Analysis of synthetic dyes in food samples by capillary zone electrophoresis

Application Note

Foods and Flavors

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Abstract

Synthetic food dyes were separated by capillary zone electrophoresis using an alkaline phosphate buffer as background electrolyte. The precision had a relative standard deviation of less than 0.5 % for the run-to-run migration times and 2 % for the peak areas with buffer replenishment after each run. The detection limit for the individual dyes was about 1 ng using a 50-µm internal diameter Agilent extended path length capillary. Compounds were detected at different wavelengths— 215, 410, 520 and 598 nm—and the identities of the individual dyes were confirmed using peak-purity routines and a UV-visible spectral library.

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Introduction

Color is a vital constituent of foods and probably the first characteristic perceived by the human senses. Almost all foods— from raw agricultural commodities to finished products—have an associated color. Further, many tests have shown that color can organoleptically dominate the flavor of a food. Colors have been added to foods since ancient times and include chlorophylls, carotinoids, flavonoids and anthocyans extracted from different plants. Today synthetic dyes have widely replaced natural colors. These dyes are used to supplement and enhance the natural colors destroyed during processing or storage, and substantially increase the appeal and acceptability of foodstuffs where no natural colors exist, for example, soft drinks or ice cream. But, synthetic dyes are also used to redye food to mask decay, and aging effects or to disguise poor products.

Colors permitted for food use can be divided in three categories:
1. synthetic dyes—described in this study,
2. natural colors (for example, caramel or beetroot) and naturally identical colors (canthaxanthine), and
3. inorganic pigments.

Synthetic colors in food products are predominantly azo and triarylmethane dyes. These are mostly acidic or anionic dyes containing carboxylic acid, sulfonic acid or hydroxy groups, which form negatively-charged colored ions at basic pH ranges. Capillary zone electrophoresis (CZE) is an ideal tool because it can separate all the different functional groups in one analysis within a short run time. CZE separates compounds based on charge and size. The range of color shades covered by the azo group includes red, orange, yellow, brown and blue-black. These dyes are prepared by coupling diazotized sulfanilic acid to a phenolic sulfonic acid moiety that often contains unwanted byproducts from corresponding impurities. Triarylmethane colors are distinguished by their brilliance of color and high tinc- torial strength, but have poor light stability. The United States (US) and the European Community (EC) are the two major geographical areas where color regulations are enforced. The lists of admitted colors are updated continuously because of suspected carcinogenicity.

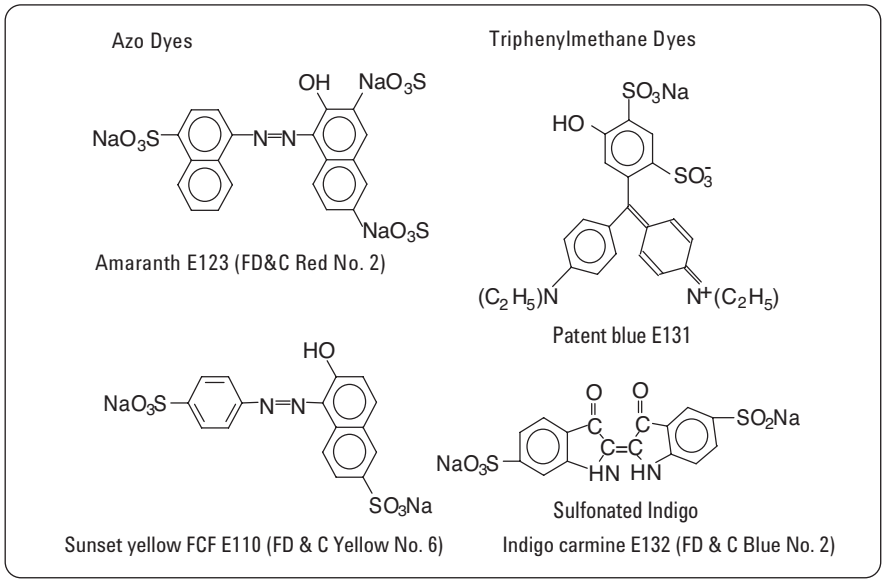


Figure 1
Chemical structures of some common synthetic dyes.

