Capillary Electrophoretic and Liquid Chromatographic Determination of Synthetic Dyes in Non-Alcoholic Drinks and Wine Samples*

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Abstract

JANČÁŘOVÁ I., VONDRÁČKOVÁ A., ŠLAMPOVÁ A., KUBÁŇ V. (2000): **Capillary electrophoretic and liquid chromatographic determination of synthetic dyes in non-alcoholic drinks and wine samples.** Czech J. Food Sci., **18**: 41–48.

Synthetic dyes in wine, juices, lemonades, instant and non-alcoholic drinks were determined by capillary electrophoresis (CE) and liquid chromatography (HPLC) with UV-VIS diode-array detection (DAD). The results were in the very good agreement (the relative deviations $\Delta < 16\%$, $\Delta < 14\%$ and $\Delta < 15\%$ between CE/UV-VIS, CE/HPLC and HPLC/UV-VIS, respectively, $\Delta < 6\%$ in majority of samples) with those obtained by UV-VIS spectrophotometry. A borate/phosphate buffer of pH 9.0 (12.5mM borate and 12.5mM phosphate) containing 40mM sodium dodecylsulfate (SDS) was selected as an electrolyte for CE. A 50mM phosphate buffer and 5mM tetrabutylammonium hydroxide (TBAOH) water solution of pH 4.2 in 38% (v/v) acetonitrile were used as a mobile phase for isocratic HPLC separation on Sepharon SGX C18 (3 × 150 mm, 5 μm) column. RSDs for individual colorants were < 0.5% for migration times, < 3% for electrophoretic peak areas, < 0.9% for retention times and < 2.4% for HPLC peak areas. Limits of quantitations (LOQs for 10 S/N) were 1.5–3.3 mg/l for CE when using 50 μm I.D. separation capillary or 0.2 to 0.4 mg/l for HPLC.

Key words: synthetic dyes; wine; non-alcoholic drinks; liquid chromatography; capillary electrophoresis; spectrophotometry

Colors are vital constituents of food and probably one of the first characteristic perceived by the human senses. Nowadays synthetic colorants have widely replaced natural colors in most areas of food industry. The usage of synthetic colorants is well regulated worldwide, but the regulations differ from one country to the next and from one foodstuff to another. To ensure compliance with the legislation, the used colorants have to be identified and quantified (NIELSEN 1998).

The use of food colorants is under strict governmental regulation also in the Czech Republic (Act No. 110/1997 on Food and Tobacco Products). The law contains so called positive list of dyes permitted for use in food industry. The application of synthetic colorants in wine is strictly forbidden while the maximum permissible amounts (NPM) according to Regulation No. 298/1997 and according to the directive of the Ministry of Health of the Czech Republic are defined for most of non-alcoholic drinks and foodstuffs.

Nowadays various HPLC procedures are in use for determination of natural and synthetic food colorants in drinks (BAKKER 1988, BAKKER & TIMBERLAKE 1985a, b; BRIDLE & GARCIA-VIGUERA 1996; CAMEIRA DOS SANTOS *et al.* 1996; CHAYTOR & HEAL 1986; DRDÁK *et al.* 1990; EDER 1990; GENNARO *et al.* 1994; WADDS 1984; ŠLAMPOVÁ *et al.* 2000), based on ion-pair reversed-phase or ion-exchange chromatography. Reversed phases on the basis of alkylated silica gels (C18, ODS, RP) are mostly applied. Mobile phases containing methanol or acetonitrile with electrolytes (Na_2SO_4) or suitable buffers (phosphate, borate, ammonium acetate) of appropriate pH (7–9) are used for isocratic or gradient elution. In many cases quaternary ammonium ions (i.e., tetrabutylammonium bromide or hydroxide, cetyltrimethylammonium chloride, tetra-*n*-hexylammonium bromide etc.) are added as the ion-pairing reagents.

Recently capillary electrophoresis (CE) has been recognized as a rapid and highly efficient method. Electrophoretic mobility of any specie is a function of net charge and molecular size (chemical structure) of the substance. Thus CE is very suitable for separation of charged synthetic colorants from the other food constituents (sugars,

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phenolic compounds, natural colorants etc.) and their subsequent determination. The majority of synthetic colorants contain one or more carboxylic or sulfonic acid groups, as well as hydroxygroups, in their molecules. Only several papers dealing with the CE determination of synthetic food dyes have appeared until now (KUO *et al.* 1998; LIU *et al.* 1995; MASÁR & KANIANSKÝ 1996; MASÁR *et al.* 1996; RAZEE *et al.* 1995; SUZUKI *et al.* 1996; ŠLAMPOVÁ *et al.* 2000). Unfortunately most of them deal with the artificial mixtures of synthetic dyes and only few were applied to analysis of real samples.

In this work, we compare the basic parameters of CE and RP HPLC methods for separation and quantitation of synthetic colorants in wine, wine drinks, lemonades, juices and other non-alcoholic drinks and compare the results with those obtained by UV-VIS spectrophotometry. The main aim of the study was to elaborate a simple and reliable method for identification and quantitation of synthetic dyes in drinks.

MATERIAL AND METHODS

Instrumentation: An HP^{3D}CE electrophoretic system with on-capillary UV-VIS diode-array detector (190 to 600 nm) controlled by HP^{3D}CE ChemStation software (Hewlett Packard) was used for CE separation of colorants. Polyimide coated capillaries (50 μm I.D., Polymicro Technologies, AZ, USA) were used. The total length of the capillaries was 50 cm (41.5 cm effective length). Constant high voltage separation potential of 30 kV was applied during all separations. Analyses were performed at 30°C. A 12.5mM borate and 12.5mM phosphate buffer containing of 40mM sodium dodecylsulfate (SDS) at pH 9.0 was used as an electrolyte. The electrolyte was prepared daily from stock solutions of the buffers and SDS. Capillary was conditioned with 1.0M NaOH for 5 min and buffer solution for 10 min before analyses and washed with 0.1M NaOH for 2 min and buffer solution for 5 min after each analysis. Samples were introduced by the hydrodynamic injection mode at 50 mbar for 10 seconds. Individual synthetic colorants were monitored at 410 nm (50 nm SBW – spectral band width), 520 nm (50 nm SBW) and 599 nm (2 nm SBW) for yellow, red and blue colorants, respectively. Sugars and other substances in foodstuffs (vanillin, *p*-hydroxybenzoic and *o*-hydroxyphenylacetic acids etc.) were measured at 220 nm. Natural colorants were monitored at 520 nm (50 nm SBW).

