Determination of sulfonated water-soluble azo dyes in foods by SPE coupled with HPTLC-DAD

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Determination of Sulfonated Water-Soluble Azo Dyes in Foods by SPE Coupled with HPTLC-DAD

Tomasz Tuzimski

Key Words

Diode array scanning densitometry
Drinks and drops
Azo dyes
Solid-phase extraction
High-performance thin-layer chromatography

Summary

The purpose of the present work is to demonstrate an application of high-performance thin-layer chromatography-diode array scanning densitometry (HPTLC-DAD) after solid-phase extraction (SPE) for the identification and quantitative analysis of azo dyes in samples of drinks and drops. To prepare the samples for SPE, diethylamine was added to a final concentration of 0.025 M. For analysis of dyes, the samples were applied to Bakerbond Octadecyl C18 SPE columns. After being loaded with the samples, the C18 SPE columns were eluted with different volumes and concentrations of mixtures (*v*/*v*) of methanol–acetate buffer, pH 3.5. The procedure described for the determination of compounds is inexpensive and can be applied to routine analysis of analytes in water-soluble samples of drinks and drops after preliminary cleanup, concentration, and fractionation by SPE. Limits of detection and limits of quatitation values were satisfactory, in the range 31.5–119.4 and 95.3–361.8 ng per zone, respectively, for all determined dyes.

1 Introduction

Some azo dyes pose a potential risk to human health and can even be carcinogenic especially, if they are excessively consumed. Therefore, the development of a simple, sensitive, and accurate test method for the analysis of these chemicals in foodstuffs is an important requirement. Many countries have regulated the use of some azo dyes in foodstuff. For these reasons, safety data have been repeatedly determined and evaluated by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) [1]. Azorubine, Quinoline yellow, and Patent blue V are permitted and frequently used in the European Union (EU) countries; however, they are not permitted in other countries including USA and Japan [2]. EU by formulation of the directive 94/36/EC has controlled the use of synthetic colorants in foods [3].

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Generally, synthetic dyes can be classified into water-soluble and fat-soluble colorants based on their solubility. Synthetic azo dyes are ionic, basic, or acidic compounds. Chromatographic techniques have been used for the analysis of highly polar components, but use of normal-phase adsorption methods is complicated by high solute retention coupled with poor peak shape and resolution. The application of a high-performance liquid chromatographic method with evaporative light scattering detection (HPLC-ELSD) has been developed for the simultaneous determination of multiple sweeteners in carbonated and noncarbonated soft drinks, canned or bottled fruits, and yoghurt [4]. Analysis of sulfonated compounds by ion-exchange HPLC-MS on an aminopropyl column applying a gradient with increasing concentration of a buffer of ammonium acetate–acetic acid and acetonitrile has been described [5, 6]. Another successful approach is the application of ion-pair chromatography [7]. Determination of dyes in food by HPLC-DAD (or photodiode array detection (PAD)) has also been described [8, 9]. A C18 stationary phase was used and the mobile phase contained an acetonitrile–methanol (20:80, *v*/*v*) mixture and a 1% (*m*/*v*) ammonium acetate buffer solution at pH 7.5 to determination of the analytes. Successful separation was obtained for all the compounds using an optimized gradient elution within 29 min. The DAD was used to monitor the colorants between 350 and 800 nm [8]. Forty synthetic food colors were determined in drinks and candies by reversed-phase high-performance liquid chromatography with PAD. The 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 [9].

Solid-phase extraction (SPE) aims at reducing the complexity of the original matrix by separating several simpler fractions of the original matrix. The fractions should contain the same amounts of analytes as in the whole sample, ready for analysis, and free from substances that can interfere during the chromatographic analysis. Fractionations of complex mixtures of analytes by SPE combined with HPLC-DAD and TLC-DAD for determination of pesticides in water have been described [10–14]. In another paper [15], SPE was used not only to preconcentrate the analytes but also for partial purification of the samples. In this paper, the application described relates to a method for the determination of azo dyes in

soft drinks and drinks with various solvents with C18 cartridges, which permits the refinement, reduction of impurities, and purification of the matrix as well as separation of analytes in samples by their fractionation and elution of the analytes in separate steps. Different volumes and concentrations of alcohol in mixtures of methanol–acetate buffer, pH 3.5, were used to elute separate analytes and matrix in the SPE experiments.

2 Experimental

2.1 Chemicals and Reagents

Standards of the dyes investigated **(Table 1)** were obtained from the Institute of Organic Industry (IPO, Warsaw, Poland). All standards were dissolved in methanol–acetate buffer, pH 3.5 (80:20, *v*/*v*) and a final solution of pH 5.8, whereas the concentration of the dyes was 0.1 g L^{-1}.

