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Analysis of food colorants by capillary electrophoresis with large-volume sample stacking

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Abstract

A technique combining an on-capillary concentration method known as large-volume sample stacking and high-efficiency CE separation has been developed to analyze and detect colorants in several food samples, such as soft drinks, jellies and milk beverages. Following optimization, this technique significantly reduced the limits of detection of eight food colorants commonly used in food products by up to two orders of magnitude when compared with the conventional capillary electrophoresis method. The developed technique was able to successfully determine colorants in food samples that had concentrations as low as 0.1–0.5 μg/ml.

Keywords: Food analysis; Large-volume injections; Sample stacking; Dyes

1. Introduction

Color is an important characteristic of foods because it enhances their visual aesthetics and promotes their sale. Food colorants, natural or synthetic, are commonly added to foods in order to compensate for the loss of natural colors that are destroyed during processing and storage, and to provide their desired colored appearance. The allowable amount of synthetic colorants, however, is strictly regulated for food safety reasons because of their potential toxic nature, especially when consumed in excess.

Presently, capillary electrophoresis (CE) is employed as a major separation tool for the analysis of food colorants [1–8]. The narrow internal diameter of the capillary tube, however, means that the CE technique generally has a lower sample capacity and a shorter optical pathlength for on-capillary UV detection. Therefore, a suitable sample concentration step prior to separation is necessary to improve the detection of commercial CE instruments commonly equipped with UV–visible detection [9,10].

Sample stacking is an on-capillary sample concentration method that can be utilized directly in commercially available CE instruments without alteration, and it can concentrate samples readily on the capillary prior to the CE separation step [11–15]. Large-volume sample stacking (LVSS) is a common sample stacking technique. The LVSS method has been used to concentrate and investigate charged analytes, such as drugs, dyes, chemicals of environmental concern, etc. [13–22]. With food containing special flavors being popular in Asia, and the permissable amount of colorants being strictly reg-

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ulated, it is worthwhile to find ways to improve the detection sensitivity for colorants in CE technology.

In this study a method combining the LVSS technique with high-efficiency CE separation was developed in order to analyze and detect eight common food colorants. Sample stacking combined with the optimum separation conditions for CE were employed to determine the levels of these colorants in real food samples, including soft drinks, jellies and milk beverages. The feasibility of the application of the developed method for food sample analysis is also discussed.

2. Experimental

2.1. Colorant standards

Brilliant blue FCF, carminic acid, Allura red AC and indigo carmine were obtained from TCI (Tokyo, Japan). Sunset yellow FCF was obtained from Aldrich (Milwaukee, WI, USA). Tartrazine and Fast green FCF were purchased from Sigma (St. Louis, MO, USA). New coccine was purchased from Acros (NJ, USA). These standards were dissolved individually in deionized water at a stock concentration of 2 mg/ml.

2.2. Chemicals and extraction column

Disodium tetraborate, ammonia solution (25%), and ethanol (absolute) were purchased from Merck (Darmstadt, Germany). β-Cyclodextrin (β-CD) was obtained from Calbiochem (La Jolla, CA, USA). Methanol was purchased from Pharmco (Brookfield, CT, USA). Sodium hydroxide and hydrochloric acid were obtained from J.T. Baker (Phillipsburg, NJ, USA). Polyamide cartridges (DPA-6S, 3 ml, 250 mg), used as the extraction column for solid-phase extraction (SPE), were purchased from Supelco (Bellefonte, PA, USA).

2.3. Food samples

Several food samples, soft drinks, milk beverages and jellies used as testing samples, were obtained from supermarkets in Taiwan. Orange-, apple-, and grape-flavored carbonated beverages mostly consisted of concentrated syrups, sugar, colorants and other additives. Grape-, pineapple-, and peach-flavored jelly samples were usually composed of agar, fruit juice, sugar, colorants and other additives. Apple-flavored milk samples usually contained at least 50% fresh milk in addition to ingredients such as fruit juice, sugar, colorants and other additives.

2.4. Sample pretreatment

Carbonated beverages were degassed by ultrasonic vibration for 10 min and then analyzed directly by CE without any other treatment. If needed, the SPE procedure described below was used for further removal of interferences.