The HPLC separations were performed on a PU 4100 M liquid chromatograph equipped with a PU 4021 Multi-Channel DAD Detector, PU 6030 DCU A/D Converter, PU 6000 Integration Software and PU 6003 Operation Software (Philips Scientific, Great Britain). Portions of a sample solution (20 μl volume) were injected on Sepharon SGX C18 analytical glass column (3 × 150 mm, 5 μm, Tessek Ltd., Prague, CR). The column was conditioned for 30 min with the mobile phase before starting separations. Flow rate of the mobile phase (5 mmol/l TBAOH and 50 mmol/l NaH$_2$PO$_4$ water solutions in 38% (v/v) acetonitrile) was 0.5 ml/min. TBAOH and NaH$_2$PO$_4$ × 2H$_2$O were dissolved in water and pH 4.2 was adjusted with H$_3$PO$_4$. Individual synthetic colorants were monitored at their actual absorption maxima E102 (Tartrazine 435 nm), E104 (Chinoline Yellow 420 nm), E110 (Yellow SY 490 nm), E122 (Azorubine 520 nm), E124 (Ponceau 4R 505 nm), E132 (Indigotine 590 nm) and E151 (Brilliant Black BN 580 nm), respectively. Natural colorants were monitored at 520 nm.

Vacuum rotary evaporator Model 350 (UNIPAN, Poland) and ultrasonic bath (Cole-Parmer Instrument Comp., Chicago, USA) were used. Acidity of solutions was measured by a pH meter PHM 64 equipped with G 202B glass and K 401 saturated calomel electrodes (Radiometer, Copenhagen, Denmark). The instrument was regularly controlled with a set of standard buffer solutions S 1306, S 1326, S 1336 of pH 2.18, 7.00 and 9.18 (± 0.01) at 25°C (Radiometer, Copenhagen, Denmark). Helium 4.8 (SIAD TP Ltd., Most, CR) was used for degassing of the mobile phase. All spectrophotometric analyses were performed on HP 8453 DAD UV-VIS spectrophotometer (Hewlett Packard).

Chemicals: A red synthetic food colorant ZAN AROMI (a mixture of Tartrazine, Black BN, Azorubine and Patent Blue V) was from Vinopol (Velké Bílovice, CR). Tartrazine (E102), Chinoline Yellow (E104), Yellow SY (E110), Azorubine (E122), Ponceau 4R (E124), Indigotine (E132), Brilliant Blue (E133), Green S (E142) and Brilliant Black BN (E151) were from Fluka and Sigma-Aldrich. Stock solutions, 1.00 g/l (0.01–2.00 g/l for HPLC), were prepared from the corresponding standard substances dissolving in water. Working solutions were prepared by mixing the stock solutions at appropriate proportions and diluting with deionised water.

Tetrabutylammoniumhydroxide (40% [m/m] water solution, C$_{16}$H$_{37}$NO, TBAOH) was from Sigma-Aldrich. Sodium dodecylsulfate (SDS), sodium hydroxide and other chemicals of reagent grade were from Fluka. Methanol, ammonia and other chemicals of reagent grade purity were from Merck, acetonitrile (called ACN) was from Laborchemie (Apolda, Germany). Deionised water from AQUA-Dem-2 (AQUA-Osmotic, Tišnov, Czech Republic) which was further purified on MILLI-Q RG (Millipore, Bedford, USA) was used throughout.

Solid phase extractions (SPE) were performed on Spe-ed™ SPE Aminopropyl NH2, 200 mg SPE columns (Applied Separations, Allentown, USA) and using an Altech manifold system (Altech, Deerfield, USA). The columns were preconditioned by washing with 3 ml of methanol and 3 ml of water.

All samples of red wines, wine juices, lemonades, juices, soft and instant drinks were obtained directly from wine producers or collected in shops in the city of Brno according to their color (to cover application of all colorants) and frequency of use.

Sample preparation: Exactly weighed amounts of homogenized samples of instant drinks (28–50 g usually) were dissolved ultrasonically in water and diluted up to 100 ml with water. A constant aliquot of a sample solution according to the content of colorants (7–9 ml for wines or 3.5–8.5 ml for lemonades, and 7–8 ml for instant drinks) and solutions of colorant standards (0, resp. 0.25–2.00 ml) were pipetted into volumetric flasks and diluted up to 10 ml with water. Sample solutions were directly injected on an analytical separation column after degassing (without previous treatment) for direct HPLC or CE analyses.

Exactly weighed amounts of homogenized samples of instant drinks (28–45 g usually) were dissolved ultrasonically in water, standard solutions of synthetic colorants were eventually added and the mixture was diluted up to 50 ml with water. Sample solutions of the volume 5–7 ml (with or without standard addition of synthetic colorants) were loaded on the preconditioned SPE Spe-ed (Aminopropyl NH2) columns. After washing with 10 ml of water, the adsorbed colorants were washed out with 1 ml of conc. ammonia or 1 ml of a mixture of CH_3OH:ammonia = 90:10 (v/v). Eluates were evaporated to dryness at 80–90°C using rotary vacuum evaporator. The residue was dissolved in 0.5–3 ml of mobile phase and 20 µl portions were injected on a Sepharon SGX C18 analytical column for HPLC method with SPE separation.

Samples of instant drinks and juice concentrates were dissolved in water according to the recommendation of the producer (usually 1:6–1:10). Sample solutions were analyzed directly by HPLC or CE procedures after degassing. A portion of the sample was eventually loaded on a separation SPE column (separation, cleaning and elution, if needed). The adsorbed colorants were preconcentrated as above and introduced into the separation CE capillary for CE analysis.

Identification of colorants: The spectra of the standard substances (40–100 mg/l) were obtained in the same experimental conditions of the isocratic HPLC elution and the CE separation, respectively. The spectra were stored in user's spectral libraries. All analytes were identified by comparing migration (CE) or retention (HPLC) times and UV-VIS spectra (using match factor) with those for authentic standards analyzed in the same batch as the drinks. The match factor is a number indicating how closely sample spectrum matches the one in the spectral library. The numbers over 990 indicate good match, numbers between 950 to 990 indicate the possibility of match and numbers below 950 indicate no match. In addition, the results were confirmed by the addition of the standard solution of the colorant(s).

Quantitation: Calibration curves were obtained for each standard by plotting the peak areas as a function of standard concentrations in CE. For this purpose the stock solutions of the colorant standards were diluted to six different concentrations. Each concentration was analyzed by triplicate hydrodynamic injections. Calculation of colorant concentrations was performed by using appropriate peak areas and statistical parameters of the calibration curves.