For example:
- amaranth (E123, FD&C Red No. 2)—one of the most widelyused red color—was delisted in the US in 1970,
- indigo carmine (E132, FD&C Blue No. 2) was delisted in 1980,
- tartrazine (E102, FD&C Yellow No. 5) was subjected to rigorous tests, and
- in 1990 the use of erythrosine (E127, FD&C Red No. 3) was discontinued.

Figure 1 shows the chemical structures of some common synthetic dyes. In the EC colors are regulated by council directives on coloring matters. Of relevant interest are the proposals in foodstuff directives for international harmonization of colorant regulations.

Table 1 shows some of the most widely used synthetic dyes with their EC name (E-number), the US name (FD&C) and the color index number (CI).

To ensure compliance with regulatory requirements, analytical methods are required to determine nature and concentration of a colorant in a food product. Paper chromatography, thin-layer chromatography, and high-performance liquid chromatography (HPLC) have helped the analyst in the examination of synthetic colors.[1,2,3]

Conventional HPLC separations use reversed-phase ion-pair chromatography with silica ODS or RP-2 material and a quaternary ammonium salt as the counterion.[4,5]

Several workers have adopted this approach for different applications using different sample-preparation techniques.[6,7,8,9] For example, in the wool-fiber method the sample is extracted in a tartaric acid solution and a wool fiber is used to absorb the colors of interest.[8] After washing with water and methanol the colors are washed off with a diluted ammonia

solution, evaporated to dryness, and dissolved in water or ethanol.

The polyamide method involves extracting the sample with acetic acid and polyamide powder, whereby a chromatographic column is filled with polyamide and washed with water and methanol.[9] The colors are extracted with an ammonia solution, evaporated to dryness, and dissolved in water or ethanol.

Both wool-fiber and polyamide extraction are official methods in Germany and described in detail in §35 LMBG.[10] In the US individual methods for colors in food are described in the handbook of official methods of analysis of AOAC.[11] Ion pair extraction with tetrabutyl-ammonium phosphate and back extraction using perchlorate has been reported by Puttemans et al.[12]

Experimental

Separations were performed using an Agilent CE system with a builtin diode-array detector and Agilent ChemStation software. Separations were achieved with fused-silica 50-µm id capillaries (64.5 cm total length, 56 cm effective length) with an extended path length or bubble cell at the detector end. All separations were performed at 30°C using a 10 mM sodium phosphate with 5 mM sodium hydrogen carbonate buffer at pH 10.5. Capillaries were preconditioned by flushing with 1 M sodium hydroxide for 3 minutes followed by running buffer for 10 minutes. Samples were introduced hydrodynamically with 100 and 200 mbars followed by a 200 mbars buffer plug. The samples were analyzed with an applied voltage of 30 kV and detected at 215/50 nm, 520/60 nm, 410/60 nm and 598/4 nm. After each run the inlet and outlet vials were replenished and the column was rinsed with the separation buffer for 1 minute. A voltage ramp from 0 to 30 kV within 0.5 minutes was performed to avoid possible thermal expansion and loss of sample. Details of the separation conditions are listed alongside figure 2 which shows the separation of common synthetic dyes: patent blue E131, acid brilliant green E142, erythrosine E127, indigo carmine E132,