The mobile phase components acetonitrile (MeCN), dioxane (Dx), methanol (MeOH), and tetrahydrofuran (THF) were pro chromatography grade from E. Merck (Darmstadt, Germany). Acetic acid, ammonia, and acetone were pro analysis grade from Polish Reagents (POCh, Gliwice, Poland). Diethylamine (DEA) and octane-1-sulfonic acid sodium salt (OSA-Na) were from Fluka (Buchs, Switzerland). The pH of acetate buffers, used in experiments at 0.2 M concentrations, were measured in aqueous solutions, whereas the pH of citrate buffers used in experiments at 0.2 M (Na_2HPO_4) and at 0.1 M (citric acid) concentrations were measured in aqueous solutions. Aqua purificata was from F.P.P.A.H. PROLAB (Nakło, Poland).

2.2 Sample Preparation

Samples of eight drinks were purchased locally. If the sample was carbonated, it was degassed by ultrasonication for 15 min. Drops were weighted, dissolved in warm aqua purificata water, and then filtered. To prepare the samples of drinks or drops for SPE, diethylamine was added to a final concentration of 0.025 M and a final solution of pH 5.8.

Bakerbond Octadecyl C18 SPE columns (2000 mg/6 mL, no. 7020-08; 500 mg/6 mL, no. 7020-06) and a Baker SPE-12G SPE chamber (J.T. Baker, Philipsburg, USA) were used. The columns were conditioned by washing with methanol (3 × 2 mL) and then aqua purificata water (3 × 2 mL). For the analysis of the dyes, the beverages were applied to the C18 SPE columns. After being loaded with the drinks or aqueous solution of drop samples (5 or 10 mL, flow rate 7.5–10 mL min^{-1}, pressure 75–85 mmHg), the SPE columns (C18) were eluted with different volumes and concentrations of both compounds of a mixture methanol–acetate buffer, pH 3.5 **(Table 2).**

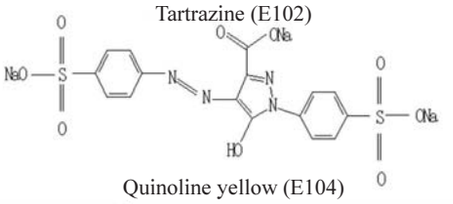
2.3 Chromatography

Thin-layer chromatography was performed on 20 cm × 10 cm glass-backed precoated with octadecyl silica gel wettable with water RP-18W F_{254S} HPTLC plates (E. Merck #1.14296.0001) and on 20 cm × 10 cm glass-backed precoated CN F_{254} HPTLC plates (E. Merck #1.12571.0001). Plates were developed to a distance of 9 cm in horizontal Teflon DS chambers (Chromdes, Lublin, Poland) [16].

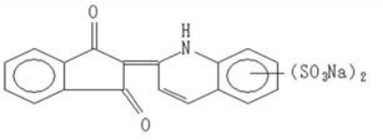
Table 1

Dyes investigated.

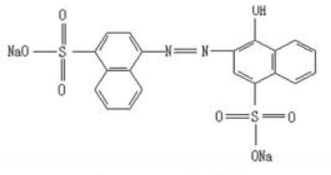
Tartrazine (E102)



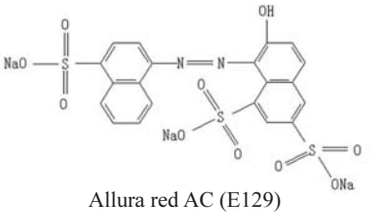
Quinoline yellow (E104)



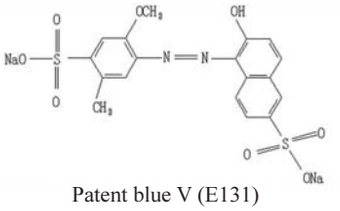
Azorubin (E122)



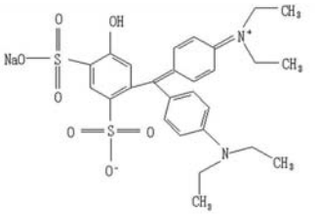
Ponceau 4R (E124)



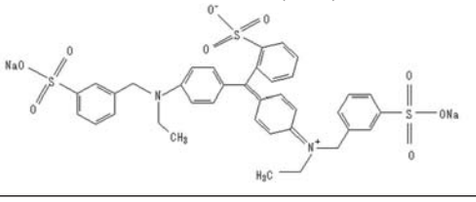
Allura red AC (E129)



Patent blue V (E131)



Brilliant blue FCF (E133)



2.4 General Investigation of the Dye Behavior

In initial work, the dyes, as 0.01% solutions, were applied to the plates as spots, 1 cm from the edge of the plate.

2.5 Analysis of Dyes in Drinks and Drops

Samples were applied to the plates as 5-mm bands by means of a CAMAG (Muttenz, Switzerland) Linomat V applicator

Table 2

Examples of fractionation of samples for the determination of dyes in foods (colorless samples without dyes are not included).