Standard addition method was used for quantitation of synthetic colorants by HPLC (and CE in some cases). Three or four precisely known volumes (0, resp. 0.10–5.00 ml) of the standard solution of individual colorant of the known concentration were added to the constant volume of samples (3.5–9 ml) and the mixtures were diluted up to 10 ml with distilled water. Known amounts (0, 0.10–1.00 ml) of each colorant (or a mixture of colorants) were added (2–4 concentrations) to 24 ml of wine and the samples were diluted up to 25 ml with distilled water. The same volumes of standards (individual colorant or a mixture of colorants) were added to the sample before the sample sorption on SPE column when pre-separation of ballast compounds was needed. The procedure allows to control the procedure for sample preparation during the measurement. Portions of 20 µl were in triplicate injected on the column. Linear least squares regression using a UNICAL computer program (JANČÁŘ & LANGOVÁ 1992) was used for statistical treatment of the data (peak areas vs. concentrations). Concentrations of the colorants were recalculated using original weights (volumes) of the samples. If two peaks appear as a result of HPLC separation of a "pure" synthetic colorant (as for Indigotine – a mixture of disodium 3,3´-dioxo-2,2´-bi-indoliden-5,5´-disulfonate and less than 18% of disodium 3,3´-dioxo-2,2´-bi-indoliden-5,7´-disulfonate), the total content of the colorant was calculated as a sum of areas of both peaks either for standard solution or for real sample solution.

RESULTS AND DISCUSSION

High-performance Liquid Chromatography (HPLC)

Unsatisfactory results (tailing of chromatographic peaks, decrease of peak heights and peak areas and changes in retention times) were obtained when the mobile phase containing 5 mmol/l TBAB in 60% (v/v) methanol was used (ŠLAMPOVÁ *et al.* 2000). Very satisfactory results were obtained when using the mobile phase containing 5 mmol/l TBAOH and 50 mmol/l NaH_2PO_4 in 30–40% (v/v) ACN. The higher buffer capacity of the mobile phase was very suitable for determination of colorants either in model or real samples using isocratic elution even in the case when no sample pre-treatment or complicated pre-separation of colorants was done. Any change in the volume ratio of individual solutions leads to the shortening of retention times when content of ACN was increased. The mobile phase with 38% of ACN was used as the best for all further experiments.

The most widely used HPLC method with SPE was tested using Dolly lemon juice, containing Tartrazine. The method leads to unacceptable repeatability due to increasing amount of salts in the mobile phase when elution with the mixture of CH_3OH:ammonia = 90:10 (v/v) or ammonia was used. The salts retained in the analytical column (salting effect) continuously reduced the peak heights and the peak areas. All the excessive operations done before the analysis introduced serious errors to the procedure under the similar sensitivity. Thus the simpler direct analysis was used for all analyses.

Collection of 14 non-alcoholic drinks used for direct consumption (lemonades, fruit juices), juice concentrates and instant drinks (Table 1) were analyzed. Qualitative analysis is demonstrated on a sample of instant drink Vitacit Jahoda containing a mixture of two colorants (Ponceau 4R and Yellow SY, Fig. 1). Very good repeatability can also be seen from triplicate injections. From the results of the method of standard addition (additions of 2 and 3 mg/l for Yellow SY and 40 mg/l and 60 mg/l for Ponceau 4R) the contents of 111.0 ± 4.3 mg/kg of Ponceau 4R and 4.1 ± 0.3 mg/kg of Yellow SY were found. The concentration was lower than the maximum permissible amount (NPM) according Regulation No. 298/1997 and according to the directive of the Ministry of Health of the Czech Republic in all 14 tested samples. LOQ was 0.2–0.4 mg/l for individual synthetic colorants. Repeatability of the complete procedure for determination of colorants was verified on 10 parallel measurements of Santa Jahoda juice concentrate containing E124 (Ponceau 4R). Relative standard deviation of peak areas was lower than 2.35%.

The model samples of red wines were prepared by mixing the aliquot volumes of wine and the mixed red food colorant (ZAN AROMI) diluted 1:50 with water. As it was observed from the chromatograms of model samples of red wines from Podluží and Brno areas (Frankovka, Svatovavřinecké, Cabernet Sauvignon a André), matrix substances and all natural colorants are eluted at the beginning of the separation step (in the first three minutes) while the synthetic red colorants (Tartrazine, Black BN, Azorubine and Brilliant Blue, ZAN AROMI mixture) between 3rd and 12th minutes (in the above mentioned order