EC	Name	Food, Drug & Cosmetics	CI
E102	Tartrazine	FD&C Yellow No. 5	19140
E103	Chryosine		14270
E104	Quinoline yellow	FD&C Yellow No. 10	47005
E105	Yellow		13015
E110	Sunset yellow FCF	FD&C Yellow No. 6	15985
E111	Sunset yellow	FD&C Orange No. 2	15980
E122	Azorubine	Carmoisine	14720
E123	Amaranth	FD&C Red No. 2	16185
E124	Ponceau 4R	Ponceau 4R	16255
E125	Scarlet red	Scarlet red	14815
E126	Ponceau 6R		16290
E127	Erythrosine	FD&C Red No. 3	45430
E131	Patent blue V	FD&C Violet No. 1	42051
E132	Indigo carmine	FD&C Blue No. 2	73015
E142	Acid brilliant green	FD&C Green No. 3	
E151	Black PN		28440
	Ponceau SX	FD&C Red No. 4	14700

Table 1
List of commonly-used colorings in EC and US classifications with color index numbers (CI).

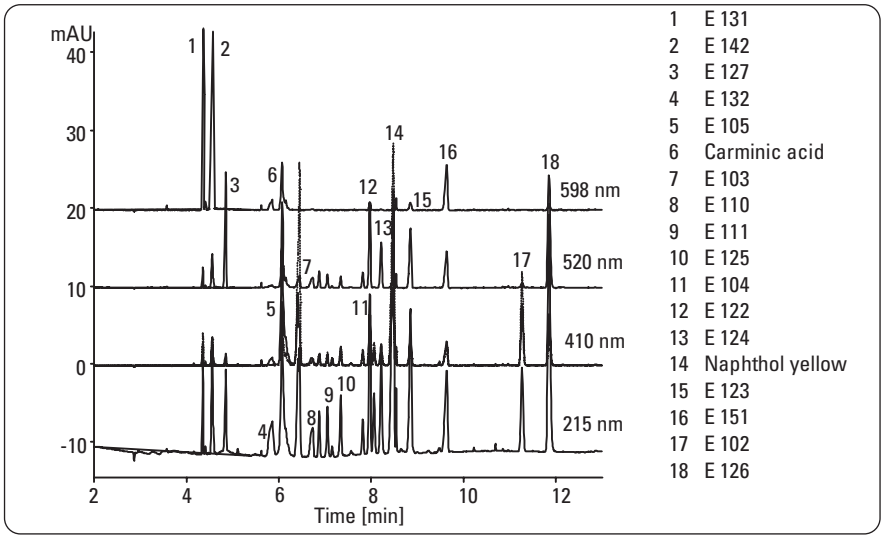


Figure 2
Electropherogram of a standard sample.

Chromatographic conditions	
Buffer:	10-mM sodium borate with 5-mM sodium hydrogen carbonate at pH 10.5
Electric field:	465 V/cm
Capillary:	l = 56 cm, L = 64.5 cm, id = 50 µm
Injection:	100 mbar x s
Temperature:	30 °C
Detection	
Signal:	215/50 nm, 520/60 nm red, 410/60 nm yellow, 598/4 nm blue
Reference:	off

chryosine E103, sunset yellow FCF E110, sunset yellow E111, scarlet red E125, quinoline yellow E104, azorubine E122, ponceau 4R E124, amaranth E123, black PN E151, tartrazine E102 and ponceau 6R E126. The E numbers correspond to the EC regulations for additives in food.

Results and discussion

Classical methods still used for determination of synthetic dyes are thin-layer chromatography (TLC), paper chromatography, and HPLC together with diode-array detection (DAD) system.

In HPLC separation is based on ion-exchange or reversed phase using ion-pairing reagents— separation modes which require time (ion exchange) or chromatographic skill (ion pairing). Further HPLC can only analyze some of the existing colors.

The use of CZE with a diode-array detector enabled us to separate most of the synthetic dyes—legal and illegal ones—in one run and detect them at their individual wavelength: 410 nm for yellow, 520 nm for red, and 598 nm for blue.

A wavelength of 215 nm was used as a pilot wavelength for spectral acquisition and as a universal monitoring wavelength (for example, sweeteners and preservatives).

The spectral information of the compound of interest was compared with the spectra in a library—compounds were analyzed not only by migration time but also by spectral comparison—and a peak-purity check was performed by overlaying spectra taken in the peak. With the new software generation just one step was required to combine all these capabilities (migration time, spectral identification and peak purity) to produce quantitative reports based on three-dimensional data.