Each jelly sample (1.0 g) was blended with 10 ml of 50% aqueous ethanol solution, mixed with a magnetic stir bar at 65 °C for 4 h, and the aqueous phase containing the colorants was decanted and collected, and was ready for the SPE procedure described below.

Milk samples diluted with ethanol in a volume ratio of 1:1 were mixed for 10 min with a magnetic stir bar, and the pH was then adjusted to 2.0 if needed. The milk solutions were centrifuged for 60 min at 16 000 rpm, then the clear centrifuged liquids were either collected and analyzed directly by CE, or were ready for the SPE procedure described below.

The polyamide column used in SPE was conditioned prior to use by washing with methanol (2 ml) then with deionized water (2 ml). After addition of the centrifuged liquid or the extracted aqueous phase, the extraction column was washed with deionized water (1 ml) followed by methanol (1 ml). The absorbed colorants were then eluted from the column with 1 ml of eluting solution (0.5% ammonia solution–methanol, 1:1, v/v) at a rate of approximately 0.5 ml/min. The eluted solution containing the colorants was then ready to be analyzed by CE.

2.5. Running buffer for CE

A running buffer of pH 10.0 was prepared by adding 20 m*M* sodium hydroxide solution to 15 m*M* disodium tetraborate (borax) until the desired pH was achieved. β-Cyclodextrin with a final concentration of 7 m*M* was added to the running buffer.

2.6. Apparatus and operating conditions for CE

All experiments were performed with a Beckman Coulter MDQ capillary electrophoresis system equipped with a photodiode-array detector (Fullerton, CA, USA). Beckman Coulter MDQ 32 Karat software was used for instrumental control and data analysis. Separations were performed using 50.2 cm (40 cm to detector)×50 μm I.D. uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). The capillaries were conditioned prior to separation by washing with 1 *M* sodium hydroxide (3 min), followed by deionized water (7 min), and then running buffer (5 min). Separations were carried out in normal mode of electrode polarity, and the applied voltage was maintained at 25 kV. The temperature of the capillary was maintained at 25 °C and 200 nm was selected as the detection wavelength.

2.7. Operating conditions for large-volume sample stacking

All samples or standards used in LVSS were prepared in a low conductivity matrix (50% aqueous ethanol or 0.5% ammonia solution–methanol, 1:1, v/v). The LVSS method consisted of three steps: (1) standards or samples were pressure-injected into the capillary column at 2 p.s.i. for 55 or 25 s, respectively; (2) after the sample solution had been introduced into the column inlet, a −5 kV voltage was applied to stack sample ions (1 p.s.i.=6894.76 Pa). When the current reached approximately 95% of the original value (or the maximum value when the whole capillary was filled with running buffer), the stacking process was stopped, which took approximately 3.0 min; (3) the running buffer vial was switched to the column inlet, and then a 25 kV separation voltage was applied to the column.

3. Results and discussion

3.1. Optimum LVSS conditions

In order to improve the detection limits for the eight colorants, optimum times for colorant stacking and for sample matrix removal from the capillary had to be determined. The colorant standards were dissolved in 50% aqueous ethanol, the conductivity of which was lower than that of the running buffer in the capillary. The colorant standard solution was injected hydrodynamically into the capillary for 55 s (2 p.s.i.), then −5 kV applied voltage was used for stacking and matrix removal.

The current was also recorded during stacking, and reached a maximum value of 8.6 μA at 5 kV as the entire capillary was filled with running buffer due to the aqueous sample matrix having been completely pushed out by electroosmotic flow. The experimental result indicated that the separation efficiency was poor when the stacking time was less than 2.8 min, where the peaks of the colorants were broad and distorted. It was also noted that the current only returned to 85% of its maximum value when the stacking step was stopped, which indicates that some of the longer sample zone remained in the capillary after 2.8 min of stacking. The first compound, Brilliant blue FCF, was eluted from the capillary inlet end when the stacking time was over 3.0 min. In this case, the current reached 99% of its maximum value, and the entire capillary was filled with running buffer. Some anion analytes were pumped out of the capillary after an excessive stacking time (greater than 3.0 min). Therefore, this left only the anions that had higher electrophoretic mobilities towards the positive electrode in the stacked sample zone and allowed them to be detected after separation.