Colorant	SPE column	Volume aqueous solution of drops samples (mL) or volume of drinks samples (mL) loaded on SPE column	Concentration of methanol in mobile phase (methanol–buffer acetate, pH 3.5, *v*/*v*) used to elute of analyte	Volume fraction (mL) (after SPE)	Colours	Fraction
			Sample: Drink "IZZY ICE GREEN"			
E102	Octadecyl	10	35	3.0	Yellow	1
	(7020-08)		35	0.5	Light yellow	2
			Sample: Drink "IZZY ICE RED"			
E102	Octadecyl	10	10	5.0	Yellow	3
	(7020-08)		15	3.6	Yellow	4
			80	0.7	Light yellow	5
			80	0.7	Colorless	6
			Sample: Drops "Frutis" with strawberry flavor			
E129	Octadecyl (7020-08)	10	50	5.0	Pink	7
			Sample: Drink "IMPRESS Mandarin"			
E129	Octadecyl (7020-08)	10	80	3.0	Pink	8
			Sample: Drink "Mocny Fajrant" with raspberry flavor			
E124	Octadecyl (7020-06)	5	40	6.1	Red	9
			Sample: Drops "Brando" with strawberry flavor			
E124	Octadecyl (7020-06)	5	60	4.0	Red	10
			Sample: Drink "IZZY ICE BLUE"			
E133	Octadecyl (7020-08)	10	90	3.0	Blue	11
			Sample: Drops "Frutis" with currant flavor			
E133	Octadecyl (7020-08)	10	80	3.0	Blue	12
			Sample: Drink with forest fruits flavor			
E122	Octadecyl (7020-08)	10	70	1.8	Pink	13
			70	1.0	Pink	14
			Sample: Drops "Brando" with lemon flavor			
E104	Octadecyl (7020-08)	10	45	5.0	Yellow	15

equipped with a 100-µL syringe. Quantitative HPTLC analyses of dyes were performed on octadecyl silica gel wettable with water RP-18W F_{254S} HPTLC (RP-18W) or CN F_{254} HPTLC (CN) plates with methanol–acetate or citric buffer (*v*/*v*) containing DEA or OSA-Na as mobile phases **(Table 3).**

After chromatography, the chromatograms were scanned in the range 200–800 nm (TLC-DAD scanner, J&M, Aalen, Germany) and peak areas of extracted substances and standards were determined by scanning densitometry. Quantification was performed by the use of calibration plots in the ranges shown in **Table 4** for analysis of dyes in foods.

2.6 Validation of the HPTLC Method

Limits of detection (LOD) and quantification (LOQ) were calculated by use of the formulas LOD = 3.3(*SD*/*S*) and LOQ = 10(*SD*/*S*), respectively, where *SD* is the standard deviation of the response and *S* is the slope of the calibration plot (Table 4).

Table 3

Chromatographic systems used for the determination of dyes in food samples.

Colorant	Fraction (no., Table 2)	Stationary phase (HPTLC plate, Merck, Germany)	Mobile phase [*v*/*v*]
E102	1–6	HPTLC RP-18W F_{254s}, 10 cm × 10 cm, 0.2 mm (1.13124.0001)	Methanol–citric buffer, pH 3.5 (45:55, *v*/*v*)
E129	7, 8	HPTLC RP-18W F_{254s}, 10 cm × 10 cm, 0.2 mm (1.13124.0001)	Methanol–citric buffer, pH 3.5 (45:55, *v*/*v*)
E124	9, 10	HPTLC RP-18W F_{254s}, 10 cm × 10 cm, 0.2 mm (1.13124.0001)	Tetrahydrofuran–acetic buffer, pH 3.5 (45:55, *v*/*v*)
E133	11, 12	HPTLC CN F_{254s}, 20 cm ×10 cm, 0.2 mm (1.12571.0001)	Dioxane–acetic buffer (pH 3.5)–H_2O (25:20:55, *v*/*v*) + 0.025 mL^{-1} octane-1-sulfonic acid sodium salt
E122	13, 14	HPTLC RP-18W F_{254s}, 10 cm × 10 cm, 0.2 mm (1.13124.0001)	Acetone–acetic buffer, pH 3.5 (40:60, *v*/*v*)
E131	Not included	HPTLC CN F_{254s}, 20 cm × 10 cm, 0.2 mm (1.12571.0001)	Tetrahydrofuran–acetic buffer (pH 3.5)–H_2O (25:20:55, *v*/*v*) + 0.025 mL^{-1} diethylamine
E104	15	HPTLC RP-18W F_{254s}, 20 cm × 10 cm, 0.2 mm (1.14296.0001)	Dioxane–acetic buffer, pH 3.5 (10:90, *v*/*v*) (conditioned 20 min before developed plate)

Table 4

Method validation parameters for quantification of dyes by the proposed SPE/HPTLC-DAD method.

