tended to elute earlier than triphenylmethane and xanthene groups.

By using the conventional analytical column (STR ODS-II, Shinwa Chemical, Kyoto, Japan, 150 mm × 4.6 mm i.d., 5 μm) at flow rate 1.0 ml/min, total analysis time of these 40 colors was 39 min. In order to shorten the analysis time, we tried to use a short analytical column. The column we used was a ZORBAX Eclipse XDB-C18 Rapid Resolution HT (50 mm × 4.6 mm i.d., 1.8 μm), whose maximum operating pressure and temperature are 60,000 kPa and 60 °C, respectively. The initial column pressure was 18,100 kPa at flow rate 1.5 ml/min. In spite of the small particle and high flow rate, the column pressure was low enough to be used for conventional HPLC system not for ultra-high-pressure or ultra-fast LC. Forty food colors were analyzed within 19 min by using this short column. This analytical method is more efficient than other methods [12,14,15,17] in terms of analysis time and the number of analytes.

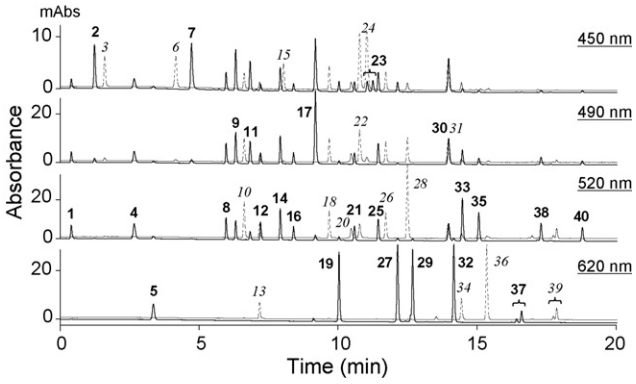

Fig. 1. HPLC chromatograms of mixed food color standard solutions (5 μg/ml). The solid lines with bold numbers indicate chromatograms of group A and the dotted lines with italic numbers indicate those of group B. Peak numbers correspond to food colors listed in Table 1.

Loading the mixed standard solution onto HPLC, some peaks in the chromatogram were partly overlapped and not separated completely. Therefore, 40 food colors were divided into 2 groups in order to avoid overlapping of peaks for the recovery test. HPLC chromatograms of mixed food color standard solutions are shown in Fig. 1.

Orange II in group A and Orange RN in group B are isomers, giving similar retention times. However, since the spectra around 410 nm were slightly different [21], they were distinguishable. Other food colors except Orange II and Orange RN were identified by their retention times.

As shown in Fig. 2, setting Column temperature at 40 °C, Orange II and Acid blue 1 had similar retention times. Increasing the column temperature to 45 °C, the peak had a shoulder and started to separate. At 50 °C they were separated (resolution, Rs = 1.67), and at 55 °C completely separated (Rs = 2.47). We chose the column temperature 50 °C in terms of the column lifetime. High temperature increased resolution and decreased the column pressure reducing viscosity of the solvent.

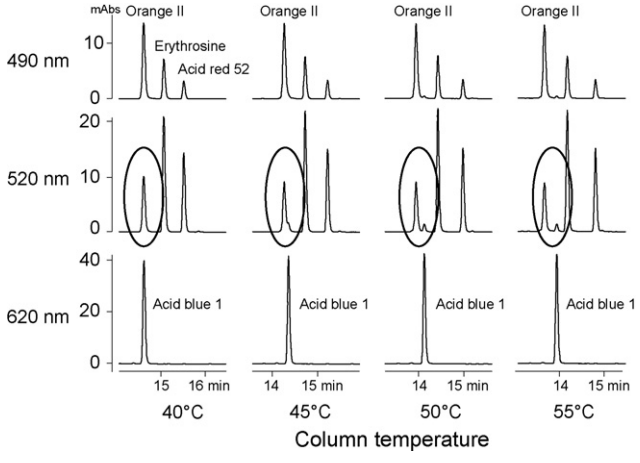

Fig. 2. Effect of column temperature in separating Orange II and Acid blue 1.

