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Assay of phenolic compounds in red wine by on-line combination of capillary isotachophoresis with capillary zone electrophoresis

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Abstract

The on-line combination of capillary zone electrophoresis (CZE) with capillary isotachophoresis (ITP) increases significantly the separation capability and sensitivity of capillary electrophoresis. This technique was used for separation and quantification of fourteen selected natural constituents in red wine belonging to flavonoids and phenolic acids. The leading electrolyte (LE) in the ITP pre-separation step was 10 mM HCl of pH* 7.2 with Tris as counterion, the terminating electrolyte (TE) was 50 mM boric acid of pH* 8.2 (adjusted with barium hydroxide). The background electrolyte in the electrophoretic step contained 25 mM β-hydroxy-4-morpholinopropanesulfonic acid (MOPSO), 50 mM Tris, 15 mM boric acid and 5 mM β-cyclodextrin of pH* 8.5. The content of methanol in all electrolytes was 20% (v/v). For exact timing of the transfer of isotachophoretically stacked analyte zones into the CZE column and for the control of the residual amount of leading and terminating ITP electrolytes picric acid was used as coloured marker. The R.S.D. values ($n = 6$) ranged between ∼0.1% (for 0.25 μg ml^{-1} rutin) and ∼11% (for 0.25 μg ml^{-1} of quercitrin). Detection limits were 30 ng ml^{-1} for phenolic acids, quercitrin and rutin, 100 ng ml^{-1} for quercetin, kaempferol and epicatechin and 250 ng ml^{-1} for catechin. A single analysis took 45 min.

Keywords: Wine; Food analysis; Isotachophoresis–capillary zone electrophoresis; Phenolic compounds

1. Introduction

The content of phenolic compounds such as phenolic acids and flavonoids is an important indicator of wine quality. These substances influence sensory attributes of wine and they also play principal role in the colour chemistry of red wine during ageing [1]. Besides, phenolic compounds present in red wine influence favourably numerous biochemical systems in human organism due to their antioxidant and chelating abilities. They decrease platelet aggregation and adhesion to endothelium, increase the level of high-density lipoprotein (HDL) cholesterol and inhibit oxidation of low-density lipoprotein (LDL) cholesterol. Flavonoids also suppress cancer cell growth in vitro [2,3].

Similar chemical properties of these compounds require application of selective and sensitive methods for their separation and assay. HPLC with spectrophotometric [4–8] and fluorimetric [9,10] detection is the most frequently used method followed by capillary zone electrophoresis (CZE) [11–14]. A paper dealing with the comparison of HPLC and CZE in the analysis of wine has been published [15]. Among other methods used for the analysis of red wine micellar electrokinetic capillary chromatography (MECC) [16] and LC–MS [17] can also be found. Several approaches to wine sample preparation before the separation step have been utilized by various authors. They usually involve solid-phase extraction (SPE) [6] or liquid–liquid extraction (LLE) [13]. A direct injection of wine sample often results in rather complicated records that usually do not allow reliable identification of peaks of analytes. Nevertheless a paper employing direct injection of red wine sample without pre-concentration has been published recently [18]. Another working strategy is based on using on-line coupling of two techniques mentioned above. Ollanketo and Riekkola described HPLC method with column-switching configuration and diode array detection for the determination of flavonoids in Finish berry wines [19]. In this case the wine sample was merely subjected to filtration before injection into a chromatographic system. Arce [20] used a continuous-flow sample clean-up system followed by capillary electrophoresis separation (FI-CE). The flow system worked as a sample preparation unit with SPE cartridge

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and the extract was directly introduced into a CZE analyser through a programmable arm.

The aim of the present work was the development of a method suitable for analysis of red wine by on-line coupling of isotachophoresis (ITP) and CZE techniques. Such an approach leads to the improvement of limits of detection, selectivity and separation efficiency [21]. It has been used recently for the separation and determination of phenolic compounds in selected medicinal plants with favourable results [22,23].

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. Barium hydroxide, boric acid, hydrochloric acid, picric acid and tris(hydroxymethylamino)methane (Tris) were from Lachema (Brno, Czech Republic). β-Hydroxy-4-morpholinopropanesulfonic acid (MOPSO) and *N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS) were from Fluka (Buchs, Switzerland). 2-Hydroxyethylcellulose (2-HEC), α-, β-, γ- and hydroxypropyl-β-cyclodextrins, caffeic acid, ferulic acid, gallic acid, *p*-coumaric acid, protocatechuic acid, syringic acid vanillic acid and flavonoids apigenin, epicatechin, kaempferol, catechin, quercetin, quercitrin and rutin were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Vitexin was obtained from Roth, Karlsruhe, Germany. Ultrapure water prepared with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