Dyes	Optimal λ	Limit of detection [ng per zone]	Limit of quantification [ng per zone]	Linearity *r* (correlation coefficient)	Range [mg mL^{-1}]	Second-degree polynomial regression *r* (correlation coefficient)	Range [mg mL^{-1}]	Concentration of dyes	
								Drink samples [mg L^{-1}]	Drops samples [mg g^{-1}]
E102	434	32.6	98.8	0.9970	0.0025–0.04	0.9935	0.0025–0.1	86.3–98.3	–
E104	412	54.0	163.7	0.9963	0.0025–0.07	0.9990	0.0025–0.1	18.7–119.0	0.49–1.06
E122	516	92.6	280.7	0.9895	0.0025–0.05	0.9985	0.0025–0.1	83.4–114.9	–
E124	505	119.4	361.8	0.9965	0.0025–0.05	0.9955	0.0025–0.1	52.1–133.6	–
E129	504	86.9	263.3	0.9930	0.0025–0.05	0.9970	0.0025–0.1	413.3–416.0	1.04–1.06
E131	636	31.5	95.3	0.9975	0.0025–0.04	0.9985	0.0025–0.1	46.5–47.05	–
E133	631	58.9	178.6	0.9920	0.0025–0.04	0.9973	0.0025–0.1	6.23–364.3	0.11–0.12

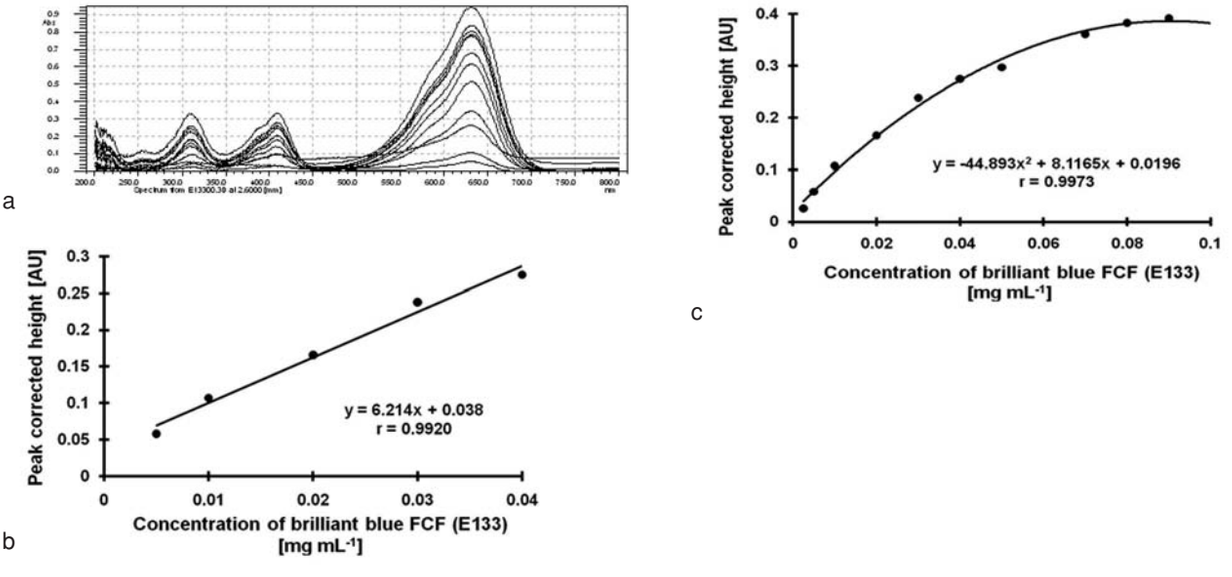


Figure 1

UV spectra of brilliant blue FCF (E133) standards at 12 concentrations (0.0025–0.1 mg mL^{-1}). The HPTLC CN plate was developed with dioxane–acetic acid buffer (pH 3.5)–H_2O (25:20:55, *v*/*v*) + 0.025 M octane-1–sulfonic acid sodium salt as mobile phase. Relationships are shown between the corrected peak height and brilliant blue concentration in the (b) linear and (c) nonlinear ranges.