Table 2 shows a report based on the analysis of figure 2 with the corresponding library search and relative standard deviation data (% RSD).

Depending on the functional groups—mostly sulfonic acid groups as shown in Figure 1—a pH in the range 8–11 was chosen for method development to separate colors as anions by CZE. Different buffers, such as borate, CAPS (3-cyclohexylalmino-1- propane sulfonic acid) and phospate were tested at different pH values and concentrations. The optimum buffer was found to be 10 mM phospate at pH 10.5.

However, the reliability data showed a clear trend to shorter migration times depending on the pH changes of the buffer even when replenishment of the two vials was used. This was mainly due to the instable pH of this buffer system. According to literature data we found at this pH sodium hydrogen carbonate was a stable alternative—a buffer system which did not give a ideal separation of the colors.

Finally a mix of 10 mM phosphate and 5 mM sodium hydrogen carbonate adjusted to pH 10.5 with sodium hydroxide gave optimum conditions for separation of all colors (figure 2). The buffer was filtered through a 0.45-µm filter and its pH value had to be checked when used over several days. The migration order of the two colors azorubine E122 and quinoline yellow E104 was reversed using different pH values (for example, 10.5–10.2) whereas migration times of other compounds were more or less stable.

Application

Library search mode: Automatic library search
Signal 1: DAD1 A, Sig=215,50 Ref=off
Meas. Library CalTbl %RSD (10 runs)

Time [min]	Timet [min]	Time [min]	Sig	Amount ppm/100m	Library		Match	Name		100 mbs 60–260ng/µl Mt	100 mbs 60–260ng/µl Area	200 mbs 6–26ng/µl Mt	200 mbs 6–26ng/µl Area
4.71	4.79	4.79	1	91.069	-	1	999	erythrosine	E127	0.2	3.3	0.08	5.0
6.45	6.71	6.71	1	84.340	-	1	975	sunset yellow	E110	0.6	2.4	0.05	2.9
6.93	7.19	7.19	1	138.535	-	1	998	scarlet red	E125	0.5	2.7	0.06	3.3
7.71	8.00	8.00	1	69.244	-	1	1000	ponceau 4R	E124	0.6	2.7	0.1	1.9
8.15	8.54	8.54	1	31.708	-	1	1000	amaranth	E123	0.8	3.1	0.07	3.2
10.23	10.81	10.81	1	84.758	-	1	1000	tartrazine	E102	1.2	3.7	0.1	5.9

Table 2
Report and standard deviation on migration time and area for some color compounds.

The method was applied to different matrices: carbonated beverages, pasta, glacé cherries, taramasalata extract and tablet capsules. The method was also tested to control quality of the colors themselves, for example, quinoline yellow.

The carbonated drink—a woodruff-ade—was injected directly and the compounds were identified using a library search, see figure 3. The "mint" impression (green color) was produced by a mix of quinoline yellow E104 monitored at 410 nm with patent blue E131 monitored at 598 nm. Other compounds such as the sweeteners labeled on the bottle could also be quantified using a library search (acesulfam and saccharine).

Figure 4 shows the electropherogram of a pasta extract containing quinoline yellow E104 with impurities (see figure 9), and sunset yellow FCF E110 to simulate the use of eggs. Both yellow compounds were selectively detected at 410 nm.

Figure 5 shows the analysis of glacé cherries containing ponceau 4R E124 which gives the intensive red color monitored at 520 nm.