In order to obtain a maximum stacking effect, the sample volume should be kept as small as possible after stacking, and no extra dispersion should be produced due to mismatch of electroosmotic flow (EOF) during separation [12,13]. In this study, a stacking time of 3.0 min, where the current returned to 95% of its maximum value, had the optimum concentration amplification. Similar to previous reports [11–15], the above results also demonstrate that careful monitoring of the current is needed to obtain optimum stacking conditions.

Electropherograms of the eight food colorant standards derived from conventional CE with 3.0 s hydrodynamic injection time, and from LVSS with 55 s hydrodynamic injection time and 3.0 min stacking time are shown in Fig. 1. The concentrations of colorants were 50 μg/ml for conventional CE and 1 μg/ml for LVSS. Concentration enhancement by LVSS was very apparent; in addition, high separation resolutions for all colorants were main-

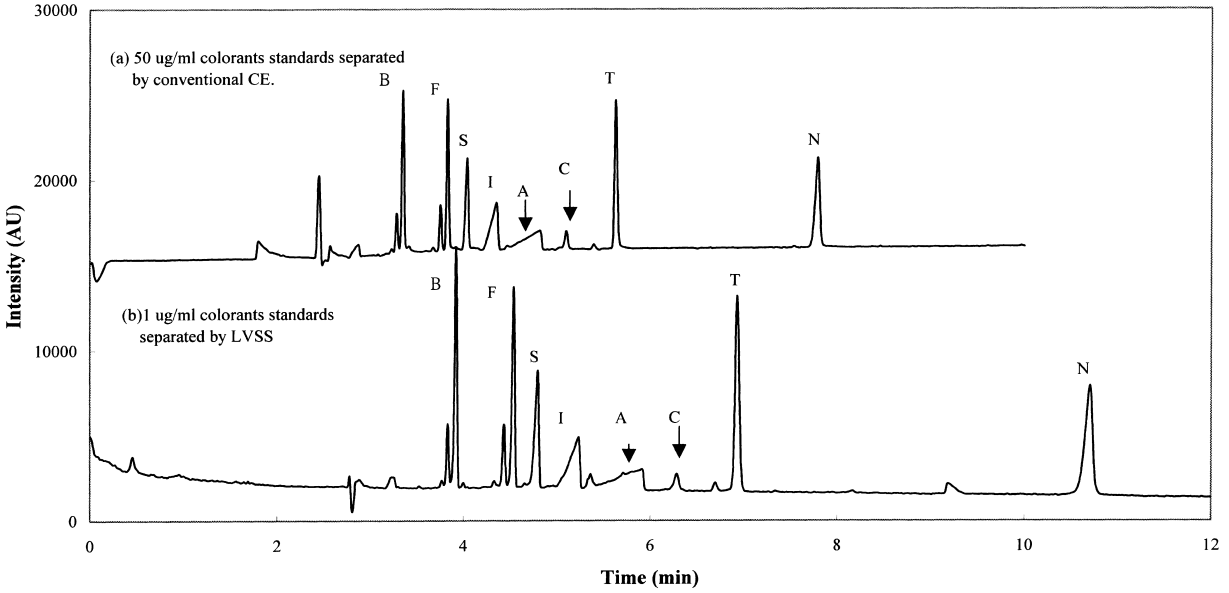

Fig. 1. Electropherograms of the eight food colorant standards: (a) using conventional CE; (b) using LVSS. Separating conditions: borax–NaOH buffer (pH 10.0) containing 7.0 m*M* β-CD was used as running buffer; sample injection time 5 s (0.5 p.s.i.) in conventional CE, and 55 s sample injection time (2 p.s.i.) and 3.0 min stacking time in LVSS, after which the current reached 95% of its maximum value; 25 kV voltage was used for the separation. The concentration of food colorant standards was 50 μg/ml (a), and 1 μg/ml (b). T, tartrazine; F, Fast green FCF; B, Brilliant blue FCF; A, Allura red AC; I, indigo carmine; S, Sunset yellow FCF; N, New coccine; C, carminic acid.

tained. The detection limits for colorant standards were reduced up to 80-fold by LVSS when compared to conventional CE without sample stacking [limits of detection (LODs) at 0.18–1.76 μg/ml, Table 1]. Calibration was performed at three concentration levels (0.5, 1.0, 5.0 μg/ml), and each concentration was analyzed three times by LVSS–CE. The correlation coefficients (*r*) of the calibration curves were above 0.996, which indicates that the concentration linearity for most colorants was still relatively good for the LVSS method.