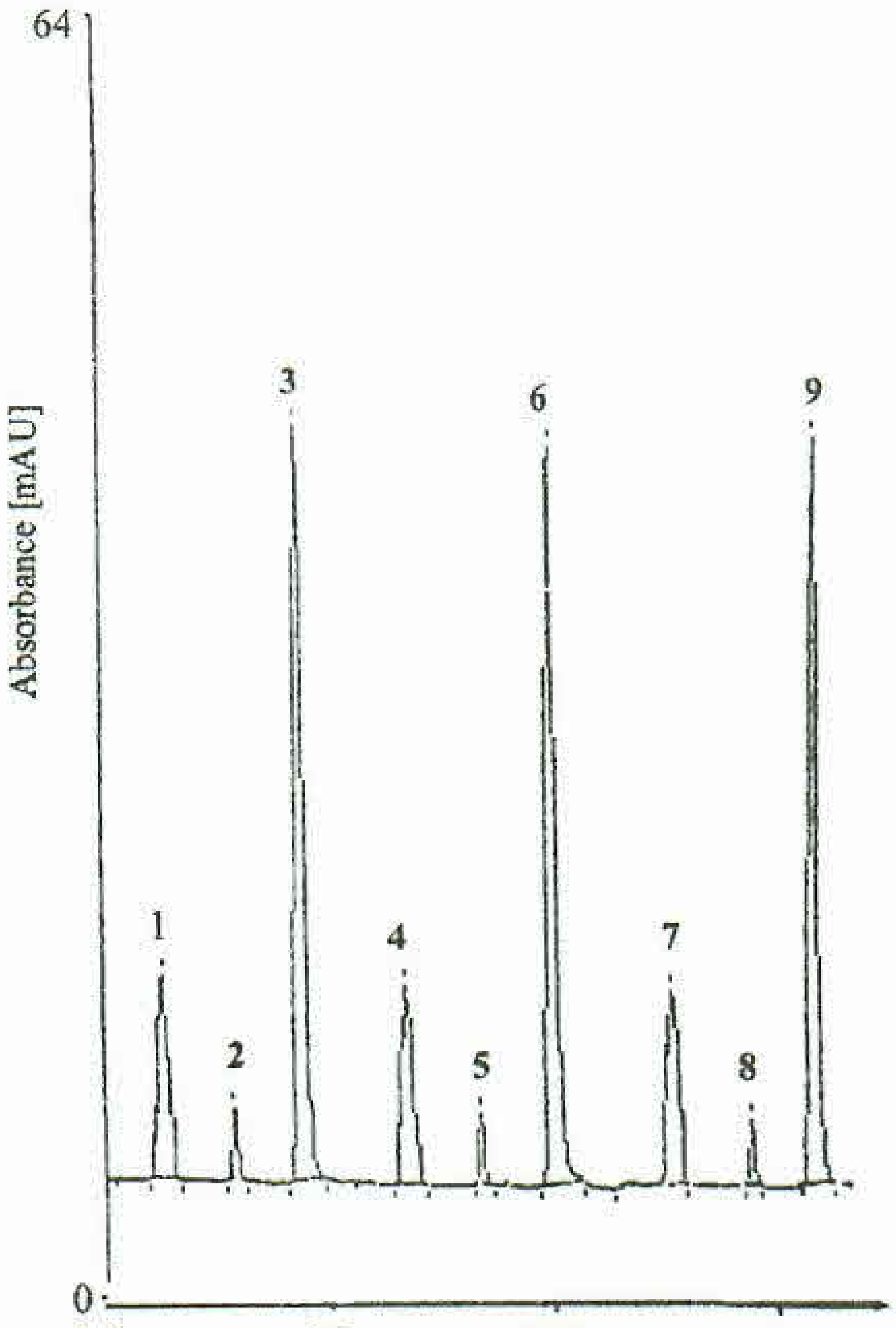

Fig. 1. Chromatogram of Vitacit Jahoda instant drink – standard addition method (sample containing Yellow SY and Ponceau 4R + addition of 2 mg/l Yellow SY and 40 mg/l Ponceau 4R, from left to right). Chromatographic conditions: pH 4.2, 50mM phosphate buffer, 5mM tetrabutylammonium hydroxide (TBAOH), 38% (v/v) acetonitrile as a mobile phase, isocratic elution, Sepharon SGX C18, 3 × 150 mm, 5 µm. 1, 4, 7 – sample matrix, 2, 5, 8 – Yellow SY, 3, 6, 9 – Ponceau 4R

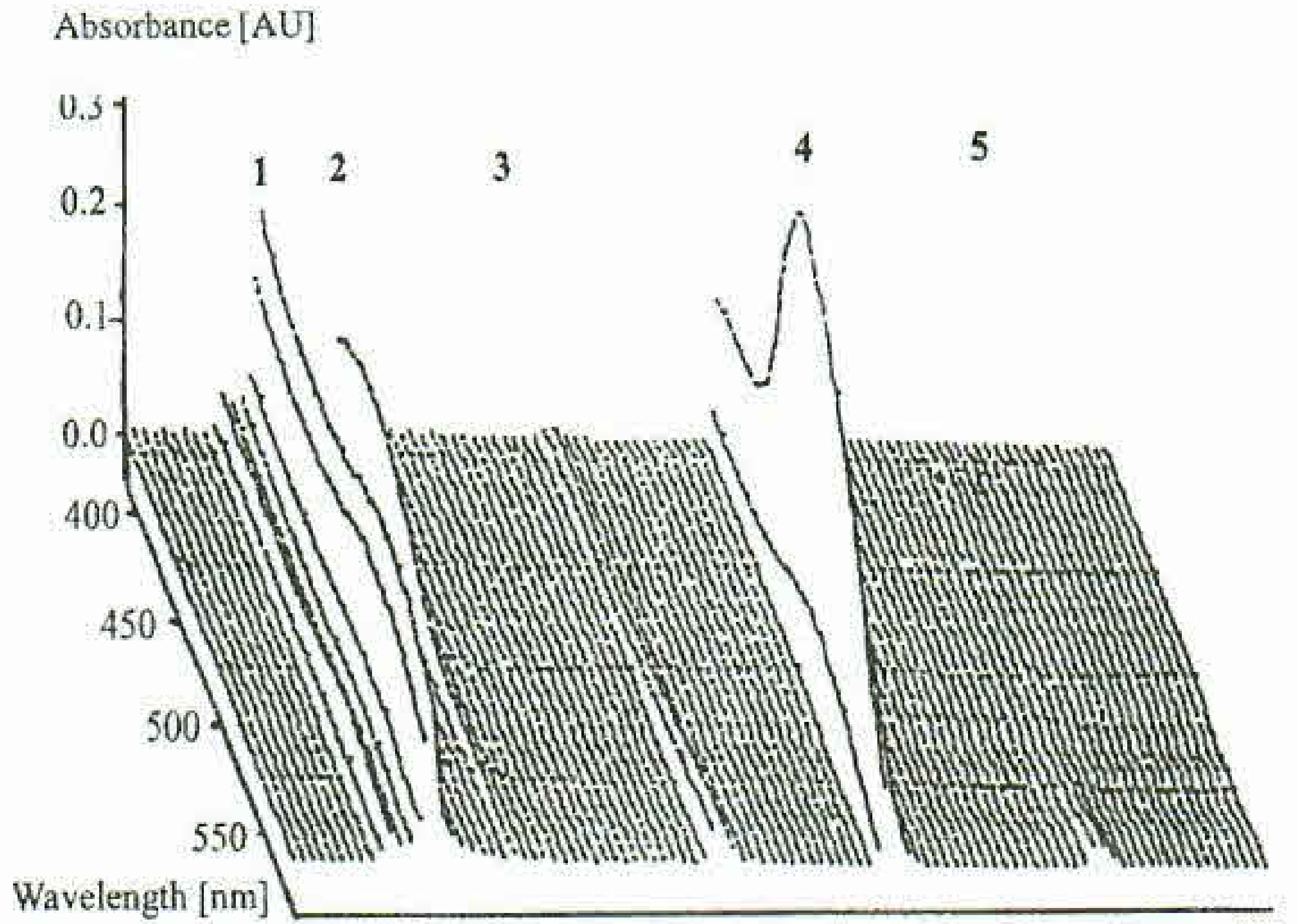

Fig. 2. 3-D Chromatogram of a model sample of the red wine (Svatovavřinecké) spiked by ZAN AROMI colorant. Chromatographic conditions see Fig. 1. 1 – Natural colorants and other constituents, 2 – Tartrazine, 3 – Brilliant Black BN, 4 – Azorubine, 5 – Patent Blue V