Figure 6 shows the analysis of tablet

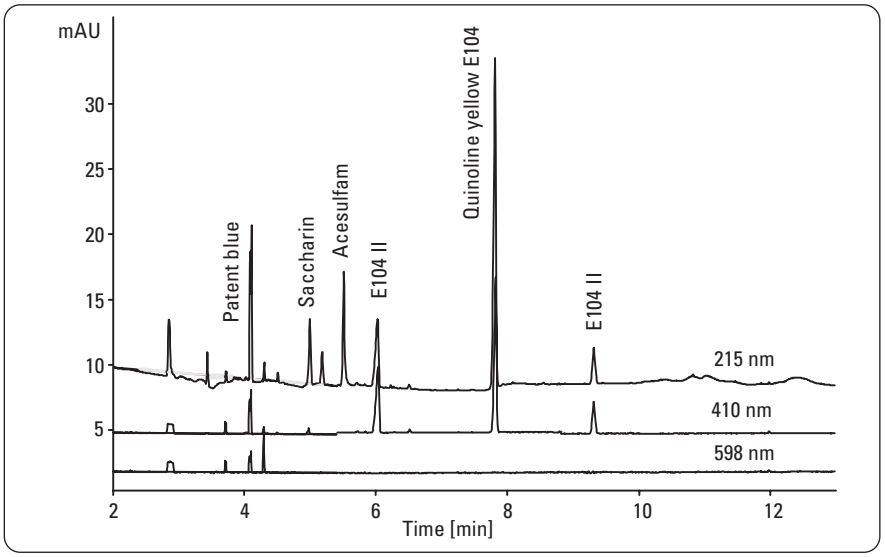


Figure 3
Electropherogram of a carbonated drink containing colors and artificial sweeteners.

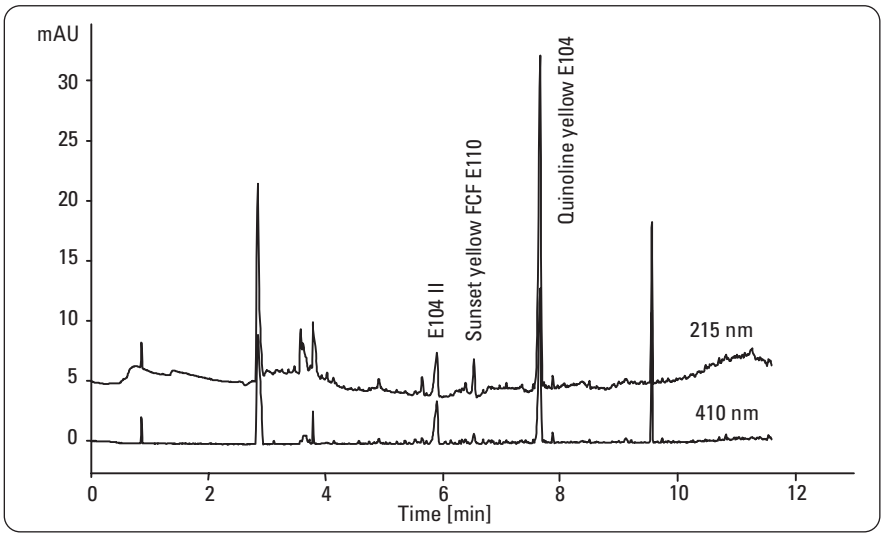


Figure 4
Electropherogram of pasta extract using wool-fiber sample preparation method.