Table 1
Comparison of detection limits of colorant standards for LVSS–CE and conventional CE methods

Colorant	Detection limit[a] (μg/ml)	
	LVSS–CE	CE
Brilliant blue FCF	0.002	0.18
Fast green FCF	0.003	0.19
Sunset yellow FCF	0.004	0.31
Indigo carmine	0.009	0.63
Allura red AC	0.021	1.68
Carminic acid	0.026	1.76
Tartrazine	0.003	0.19
New coccine	0.005	0.31

[a] Values are means of triplicate measurements.

3.2. Effect of sample matrix on colorant stacking

The feasibility of the LVSS method applied to various real food samples was then examined. Various fruit-flavored jellies, carbonated beverages, and milk beverages were tested by the stacking method. When LVSS was used to concentrate colorants, it was still difficult to determine colorants in these samples even though their peak intensities had increased markedly, as the appearance of unknown spikes resulted in overlapping peaks in the electropherograms. As most of the components in these samples were all concentrated simultaneously during the stacking procedure, the selection of one specific colorant to concentrate was difficult. These results clearly demonstrate the importance of the effect of the sample matrix on the separation ability of CE, and the inability of the LVSS–CE method developed from colorant standards to be used directly to

analyze colorants in foods due to interference from other components coexisting in the samples.

As the sample matrix altered the stacking effect of LVSS and the separation ability of CE, the LVSS method was modified in order to obtain a better separation and concentration effect for colorants. Optimum conditions of 25 s (2 p.s.i.) for sample injection and 2.9 min for sample stacking (the current was 95% of its maximum value) were determined for the application of LVSS to the analysis of food samples.

3.3. Effect of sample pretreatment on colorant stacking and separation

In order to avoid matrix interference during separation, polyamide SPE was used as a pretreatment step. Three different flavored carbonated beverages were first passed through a polyamide column SPE prior to separation by LVSS–CE. The colorants were detected successfully, demonstrating that the SPE method can effectively remove many unknowns from food samples, and these unknowns are not concentrated with the colorants during the stacking step. In the three tested samples, Fast green FCF and Allura red AC were detected in grape-flavored beverages, while tartrazine and Sunset yellow FCF were detected in orange-flavored beverages, and Brilliant blue FCF and tartrazine were detected in apple-flavored beverages (Fig. 2).

For jelly samples and milk beverages, separations were improved immediately when SPE was employed to reduce matrix compositions in the extraction solution. Brilliant blue FCF and Allura red AC were detected in grape-flavored jelly, and Sunset yellow FCF was detected in peach-flavored jelly without any interference (Fig. 3a and b).

In order to avoid the co-amplification of unknowns together with the colorants during stacking, another dilution method was examined. An apple-flavored milk sample was diluted to one-tenth of its original volume with 50% aqueous ethanol, and was then analyzed directly by LVSS without SPE pretreatment. Tartrazine and Sunset yellow FCF were clearly detected in the diluted sample without any interference (Fig. 3c). Therefore, a simple sample dilution