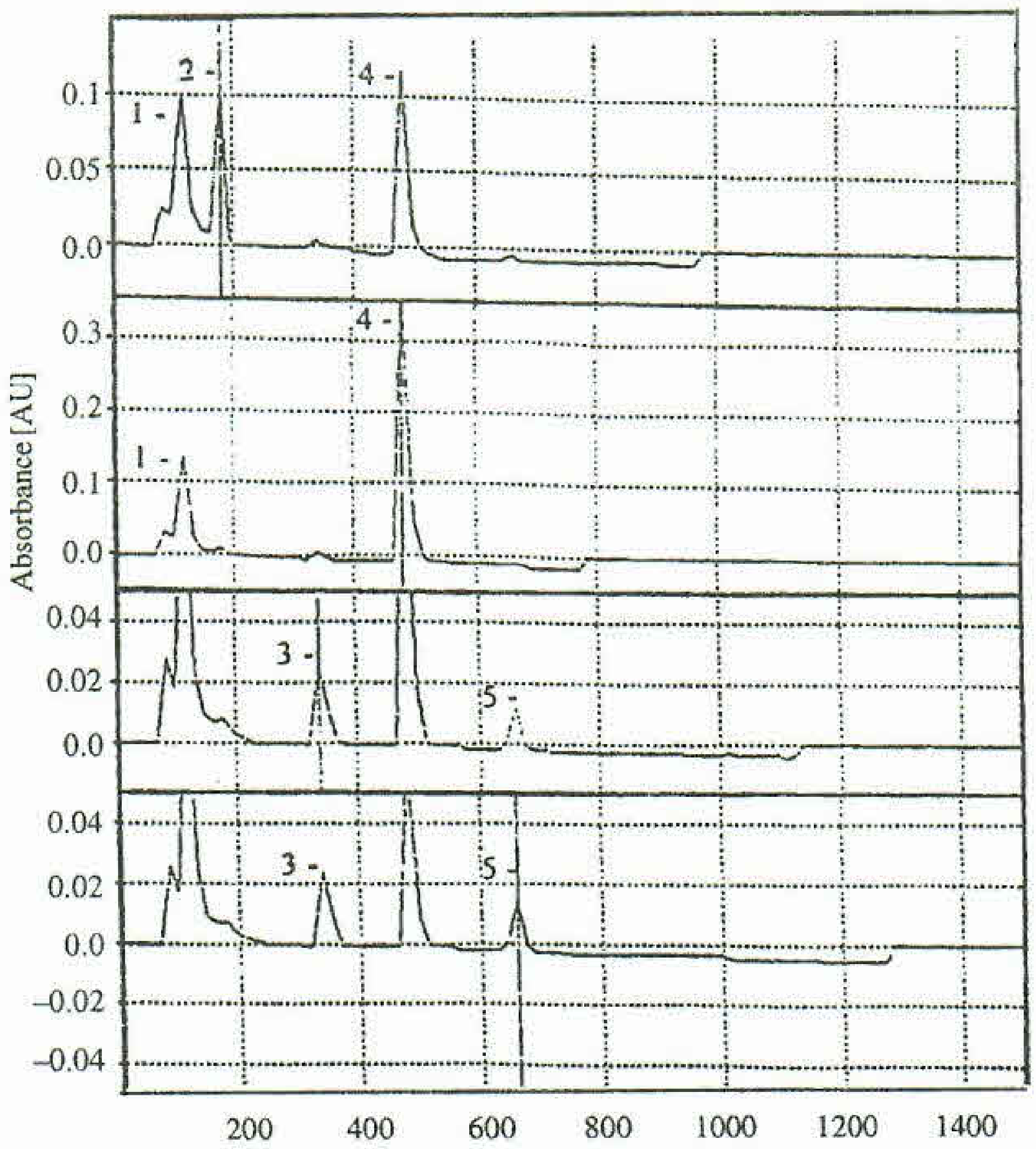

Fig. 3. Chromatograms of a model sample of the red wine (Svatovavřinecké) spiked by ZAN AROMI colorant measured at 519, 437, 579 and 590 nm (from the top to the bottom). Chromatographic conditions see Fig. 1. 1 – Natural colorants and other constituents, 2 – Tartrazine, 3 – Brilliant Black BN, 4 – Azorubine, 5 – Patent Blue V

Table 1. Content of synthetic colorants (mean ± SD, $n = 3$, 6 or 12) in non-alcoholic drinks, juices, instant drinks and juice concentrates

Sample	Colorant(s)	Content ± SD [mg/l]		
		MEKC	UV/VIS	HPLC
Ice Plosion	Brilliant Blue V	15.9 ± 0.2	15.6 ± 0.4	16.5 ± 0.5
Modrá svině	Brilliant Blue V			17.9 ± 1.1
Relax	Azorubine			18.5 ± 0.9
Limo – Semtex	Ponceau 4R	33.2 ± 1.2	34.9 ± 0.9	31.6 ± 0.9
Vitar Malina	Azorubine			17.3 ± 0.4
	Yellow SY			2.6 ± 0.4
Sirup Hello – lesní směs	Azorubine	121.2 ± 1.0	119.7 ± 3.0	141.1 ± 4.8
	Ponceau 4R	4.1 ± 0.2		4.1 ± 0.2
	Brilliant Blue V	4.4 ± 0.1	4.3 ± 0.2	4.2 ± 0.2
Sirup Dolly – citron	Tartrazine	31.1 ± 0.9	29.4 ± 0.3	29.5 ± 0.2
SirupDolly – pomeranč	Yellow SY	29.9 ± 0.1	30.0 ± 0.3	30.1 ± 1.2
Sirup Koruna – malina	Azorubine	106.9 ± 0.7	105.4 ± 0.9	109.8 ± 0.2
Vitacit Jahoda	Ponceau 4R			111.0 ± 4.3
	Yellow SY			4.1 ± 0.3
Vitacit Mandarinka	Yellow SY			86.4 ± 1.8
Dolly Malina	Azorubine			54.5 ± 3.7
Luna Pomeranč	Yellow SY			43.3 ± 2.4
Santa Jahoda	Ponceau 4R			74.9 ± 3.5
Šumák Oranž	Yellow SY	254.1 ± 4.4	257.0 ± 8.4	
Letní hit – pomeranč	Yellow SY	40.3 ± 0.2	41.3 ± 2.3	
	ChinolineYellow	26.8 ± 0.1	23.2 ± 1.1	
Letní hit-kiwi	ChinolineYellow	66.2 ± 1,1	63.8 ± 2.2	
	Indigotine	6.2 ± 0,1	6.6 ± 0.1	
Vitacit Jahoda	Ponceau 4R	62.1 ± 0.6	62.4 ± 3.3	
	Yellow SY	1.2 ± 0.1	not determined	

– Figs 2 and 3). The complete separation of individual natural colorants was not necessary since the application of synthetic colorants is forbidden by the law and the only their concentration has to be determined and their identification is necessary.