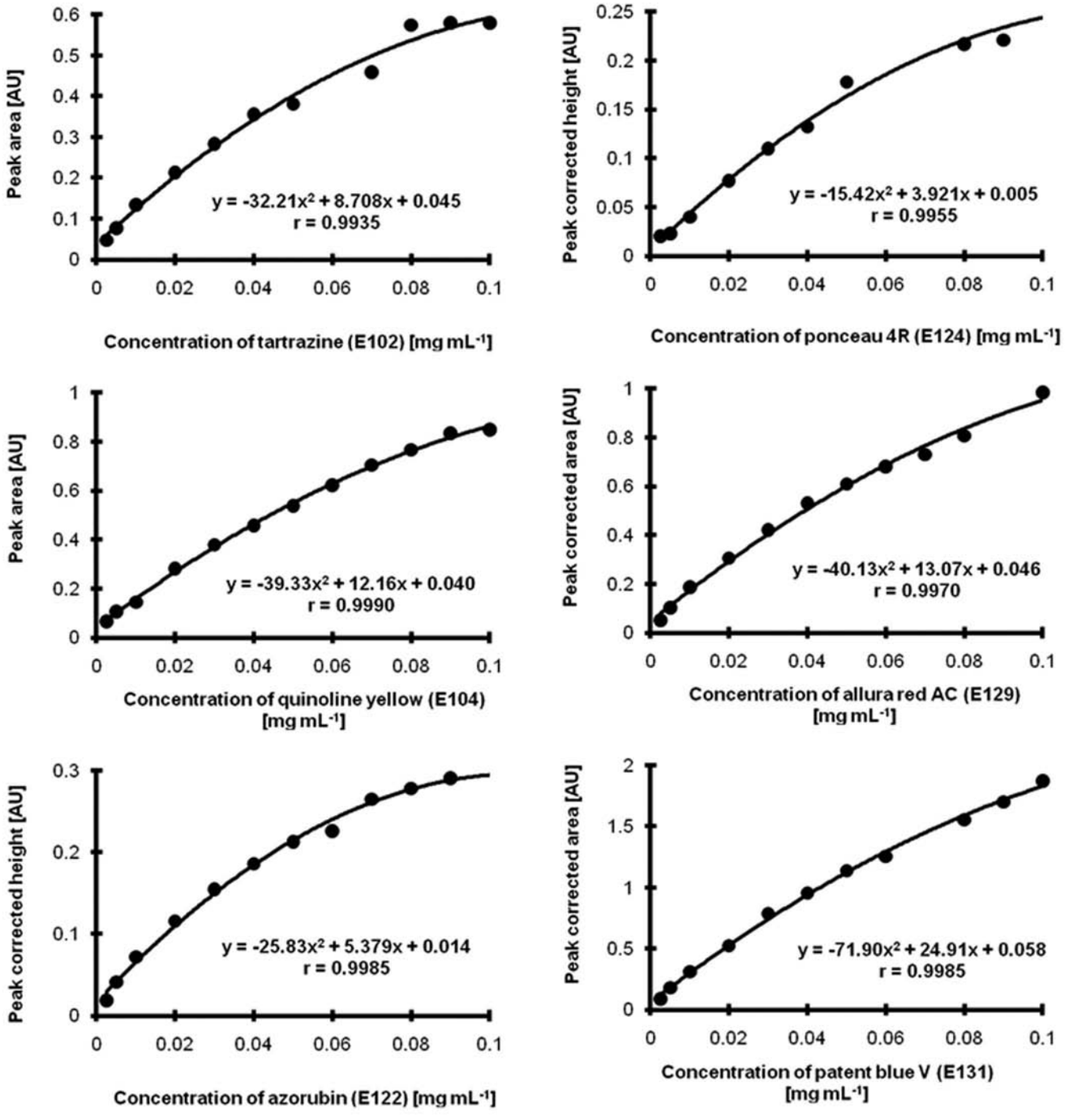


Figure 2

Relationships between peak area or peak height and concentration of standard in the range 0.0025–0.1 mg mL^{-1} for six dyes. Chromatographic systems (stationary and mobile phases) used are presented in Table 3.

3 Results and Discussion

Synthetic azo dyes are highly polar ionic compounds with especially several sulfonic acid groups and sometimes also basic groups per molecule. The synthetic azo dyes are highly water-soluble analytes. Retention of analytes on C18 SPE columns was stronger by the addition of DEA to the sample. Adsorption of dyes on surface sorbent C18 SPE columns was higher, because hydrophobic interaction of analytes was predominant in this condition.

Applications of fractionation of samples by use of different volumes and mobile phase systems for elution during a single SPE experiment enables not only the separation of analytes in a sample but also the separation of dyes from impurities and other components of the matrix. The results are presented in Table 2. Applications of different concentrations of the organic modifier in mobile phases and different volumes to elute in SPE experiment steps allow complete separation of dyes in the samples. First, volume with a lower concentration of the modifier was used to elute only impurities and other components of the matrix