Table 1
Detection wavelengths and limits of detection (LODs) of the food color standard solution

Peak number	Compound	CI number	E number	Detection wavelength (nm)	LOD (μg/ml)
1	Ponceau 6R	16290		520	0.078
2	Tartrazine	19140	E102	450	0.040
3	Fast yellow AB	13015		450	0.060
4	Amaranth	16185	E123	520	0.053
5	Indigotine	73015	E132	620	0.039
6	Naphthol yellow S	10316		450	0.063
7	Chrysoine	14270		450	0.036
8	Ponceau 4R	16255	E124	520	0.050
9	Sunset yellow FCF	15985	E110	490	0.027
10	Red 10B	17200		520	0.011
11	Orange G	16230		490	0.038
12	Acid violet 7	18055		520	0.047
13	Brilliant black PN	28440	E151	620	0.047
14	Allura red AC	16035	E129	520	0.032
15	Yellow 2G	18965		450	0.058
16	Red 2G	18050	E128	520	0.060
17	Uranine	45350		490	0.014
18	Fast red E	16045		520	0.048
19	Green S	44090	E142	620	0.013
20	Ponceau 2R	16150		520	0.086
21	Azorubine	14720	E122	520	0.051
22	Orange I	14600		490	0.017
23	Quinoline yellow	47005	E104	450	0.084
24	Martius yellow	10315		450	0.040
25	Ponceau SX	14700		520	0.038
26	Ponceau 3R	16155		520	0.015
27	Fast green FCF	42053		620	0.005
28	Eosine	45380		520	0.006
29	Brilliant blue FCF	42090	E133	620	0.006
30	Orange II	15510		490	0.040
31	Orange RN	15970		490	0.039
32	Acid blue 1	42045		620	0.007
33	Erythrosine	45430	E127	520	0.011
34	Amido black 10B	20470		620	0.019
35	Acid red 52	45100		520	0.019
36	Patent blue V	42051	E131	620	0.009
37	Acid green 9	42100		620	0.075
38	Phloxine B	45410		520	0.012
39	Benzyl violet 4B	42640		620	0.125
40	Rose bengal	45440		520	0.059

3.2. Quantitation

Each food color has different wavelength of maximum absorption. We chose four typical wavelengths, 450, 490, 520, and 620 nm. The food colors were monitored at the wavelengths nearest to their own maximum absorption in the four wavelengths. They were monitored at 450, 490, 520, and 620 nm for the yellow, orange, red, and blue colors, respectively. The detection wavelengths of the food colors are presented in Table 1.

As Quinoline yellow, Acid green 9, and Benzyl violet 4B standards we used have two major peaks in the chromatograms, sum of the peak areas were used for quantitation.

Since Ponceau 6R has four sulfonate groups in a molecule, it was eluted very early (0.39 min). However, the peak shape was good and separated from other interference peaks detected at 520 nm.

The linearity for each compound was checked by analyzing mixed standard solutions of 7 different concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, and 20 μg/ml). Calibration curves for the standard solutions except Benzyl violet 4B were linear ($r^2 = 0.999$) between 0.05 and 20 μg/ml (0.5–20 μg/ml for Benzyl violet 4B). The limit of detection (LOD) was defined as three times the peak-to-peak noise. LODs of standard solutions are presented in Table 1. The LODs ranged from 0.005 (Fast green FCF) to 0.125 (Benzyl violet 4B) μg/ml.

3.3. Extraction and purification

There are many sorbents for solid phase extraction (SPE) such as ODS [9,13,22], quaternary amine [23], NH_2 (aminopropyl) [24], polyamide [8,12] for clean-up of synthetic colors in foods. Polyamide adsorbs synthetic food colors more specifically than other sorbents, so we used polyamide column clean-up for the sample purification. The pH of the sample solution for the polyamide column clean-up was adjusted to approximately pH 3 by checking with pH indicator paper because synthetic food