Collection of 27 samples of red wines, wine juices and wine based drinks from several areas of Southern Moravia and other regions was analyzed (Table 2). Two samples of red wine Svatovavřinecké 1 and Svatovavřinecké 2 contained synthetic colorants (Table 3). Major concentration of Azorubine and Brilliant Black BN were confirmed while Tartrazine and Brilliant Blue were present at the levels close to the limits of quantitations (LOQs). The two latest colorants were not taken into account for quantitative analyses (Table 3) of the wines using standard addition method (25 mg/l and 37.5 mg/l for Azorubine and 3.6 mg/l and 6.0 mg/l for Brilliant Black BN). Since the addition of synthetic colorants is forbidden by Regulation No. 298/1997, the producers and the business organizations are not mentioned.

Capillary Electrophoresis

The tested colorants contain two to three sulfonic groups which dissociate completely into di- or trivalent anions. Additional carboxylic and phenolic groups can dissociate at the higher (alkaline) pH values thus the net charge is increased further. According to the previous results (ŠLAMPOVÁ *et al.* 2000) the 12.5mM borate and 12.5mM phosphate buffer containing of 40mM sodium dodecylsulfate (SDS) at pH 9.0 was used as the most suitable electrolyte for CE separation of natural and synthetic colorants and for the quantitation of the latter. The method was tested on the standard solutions of natural and synthetic colorants, a sample of red colorant from the wine grapes and a food colorant ZAN AROMI (containing mixture of E102 Tartrazine, E122 Azorubine, E131 Patent Blue V and E151 Brilliant Black BN) at 50-times lower concentrations compared to the expected concentrations. All natural colorants were monitored at the very beginning of the electrophoregrams while the peaks of individual synthetic colorants were located at the longer migration times (Figs 4 and 5). The fact

Table 2. List of analyzed wine samples and wine based drinks

Wine sample	Production area	Method
André	Podluží	CE/LC
Cabernet + Sauvignon	Podluží	CE/LC
Frankovka 1	Brněnská	CE/LC
Frankovka 2	Velkopavlovická	CE/LC
Frankovka 3	Podluží	CE/LC
Frankovka – R.W.	Velkopavlovická	CE/LC
Starovavřinecké 1*	Brněnská	LC
Starovavřinecké 2*	Brněnská	LC
Starovavřinecké 3	Velkopavlovická	LC
Starovavřinecké 4	Brněnská	LC
Starovavřinecké 5	Brněnská	LC
Starovavřinecké 6	Mikulovská	CE
Starovavřinecké – R.W.	not given	CE
Starovavřinecké – R.W.	Velkopavlovická	CE
Poezie red – wine drink	not given	LC
Vinedos del Litoral – P.W.	not given	LC
Vinium ad Vitam – T.W.	Velkopavlovická	CE
Vinium ad Vitam – P.W.	not given	LC
Vavřinec + Burgund – W.J.	Brněnská	CE
Zweigltrebe – W.J.	Brněnská	CE
Frankovka – W.J.	Brněnská	CE
Kláštorné červené	Slovakia, Pezinok	CE
Grenache – Syrah	not given	CE
Červená růže* – T.P.	unknown	CE

P.W. – table wine; R.W. – butt wine; T.P. – Tetrapack wine drink; W.J. – wine juice

*wine containing synthetic colorants (see Table 3 for results), all other samples contain only natural colorants, contents down NPM

allows simple identification and quantitation of synthetic colorants in wine and non-alcoholic drinks and to control the addition of colorants to the natural samples of wine.