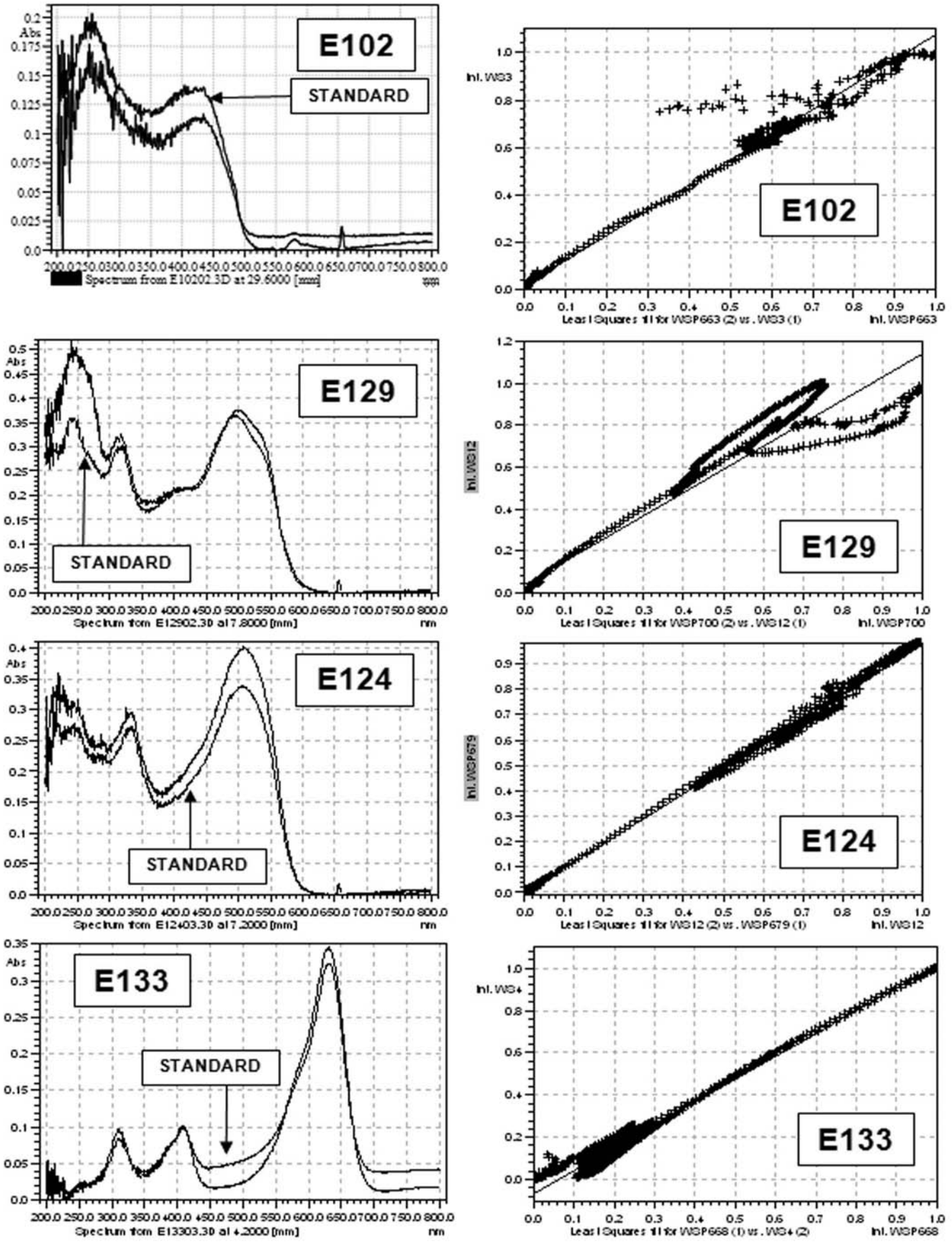


Figure 3

Determination of dyes in drinks samples (following fractions: 3, 8, 9, 11, 13 and not included Table 2): left column: comparisons of the UV spectra dyes of standards (library) and spectra found in drinks; right column: purities of peaks found in drinks samples – correlations curves of peaks purities of spectra of dyes found in drinks samples and that of dyes standards (library) after SPE and HPTLC-DAD. Pearson's *r* is in the range 0.9596–0.9985.