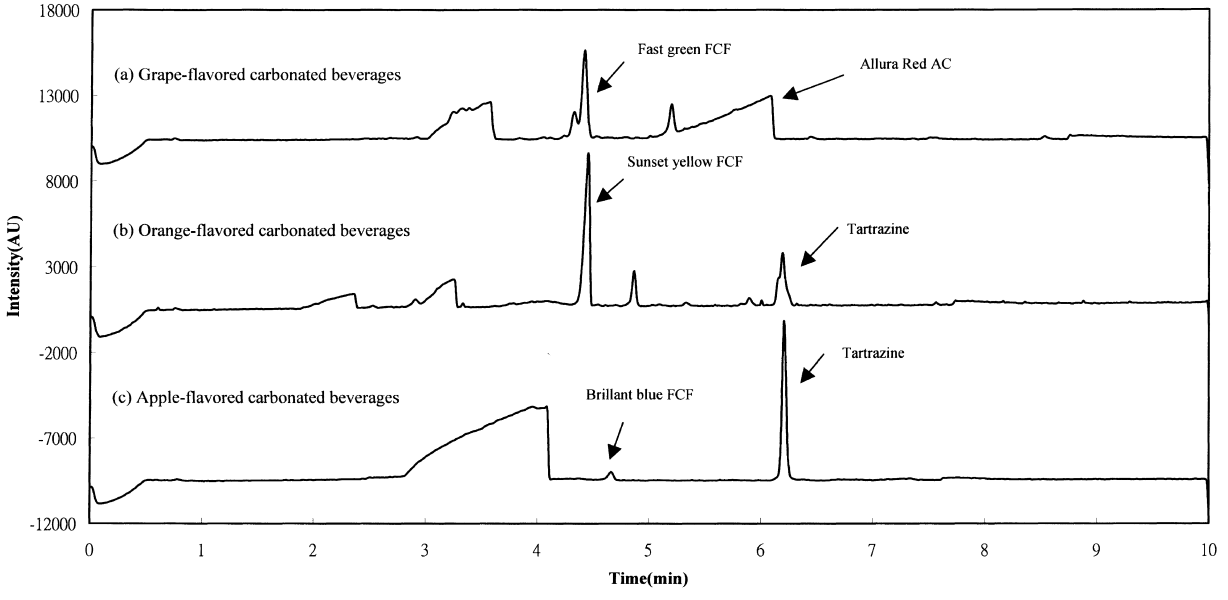

Fig. 2. Electropherograms of commercially available carbonated beverages determined by LVSS. (a) Grape-, (b) orange-, and (c) apple-flavored carbonated beverages. Fast green FCF, Allura red AC, Sunset yellow FCF, tartrazine, and/or Brilliant blue FCF were found in these products. LVSS conditions: sample injection time 25 s (2 p.s.i.); sample stacking time 2.9 min (−5 kV), after which the current reached 95% of its maximum value. Samples were treated with a polyamide column SPE, and then separated by LVSS–CE. Other separation conditions as in Fig. 1.