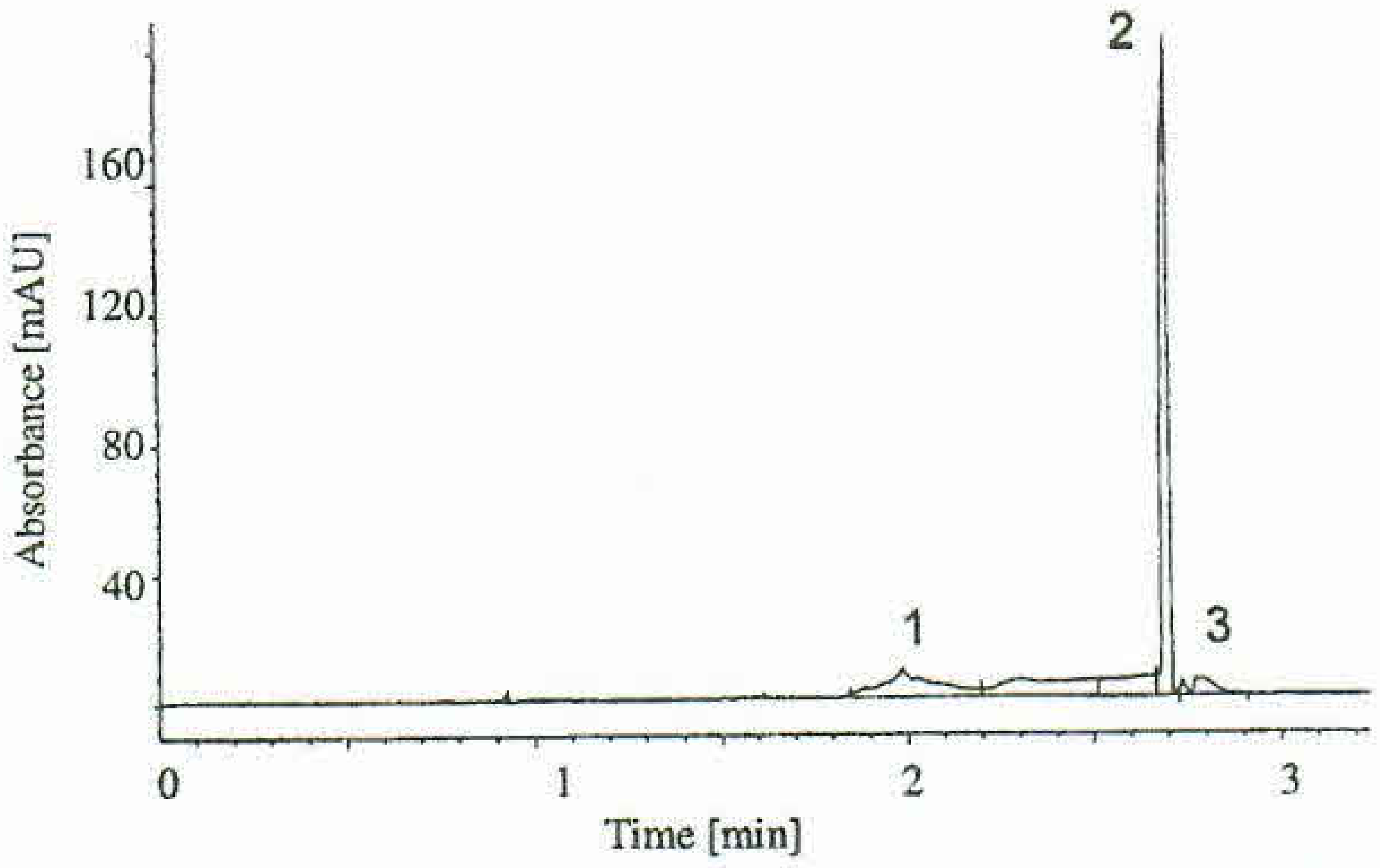

Fig. 4. Electrophoregram of Sirup Hello juice – lesní směs. Electrophoretic conditions: 12.5mM borate/12.5mM phosphate buffer of pH 9.0, 40mM sodium dodecylsulfate (SDS), total (effective) length of the capillary 50 cm (41.5 cm), 30 kV, 30°C, 520 nm (SBW 50 nm). Peak – colorant: 1 – Patent Blue V, 2 –Azorubine, 3 – Ponceau 4R

Table 3. Content (mean ± SD, n = 3, 6 or 12) of synthetic colorants in wine and wine based drink samples and in synthetic food colorant ZAN AROMI

Sample	Colorant(s)	Content ± SD [mg/l]
Svatovavřinecké 1[a]	Azorubine	48.2 ± 0.8
	Brilliant Black BN	7.0 ± 0.2
Svatovavřinecké 2[a]	Azorubine	55.3 ± 1.3
	Brilliant Black BN	9.6 ± 0.6
Červená růže[b, c]	Azorubine	38.2 ± 0.1
	Brilliant Black BN	1.5 ± 0.1
	Tartrazine	2.3 ± 0.1
	Brilliant Blue V	not determined[d]
ZAN AROMI[a, b, c]	Azorubine	111.8 ± 1.7 128.5 ± 1.2[e]
	Brilliant Black BN	12.3 ± 0.2 11.7 ± 0.7[e]
	Tartrazine	9.4 ± 0.8 12.5 ± 0.8[e]
	Brilliant Blue V	not determined[d, e]

[a]HPLC; [b]CE; [c]dilution 1:50 with water; [d]value close to LOQ; [e]standard addition method for CE

Methods of standard addition and calibration curve were tested for quantitation of individual synthetic colorants in artificial samples and the synthetic food colorant ZAN AROMI. The results are quite similar (Table 3) but simpler method of standard addition was applied for further measurements since no simulation of sample matrix was necessary. Each sample of wine was measured in triplicate with two or three different standard additions of each colorant (5–25 mg/l). From 15 samples tested in our study, all 14 contained only natural colorants. Only one sample of table wine based drink Červená růže contained a mixture of natural and synthetic colorants (Table 3, Fig. 5). Be-

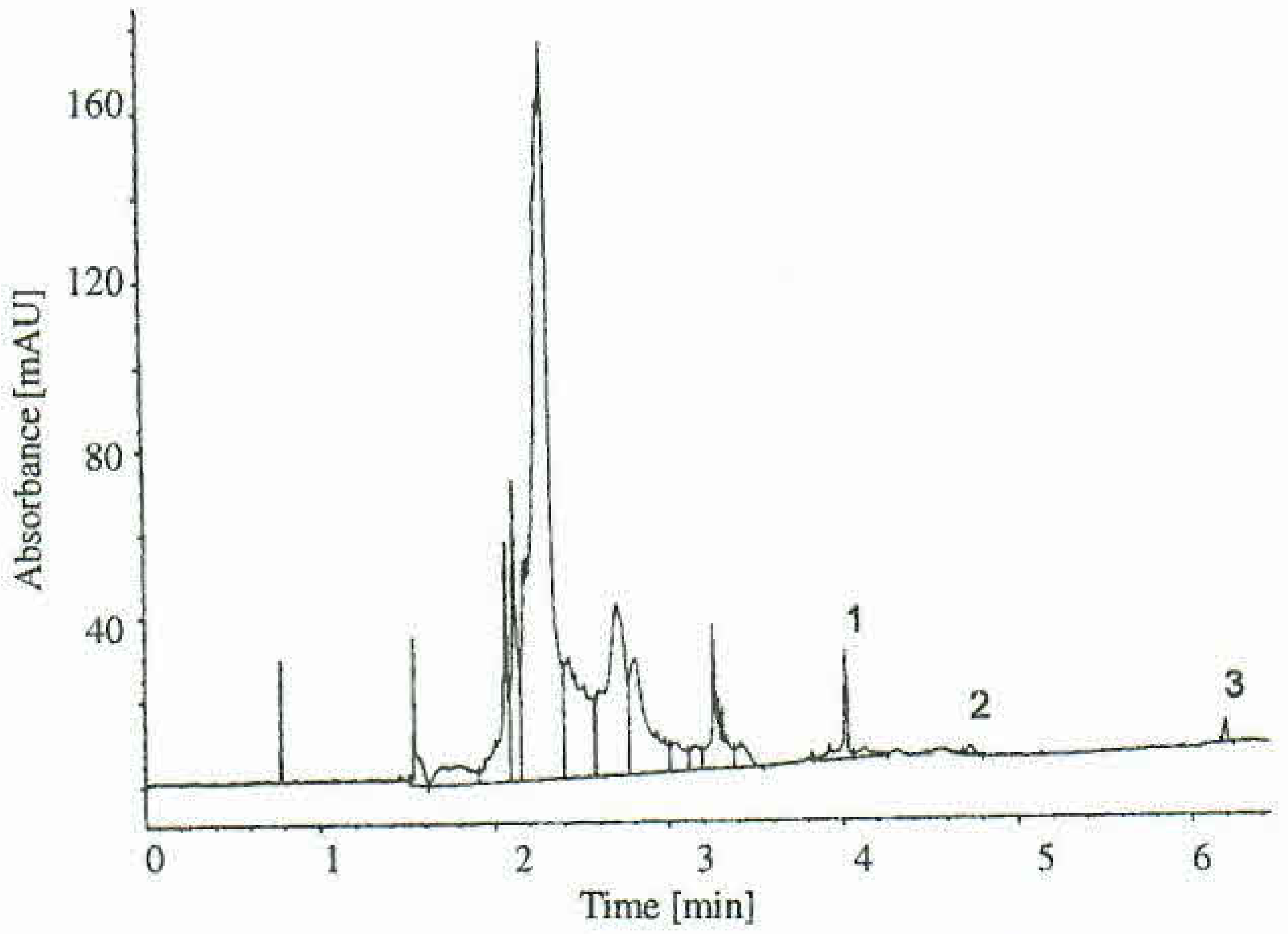

Fig. 5. Electrophoregram of wine based drink Červená růže. Electrophoretic conditions see Fig. 4.
Peak – colorant: 1 – Azorubine, 2 – Brilliant Black BN, 3 – Tartrazine, Patent Blue V not determined, peaks between 1st and 4th minute – other wine constituents (natural colorants, sugars, phenolics etc.)