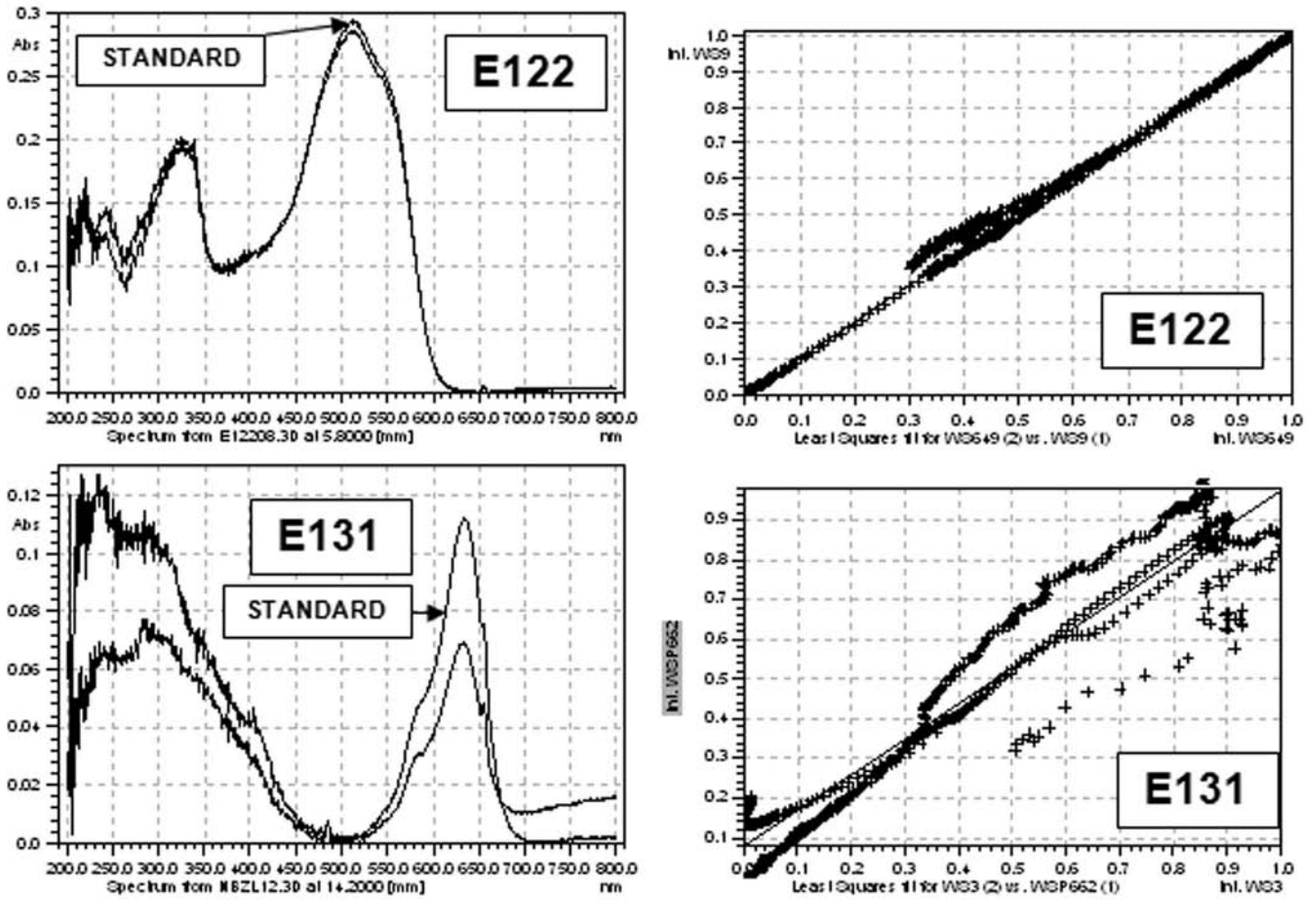


Figure 3 (cont.)

(not included in Table 2). Next, volumes with a lower concentration of the modifier were used to elute the first dye, for example, E102 (fractions 3 and 4). Next, volumes were used to obtain a colorless sample with small contents of analytes or without dyes (fractions 5 and 6). Finally, volumes of mobile phase were used with the highest concentration of organic modifier to elute the second dye (if present in sample), followed by volumes to obtain the colorless fractions without dyes (not included in Table 2). All samples and also colorless fractions were applied to the HPTLC plates and checked. Thanks to alternately colored and colorless fractions in the SPE experimental steps, the complete separation of dyes in the samples is possible.

In the chromatography experiments, the eluates were applied on HPTLC RP-18W or CN plates. On the basis of the R_F values in normal and reversed-phase systems published earlier [17], it was possible to choose the best systems to determine the analytes. The modified chromatographic systems (mobile phases) were used to study the behavior of the analytes on these adsorbents (Table 3).

Strong silanophilic interactions that can occur with these analytes and the adsorbent surface can lead to poor peak shapes. Silanol interaction can be reduced, for example, in the chromatography of compounds with basic groups by addition of a more basic compound which will interact more strongly with the residual silanols, allowing the less basic compound to interact solely with the alkyl ligand of the stationary phase. DEA as silanol blocker and acetate or citrate buffers (pH = 3.5) were added to the mobile phase (eluent containing 0.025 M DEA in the organic modifier-buffered mobile phase).

The HPTLC plates were scanned in the wavelength range 200–800 nm at the optimal wavelengths for all dyes (see Table 4). The calibration plot at 631 nm used for quantitative analysis of brilliant blue FCF (E133), shown in **Figure 1(a)**, was obtained by plotting the corrected peak height obtained with the DAD scanner against concentration. The calibration function for brilliant blue FCF (E133) (relationship between the corrected peak height and the amount of dye) was determined by linear **(Figure 1(b))** and nonlinear regression **(Figure 1(c))** over the ranges 0.0025–0.04 and 0.0025–0.1 mg mL^{-1}, respectively. Calibration functions for six dyes (E102, E104, E122, E124, E129, and E131) as a relationship between peak area or peak height and amount of standard are presented in **Figure 2**. The scanner was also used to identify the analytes in the drinks **(Figure 3)** and drops **(Figure 4)** by comparison of their spectra with those obtained for standards for all investigated dyes (Figures 3 and 4 – left parts). The purities of the peaks were also determined. Figures 3 and 4 (right parts) show the fitted values calculated by use of the cross-correlation function, which confirmed the correct identification of the dyes in foods. The peak-purity index (P) is a numerical index of the quality of the coincidence of the datasets. P has values in the range from 0 (compared spectra are different) to 1 (compared spectra are absolutely (ideally) identical). The value of P was in the range 0.9596 and 0.9985 (Figure 3, drinks samples) and 0.9300 and 0.9860 (Figure 4, drops samples) for all investigated dyes in the samples of foods.