Table 2
Recoveries of the food colors fortified to soft drinks and candies

Peak number	Compound	Intra-day recovery ($n=6$)				Inter-day recovery ($n=6$)			
		Soft drink		Candy		Soft drink		Candy	
		Mean (%)	R.S.D. (%)	Mean (%)	R.S.D. (%)	Mean (%)	R.S.D. (%)	Mean (%)	R.S.D. (%)
1	Ponceau 6R	89.9	1.9	91.3	5.7	85.1	3.6	88.9	3.1
2	Tartrazine	91.4	1.6	93.9	5.6	89.5	3.4	93.2	1.8
3	Fast yellow AB	90.9	2.1	92.2	1.5	88.3	4.3	87.6	2.8
4	Amaranth	90.6	2.1	92.8	5.4	89.1	3.2	93.0	2.2
5	Indigotine	86.3	2.0	82.9	4.3	86.5	3.3	80.0	3.8
6	Naphthol yellow S	90.1	2.2	93.2	2.3	88.0	4.1	87.8	2.7
7	Chrysoine	76.6	2.7	76.9	4.5	78.8	2.0	76.0	2.4
8	Ponceau 4R	91.5	1.5	93.6	5.7	89.9	3.4	94.4	1.9
9	Sunset yellow FCF	91.6	2.4	93.0	5.6	90.0	4.1	94.1	1.9
10	Red 10B	90.1	2.8	90.9	1.6	87.3	4.1	85.6	2.9
11	Orange G	91.9	1.9	93.7	5.9	90.0	3.7	94.0	1.9
12	Acid violet 7	90.7	1.4	93.0	5.4	89.5	3.3	94.2	2.8
13	Brilliant black PN	84.3	1.9	88.7	1.8	84.0	3.7	82.3	3.4
14	Allura red AC	91.7	1.7	93.3	5.6	90.2	3.5	94.9	2.3
15	Yellow 2G	91.7	2.2	93.7	1.7	89.3	4.6	88.1	3.1
16	Red 2G	91.5	1.8	93.9	5.8	90.0	3.9	93.3	2.4
17	Uranine	92.0	1.6	90.8	4.5	90.3	3.8	94.7	3.0
18	Fast red E	89.7	3.1	92.6	1.8	88.4	4.6	87.3	3.3
19	Green S	90.3	1.5	91.0	5.2	88.7	3.5	92.7	1.9
20	Ponceau 2R	90.2	3.6	92.7	6.0	88.1	4.1	88.5	3.9
21	Azorubine	90.5	1.7	91.6	5.5	88.8	3.2	92.8	2.0
22	Orange I	89.1	2.7	91.2	1.4	88.2	3.2	87.2	2.2
23	Quinoline yellow	88.6	2.3	87.3	4.6	88.3	1.9	92.7	3.0
24	Martius yellow	88.3	3.4	90.7	2.7	85.7	5.1	85.0	3.7
25	Ponceau SX	91.2	1.6	92.4	5.5	89.4	3.6	93.4	1.9
26	Ponceau 3R	91.7	2.3	93.1	1.6	88.8	4.5	88.2	2.9
27	Fast green FCF	92.3	1.4	92.4	5.4	90.2	3.6	94.1	2.0
28	Eosine	90.0	2.7	89.6	4.0	87.8	4.1	84.0	4.9
29	Brilliant blue FCF	92.2	1.5	92.6	5.5	90.3	3.7	94.3	2.2
30	Orange II	90.9	1.7	89.2	3.7	89.5	3.2	94.0	2.7
31	Orange RN	90.9	3.0	94.2	3.0	88.4	5.0	88.1	3.6
32	Acid blue 1	92.7	1.3	93.0	5.4	91.4	3.4	95.3	2.4
33	Erythrosine	88.3	2.1	85.1	3.6	87.0	3.1	88.5	1.8
34	Amido black 10B	83.4	2.5	87.0	2.6	83.0	4.4	80.2	4.1
35	Acid red 52	92.8	1.7	92.9	5.7	91.7	3.6	95.6	2.5
36	Patent blue V	93.2	2.0	94.8	1.7	90.6	4.4	89.7	3.1
37	Acid green 9	95.9	4.4	91.3	5.5	100.9	6.7	102.6	5.7
38	Phloxine B	89.4	1.8	85.9	4.2	88.9	2.8	87.6	1.5
39	Benzyl violet 4B	114.8	3.8	115.0	2.0	114.9	4.6	114.3	3.9
40	Rose bengal	92.3	3.1	83.9	3.7	93.8	3.2	90.1	2.3

Each food color was fortified to the food sample at 5 μg/g.

colors are adsorbed on polyamide most strongly under acidic conditions at pH 3–4.

After the column clean-up, small particles of polyamide were observed in the eluate. They adsorbed the food colors in the eluate under neutral and acidic conditions, causing low recoveries especially for Brilliant black PN, Erythrosine, Amido black 10B, and Rose bengal. Therefore, the eluate was filtrated through a 0.45 μm syringe filter and small particles were removed under basic condition before neutralization and evaporation.

3.4. Recoveries

Table 2 shows the intra-day and inter-day recoveries of 40 food colors from soft drinks and candies fortified at 5 μg/g. The intra-day recoveries ($n=6$) ranged from 76.6 to 115.0%, with relative standard deviations (R.S.D.s) ranging from 1.3 to 6.0%, while the inter-day recoveries over six working days ranged from 76.0 to 114.9%, with relative standard deviations (R.S.D.s) ranging from 1.5 to 6.7%. Both intra-day and inter-day accuracy and precision data showed good R.S.D.s and recovery values.