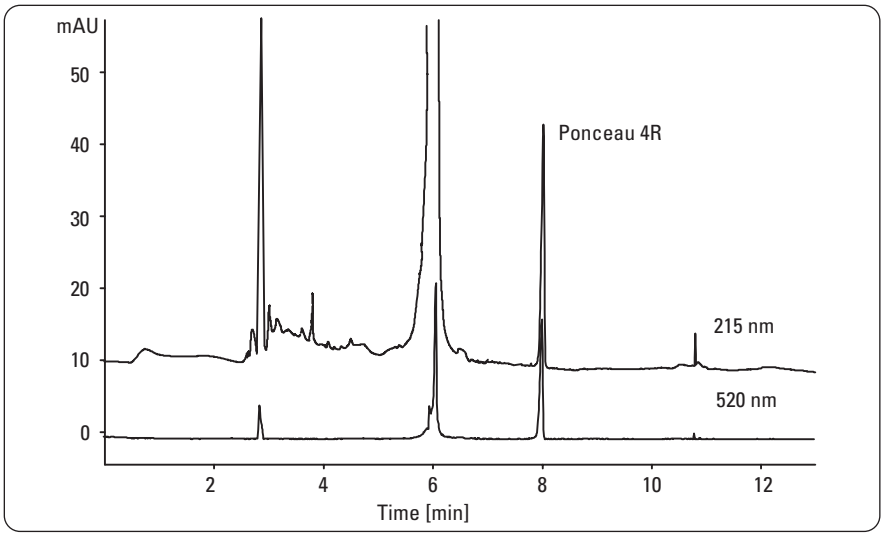


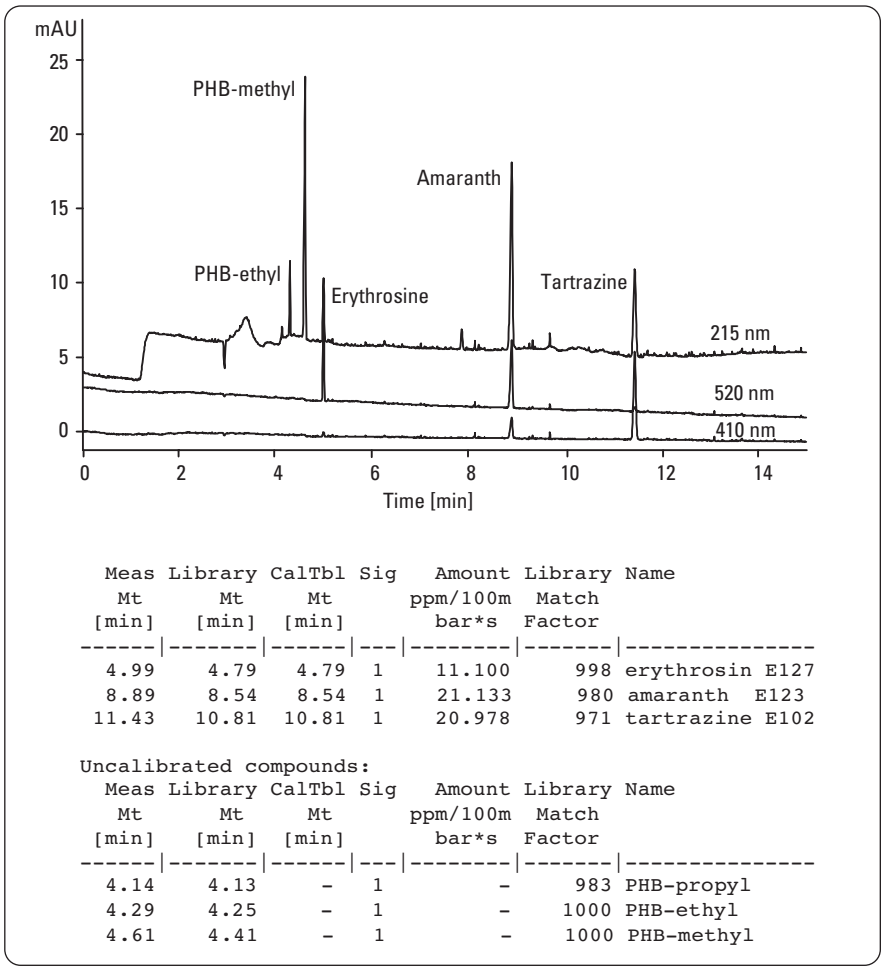
Figure 5
Electropherogram of glacé cherries colored with E124

capsules which were dissolved in water and injected directly. The red coloring of the capsule comprised two red colors erythrosine E127 and amaranth E123 (monitored at 520 nm), and a yellow color tartrazine E102 (monitored at 410 nm). The preservatives PHB-propyl, -ethyl and -methyl (215 nm) could be identified using a library search. It was found that under these conditions some of the sweeteners and preservatives could also be determined by this method.[13]

In addition to classical food applications this method could be applied to the quality control of the individual colors where the use of intermediates, containing impurities and coupling reactions, results in the formation of unwanted products.