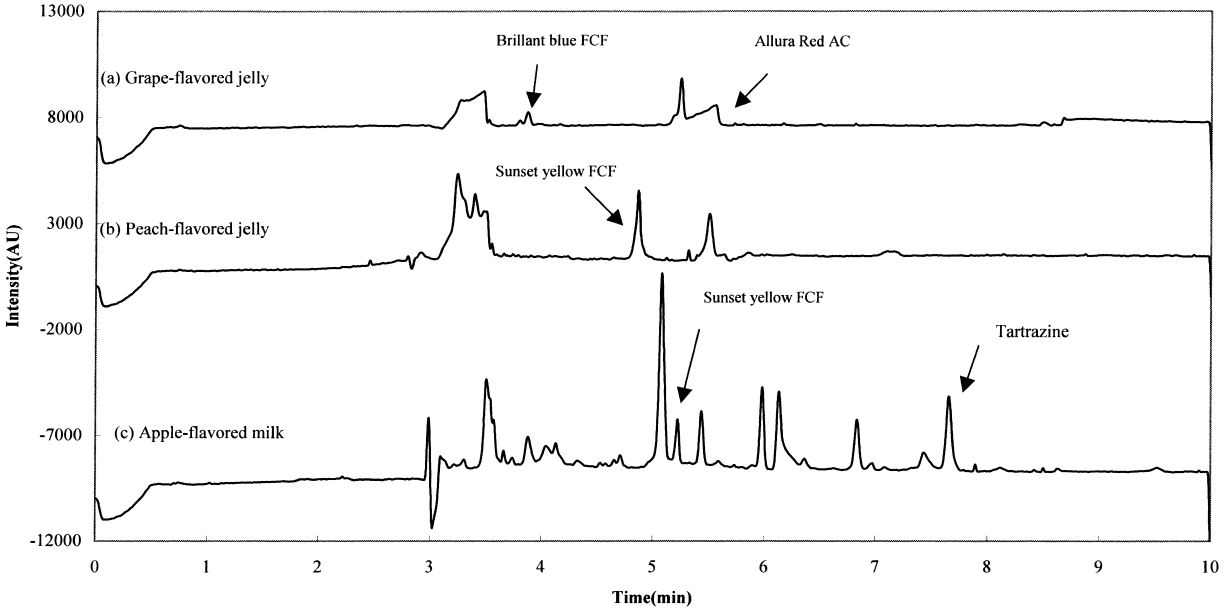

Fig. 3. Electropherograms of commercially available jelly and milk products determined by LVSS. (a) Grape-flavored jelly, (b) peach-flavored jelly, and (c) apple-flavored milk. Brilliant blue FCF, Allura red AC, Sunset yellow FCF, and/or tartrazine were found in these products. Other separation conditions as in Fig. 2.

was able to markedly reduce the interference caused by the unknown components. However, this dilution approach was only feasible for certain food samples.

In order to examine the improvement in concentration sensitivity for all colorant analytes in food samples by the developed method, colorant-free carbonated beverages spiked with eight colorant standards were separated by LVSS–CE after polyamide column SPE. Fig. 4 shows electropherograms of colorant-free carbonated beverages spiked with the eight food colorant standards at 0.1, 0.5 and 1.0 μg/ml. All colorants were clearly concentrated and detected at 0.1 μg/ml, except for carminic acid and New coccine, which were clearly detected at 0.5 μg/ml. The detection limits for most of the colorants in carbonated beverages were in the range 0.009–0.04 μg/ml based on a S/N ratio of 3, with the exception of carminic acid, which was 0.1 μg/ml. Upon further examination of Fig. 4, a sharp peak can be observed in front of the broad Allura red AC peak. We believe that this sharp peak is a result of the isomer of Allura red AC, which has a higher concentrating effect than that of Allura red AC itself under the LVSS conditions used. Therefore, the intensity of the peak for the isomer was more enhanced than that of Allura red AC.

3.4. Determination of the content of food colorants in commercial food products

Similar to previous reports that differences in the compositions of samples have a noticeable influence on the results of LVSS, we also found that the migration time of colorants in various food samples changed with sample type, and this was likely due to the different amounts of sample remaining in the capillary after stacking [16]. Therefore, besides using different migration times, a qualitative method for colorants is suggested by the standard addition method, and by comparing the UV spectra of the colorants in the food samples with those of the colorant standards. Hence, a quantitative analysis of the colorants in food samples was also carried out based on the standard addition method. Furthermore, minor peaks, which likely result from colorant isomers, co-migrate with the major peaks of Brilliant blue FCF, Fast green FCF and Allura red AC (see Fig. 1). If ignored, these minor peaks may cause