cause of the fruit wine based drink and the total concentration of synthetic colorants down the NPM value, no infringement of Regulation No. 115/1995 was registered.

UV-VIS Spectrophotometry

Content of synthetic colorants was determined simultaneously by UV-VIS spectrophotometry. A part of the results is presented in Tables 1 and 3 for samples of instant drinks and juices easily soluble in water and for wine samples. Most of the results are in the very good agreement with those obtained by CE and HPLC. The spectrophotometric determination of colorants in the samples containing yellow and green components by single component analysis after SPE separation of individual substances was more complicated than for other colorants. Direct UV-VIS spectrophotometric method gives very good results if a single colorant or a mixture of colorants of different absorption spectra (λ_{max}) is present or if the colorants can be completely separated by SPE extraction. In the opposite case, the simple multicomponent analysis leads to the deviations up to 8–15%. More complicated programs for multicomponent analysis (PLS, nonlinear regression) have to be used when a mixture of colorants of similar spectral characteristics is present or when other constituents (sugars, phenolics etc.) are present at very high concentrations (NI *et al.* 1997).

Conclusions

Twenty four wine and wine related samples and 19 non-alcoholic drinks were analyzed by isocratic elution on chromatographic column Sepharon SGX C18 using the mobile phase containing 5 mmol/l TBAOH and 50 mmol/l NaH_2PO_4 (pH 4.2) in ACN 38% (v/v) and/or by capillary electrophoresis. The methods proposed in the paper are very suitable for monitoring of synthetic colorants in different types of samples. The complete separation of synthetic colorants from natural pigments and also from other food constituents (sugars, phenolics etc.) and baseline separation of individual synthetic colorants was achieved in both cases. The proposed HPLC method is simpler than the procedures recommended in literature since no complicated sample pre-treatment is necessary. Its sensitivity (0.2–0.4 mg/l) is comparable with the procedures in literature and approximately 5-times higher than for CE. The cost of the analysis is reduced and simpler instrumentation can be applied because of the isocratic mode. The CE method developed in this work is simple, effective, inexpensive and competitive with the other methods. It shows acceptable reproducibility (RSDs < 4.5% were found) and accuracy for routine food analysis for colorant additives (the relative deviations $\Delta < 16\%$, $\Delta < 14\%$ and $\Delta < 15\%$ between CE/UV-VIS, CE/HPLC and HPLC/UV-VIS, respectively, the values down $\Delta < 6\%$ were found in the majority of samples).

The SPE procedure is preferable for MEKC determinations of synthetic colorants in samples with complicated matrices. It allows selective sorption of synthetic colorants and their complete separation from ballast substances and mechanical impurities without losses of colorants. It leads to more complicated and time-consuming procedures. Its elimination in combination with HPLC is preferred for the majority of real samples. Elution with ammonia increases the content of salts in a mobile phase and thus the salting effect influences the retention times and reduces repeatability in HPLC separations.

Quantitation of synthetic colorants was done by a standard addition method and none of the 19 non-alcoholic drinks contained an excessive amount of individual colorants or excessive total amount of colorants. No violation of the NPM according to the directive of the Ministry of Health of CR and Act No. 298/1997 and the necessity of the declaration of their origin and content on packing have been registered. No violation of the rules on the purity and identification of the used colorants has been found either.

Only two samples of table wine and one sample of Tetrapack wine based drink from all 27 tested wine related samples from different sources were contaminated by synthetic colorants which is in strict disagreement with the above mentioned directive and also with Regulation No. 115/1995 on wine production, vintage and other directives.

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Souhrn

JANČÁŘOVÁ I., VONDRÁČKOVÁ A., ŠLAMPOVÁ A., KUBÁŇ V. (2000): **Stanovení syntetických barviv v nealkoholických nápojích a vzorcích vín kapilární elektroforézou a kapalinovou chromatografií.** Czech J. Food Sci., **18**: 41–48.

Syntetická barviva v nealkoholických nápojích a ve vínech byla stanovena kapilární elektroforézou (CE) a kapalinovou chromatografií (HPLC) s UV-VIS diode array detekcí (DAD). Výsledky pro jednotlivé vzorky vín, sirupu, limonád, instantních a nealkoholických nápojů byly ve velmi dobré shodě (relativní odchylky Δ < 16 %, Δ < 14 % a Δ < 15 % mezi CE/UV-VIS, CE/HPLC a HPLC/UV-VIS, hodnoty Δ < 6 % byly nalezeny v naprosté většině případů) s výsledky získanými klasickou UV-VIS spektrofotometrií. Směs 12,5mM borátového a 12,5mM fosfátového pufru o pH 9,0 obsahující 40mM dodecylsulfát sodný (SDS) byla zvolen jako optimální elektrolyt pro CE. Mobilní fáze obsahující vodný roztok 50mM fosfátového tlumiče a 5mM tetrabutylammonium hydroxid (TBAOH) o pH 4,2 v 38 % (v/v) acetonitrilu byla použit pro isokratickou HPLC separaci na koloně Sepharon SGX C18 (3 × 150 mm, 5 μm). RSDs pro jednotlivá syntetická barviva byly < 0,5 % pro migrační časy, < 3 % pro plochy elektroforetických píků, < 0,9 % pro retenční časy a < 2,4 % pro plochy HPLC píků. Meze stanovitelnosti (LOQs pro 10 S/N) byly v rozsahu 1,5–3,3 mg/l při použití 50 μm I.D. separační CE kapiláry nebo 0,2–0,4 mg/l pro HPLC.

Klíčová slova: syntetická barviva; vína; nealkoholické nápoje; kapalinová chromatografie; kapilární elektroforéza; spectrofotometrie

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