For example, figure 7 shows the analysis of quinoline yellow E104 which contained seven impurities. After spectral analysis two different types of yellow were identified—type I with a wavelength maximum at 422 nm and type II with a maximum at 387 nm. All other compounds could be associated with either of these two spectral types. This was also observed in all samples containing quinoline yellow E104, see figure 3. Similar impurities were found in the analysis of quinoline yellow FCF E110 (FD&C Yellow No. 6), tartrazine E102 (FD&C Yellow No. 5) and FD&C Red No. 40 (allura red is not used in Europe).[11]

In recent years concern has been expressed about the safety of certain synthetic dyes and this has prompted increased consideration for the use of natural colorants in food samples. Natural and naturally-identical colors are usually mixtures of several colored as well as noncolored compounds. Figure 8 shows the analysis of an



Meas Mt [min]	Library Mt [min]	CalTbl Mt [min]	Sig	Amount ppm/100m bar*s	Library Match Factor	Name
4.99	4.79	4.79	1	11.100	998	erythrosin E127
8.89	8.54	8.54	1	21.133	980	amaranth E123
11.43	10.81	10.81	1	20.978	971	tartrazine E102

Uncalibrated compounds:

Meas Mt [min]	Library Mt [min]	CalTbl Mt [min]	Sig	Amount ppm/100m bar*s	Library Match Factor	Name
4.14	4.13	-	1	-	983	PHB-propyl
4.29	4.25	-	1	-	1000	PHB-ethyl
4.61	4.41	-	1	-	1000	PHB-methyl

Figure 6
Electropherogram of a tablet capsule and corresponding report based on a library search.

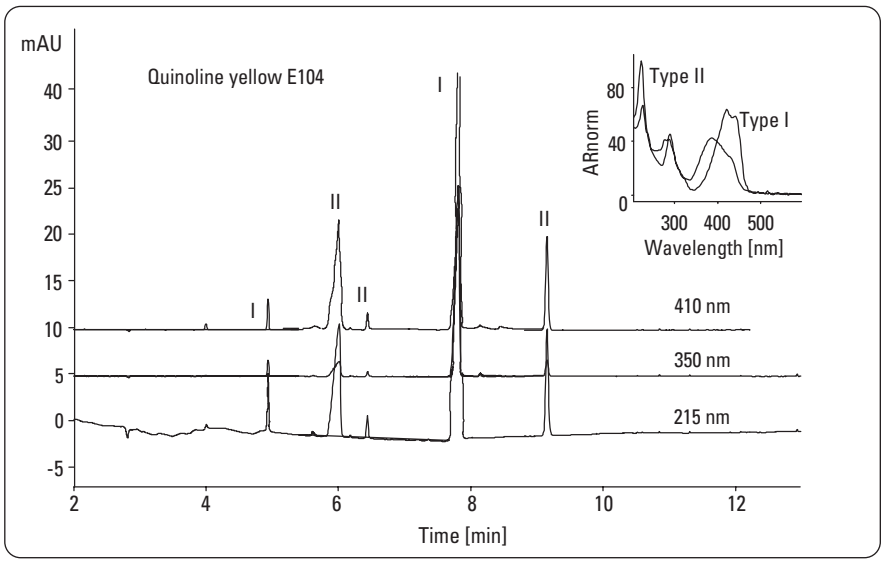


Figure 7
Electropherogram of quinoline yellow E104.

aqueous beetroot extract with two red compounds (520 nm).

Reproducibility and Linearity

The migration-time precision calulated as % RSD for all compounds was better than 0.5 % and the peak-area precision was between 2–4 %. The calculation was based on ten runs with amounts of 60–260 ng injected at 100 mbars and 6–26 ng injected at 200 mbars, see table 2. To achieve such excellent reproducibility, buffer replenishment after each run was necessary. The method was nearly linear over a range from low nanogram amounts to about 0.5 µg with variable injection volumes (figure 9).