The method was validated for precision, repeatability, and accuracy. Results of the quantitative analysis are given in Table 4. LODs and LOQs values were satisfactory, in the range

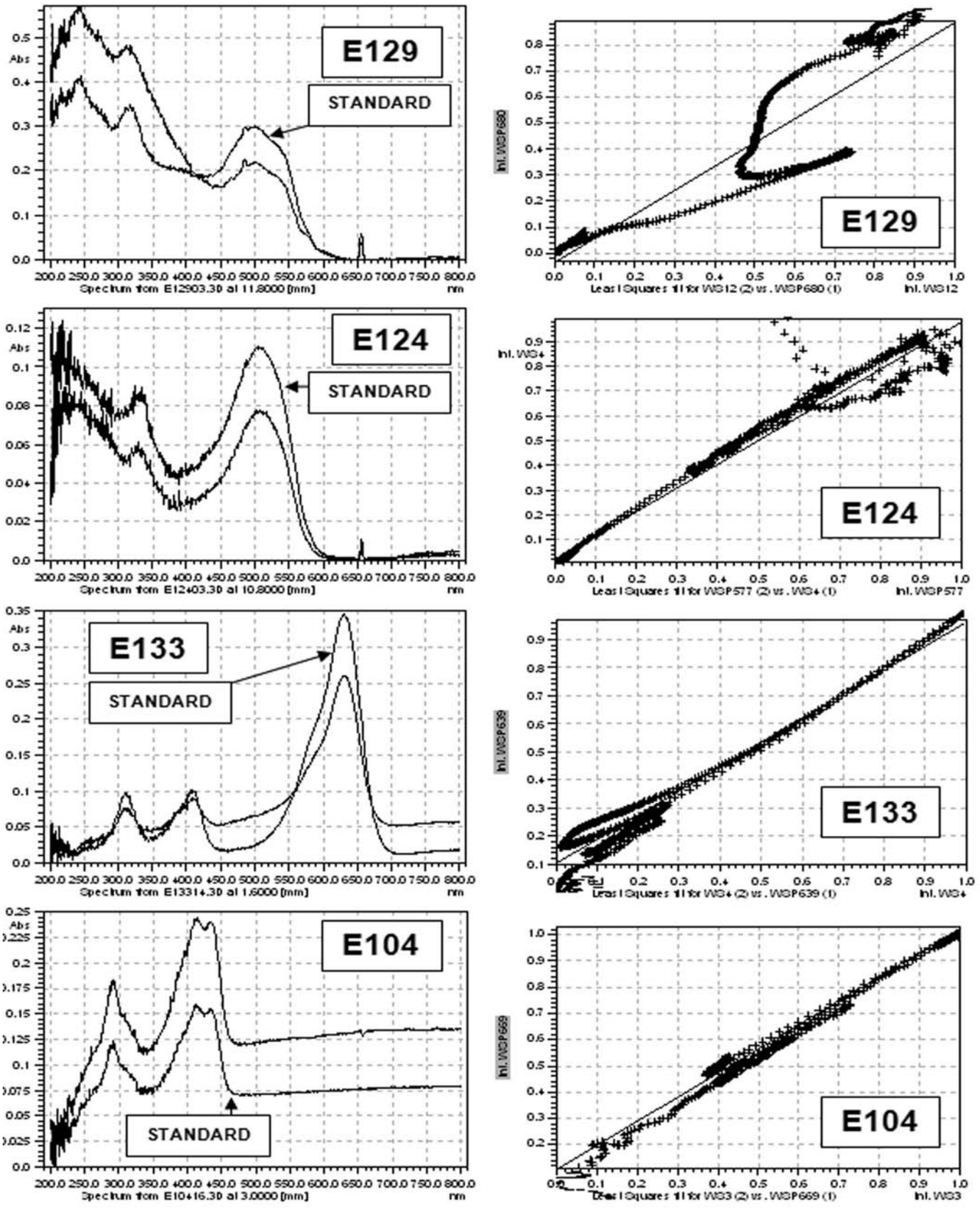


Figure 4

Determination of dyes in drop samples (Table 2 – following fractions: 7, 10, 12, 15): left column: comparisons of the UV spectra dyes of standards (library) and spectra found in drops; right column: purities of peaks found in drops samples – correlations curves of peaks purities of spectra of dyes found in drops samples and that of dyes standards (library) after SPE and HPTLC-DAD. Pearson's *r* is in the range 0.9300–0.9860.

31.5–119.4 and 95.3–361.8 ng per zone, respectively, for all determined dyes.

The suggested procedure is efficient and simple and allows the analysis of the quantity of dyes in foods.

4 Conclusion

HPTLC-DAD can be successfully applied for the correct identification of the analytes and their quantitative analysis in the food samples. Application of fractionation of the samples with different volumes and concentrations of organic modifiers in mobile phase systems used to elute analytes in the steps of SPE experiments can be useful for full separation of the analytes and also their separation from the components of the matrix in food samples. It is possible to obtain pure peaks of all dyes investigated in the HPTLC-DAD technique and acceptable values of LODs and LOQs.

Acknowledgments

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