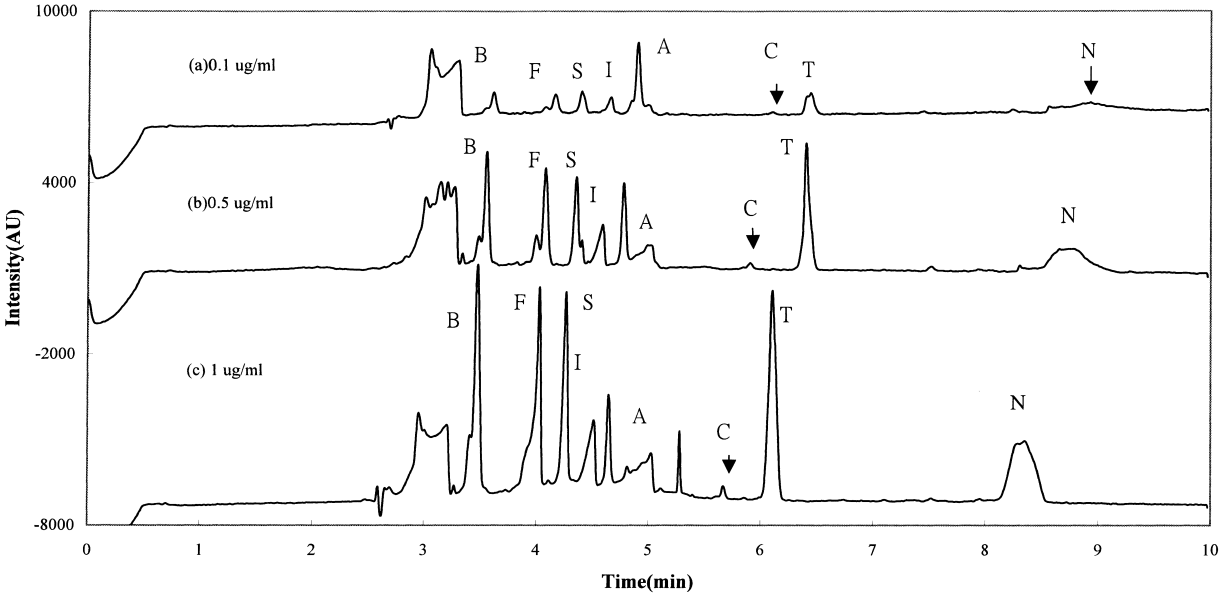

Fig. 4. Electropherograms of colorant-free carbonated beverages spiked with eight food colorant standards and separated by LVSS. Conditions: 25 s sample injection time (2 p.s.i.) and 2.9 min stacking time used in LVSS; the concentration of the added food colorants was 0.1 μg/ml (a), 0.5 μg/ml (b) and 1 μg/ml (c). The samples were first treated with a polyamide column SPE, and then separated by LVSS–CE. Other separation conditions as in Fig. 2.

problems with the quantitative analysis of these colorants. Therefore, in order to reduce the effect of this phenomenon on the quantitative analysis, the combined area of both the major and minor peaks for each dye was used when quantifying the colorant content. Table 2 summarizes the content and migration times of colorants in the real food samples. The relative standard deviations of the quantitative results

Table 2
Average migration times and contents of colorants determined in commercial food samples

Food sample	Colorant	Concentration[a]		Migration time[b]	
		μg/ml	RSD (%)	min	RSD (%)
Grape-flavored carbonated beverage	Fast green FCF	0.90	0.79	4.38	2.60
	Allura red AC	4.31	2.29	6.01	3.79
Orange-flavored carbonated beverage	Sunset yellow FCF	0.97	7.69	4.58	3.49
	Tartrazine	0.38	3.74	6.69	1.10
Apple-flavored carbonated beverage	Brilliant blue FCF	0.05	0.23	4.68	0.40
	Tartrazine	0.81	3.36	6.20	0.61
Grape-flavored jelly	Brilliant blue FCF	0.07	2.34	3.86	0.44
	Allura red AC	0.48	0.03	5.52	1.05
Peach-flavored jelly	Sunset yellow FCF	0.16	6.45	4.88	0.52
Pineapple-flavored jelly	Tartrazine	0.30	6.83	8.16	0.84
Apple-flavored milk	Sunset yellow FCF	1.67	3.45	5.15	2.71
	Tartrazine	3.46	1.70	7.49	0.47

[a] Quantitative analysis was based on the standard addition method; colorant standards were added to each food sample in the range 1 to 5 μg/ml. Values are the mean of nine measurements.
[b] Values are the mean of nine measurements.

and of the migration times were in the range 0.03–7.69 and 0.40–3.79%, respectively, based on triplicate measurements.

4. Conclusion

A method for the on-line concentration and analysis of eight food colorants commonly used in foods has been developed by the use of a LVSS method plus rapid separation by CE. When LVSS was applied to real food samples, the concentrations of the other components in the samples increased simultaneously, thus resulting in a decrease of resolution in CE separation. In order to reduce this phenomenon, a suitable polyamide column solid-phase extraction was required to clean up the sample matrix. Colorants in various food samples were concentrated and detected successfully using the same LVSS conditions after either a sample pretreatment step with SPE or applying a sample dilution method.

This study demonstrates that the use of a simple large-volume sample stacking method is effective in detecting low concentrations of food colorants in some popular foods when on-capillary UV detection is used.

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