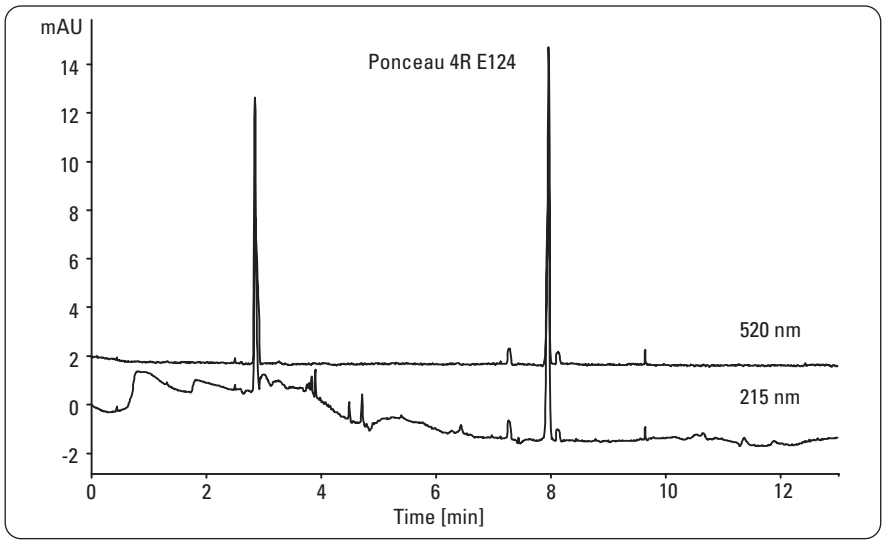


Figure 8
Electropherogram of an aqueous beetroot extract.

Conclusion

We have shown that CZE is well suited for control of synthetic dyes in food samples and for some sweeteners and preservatives. The combination of this separation method with library searches and peak-purity checks enabled separation, identification and identity confirmation of the analytes in a single run. Diodearray detection enabled selective monitoring of individual color group at their appropriate wavelength. The detection limit for most of the compounds was found to be in the low nanogram range.

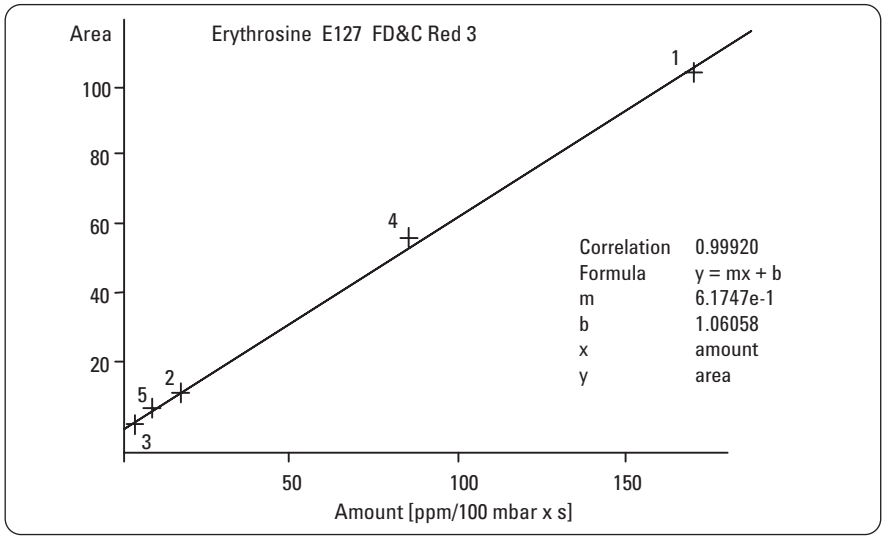


Figure 9
Linearity curve for erythrosine E127.

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