Typical HPLC chromatograms of soft drink samples are shown in Figs. 3. They were fortified with food colors at the level of 0.5 μg/g. LODs and limits of quantitation (LOQs) in real food samples depended on food matrix of the samples. LODs (S/N = 3) in soft drinks (soda) were 0.03 μg/g (0.2 μg/g for Ponceau 6R and Acid green 9), and LOQs (S/N = 10) were 0.1 μg/g (0.5 μg/g for Ponceau 6R and Acid green 9). These LOD and LOQ values were confirmed by analyzing fortified samples and calculating recoveries. Recoveries of samples fortified at LOQ levels were over 60%.

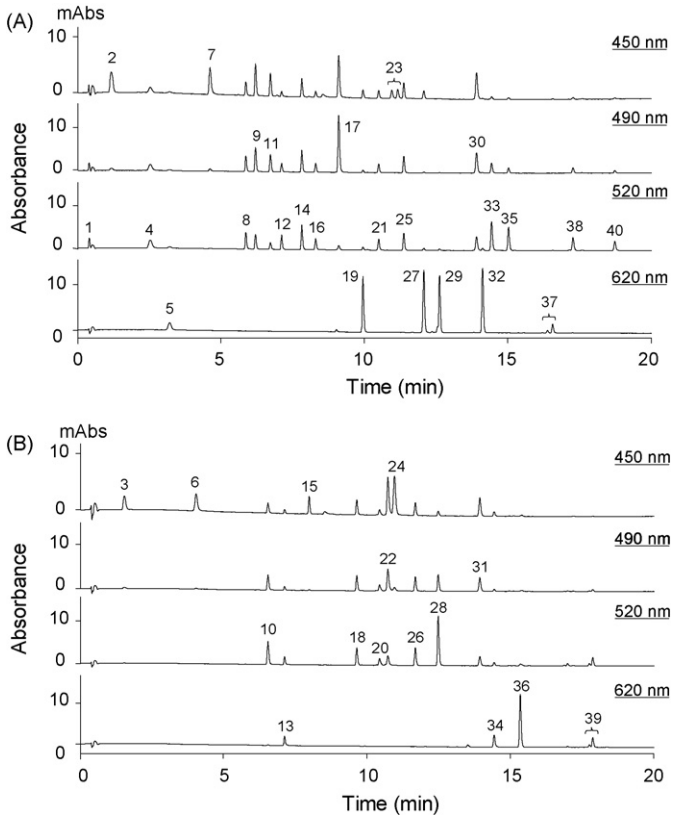

Fig. 3. Typical HPLC chromatograms of soft drink samples fortified with food colors at 0.5 μg/g. Peak numbers correspond to food colors listed in Table 1.

3.5. Application

Thirty imported commercial products (soft drinks, syrups, candies, gelatin candies, and marshmallows) colored with synthetic or natural food colors were analyzed by using this method. They were obtained from local markets at Hyogo prefecture in Japan. Quantitation was done by using calibration curves and confirmed by comparing absorption spectra of the samples with food color standards.

The food labels on 14 products out of 30 listed the names of the synthetic food colors. As their food labels, 14 food products contained some synthetic food colors: Tartrazine (1.3–21.8 μg/g, 7 samples), Amaranth (27.2 μg/g), Indigotine (trace−18.3 μg/g, 4 samples), Ponceau 4R (6.0 μg/g), Sunset yellow FCF (3.1–17.3 μg/g, 5 samples), Allura red AC (0.2–41.6 μg/g, 5 samples), and Brilliant blue FCF (0.9–5.9 μg/g, 3 samples). In addition, Fast red E which was not permitted in Japan was detected in grape-flavored soft drink at 0.7 μg/g.

Fast red E was one of the subsidiary colors in commercial Amaranth [25]. It was detected together with Amaranth (permitted in Japan), the ratio of Fast red E to Amaranth was 2.5%, which was lower than the maximum limit (3%) of total subsidiary colors regulated by JECFA [26]. It was considered to be a subsidiary color in Amaranth.

4. Conclusions

An analytical method was developed for quantitation of 40 synthetic food colors in drinks and candies by HPLC with photodiode array detection. Using a short analytical column, these food colors were analyzed within 19 min. This method gave reliable and reproducible results with satisfactory detection limits and short analysis time for the routine analysis of food colors.

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