2.2. Apparatus

The instrument used for ITP–CZE was EA100 (Labeco-Villa, Slovak Republic) analyzer with column switching system. The ITP separation was performed in a FEP (fluorinated ethylene-propylene copolymer) pre-separation capillary (9.0 cm × 0.8 mm i.d.) equipped with conductivity detector positioned at 38 mm from the bifurcation point, which was connected to CZE separation capillary made from FEP (16 cm × 0.3 mm i.d.); this capillary was operated with both conductivity and spectrophotometric (254 nm) detectors. The samples were injected via a sampling valve (30 μl). The temperature of the CZE capillary was maintained at 25 °C by using a laboratory-made thermostat based on Peltier elements. The data were collected and evaluated with use of a personal computer software package ITPWIN ver. 2.31 (KasComp, Slovak Republic). The pH was measured by PHM-220 (Radiometer, France) pH-meter equipped with pHC2401-8 combined glass electrode calibrated with standard Radiometer buffers.

2.3. Electrolyte solutions

The leading electrolyte (LE) of pH* 7.20 was 10 mM in HCl with Tris as counterion and contained 0.2% 2-HEC as additive. The terminating electrolyte (TE) of pH* 8.2 (adjusted by barium hydroxide) was 50 mM H_3BO_3. The TE was prepared fresh daily and it was kept in a well stoppered flask to minimize the adsorption of CO_2 from the air. The composition of background electrolytes (BGEs) is given in Table 1. All electrolytes contained 20% (v/v) of methanol; they were filtered through sintered glass filter No. 4 and degassed for 15 min in an ultrasonic bath before use. The pH values were measured in 20% methanol and therefore they were denoted as apparent pH*.

Table 1
The composition of BGE used in ITP–CZE

	BGE1	BGE2
Solvent	H_2O–methanol (4:1)	H_2O–methanol (4:1)
Co-ion	25 mM MOPSO	25 mM TAPS
Counter-ion	50 mM Tris	50 mM Tris
Complexing agent	15 mM H_3BO_3	40 mM H_3BO_3
pH*	8.5 (adjusted by $Ba(OH)_2$)	8.7 (adjusted by $Ba(OH)_2$)
Additive	0.2% 2-HEC	0.2% 2-HEC
Type of cyclodextrin	5 mM β-cyclodextrin	5 mM β-cyclodextrin

pH* pseudo pH value measured in 20% (v/v) methanol.

2.4. Standard solutions

The stock solution of standards contained 12.5 μg ml^{-1} of quercitrin, rutin and phenolic acids, 25 μg ml^{-1} of epicatechin, kaempferol, catechin and quercetin and 50 μg ml^{-1} of myricetin in methanol. Internal standard solutions containing 50 μg ml^{-1} of apigenin (IS 1) and 62.5 μg ml^{-1} of vitexin (IS 2) in methanol were prepared separately. The final model mixture and calibration solutions were prepared by diluting the stock solution with water, adding the appropriate amount of internal standard solution and adjusting the content of methanol to 20%.

2.5. Sample

Commercially available red wines were tested: Cabernet Sauvignon (Moravské Vinařské Závody, Hukvaldy) and Taylor's Port Special Ruby (Taylor, Fladgate &Yeatman, Portugal). The wines were diluted with water (10-fold dilution for Cabernet Sauvignon, and 30-fold for Taylor's Port); the content of methanol was adjusted to 20% and IS was added. These samples were injected directly to the system.

3. Results and discussion

The pK_a values of the compounds under study range between 4 and 5 for phenolic acids [24] and 9–11 for flavonoids [25]. The main structural attribute of these compounds is the presence of *o*-dihydroxyphenyl groups in the flavonoid structure and the presence of hydroxyl groups in the carbohydrate part of the molecules. These groups are capable of

forming negatively charged borate complexes which could facilitate the migration of flavonoids at relatively low pH* values [25].

3.1. Pre-separation stage (ITP)

At this stage, boric acid of pH 8.2 (adjusted with barium hydroxide) was used as TE to ensure that hydroxyl groups of the analytes undergo complex formation. The LE was hydrochloric acid containing Tris as counterion [22,23]. At the end of this stage the analytes were concentrated in a stack of narrow rectangular zones that are ready to be introduced into the analytical capillary.

3.2. Separation stage (CZE)

Considering the results of previous experiments, LE and TE from the pre-separation stage cannot serve as BGE due to the unsuitable effective mobilities of the leading or terminating ions. On the other hand, the effective mobilities of some sulfonic acids (cf. MOPSO, TAPS) are close to those of phenolic compounds analysed. This is the reason why the BGE-S-BGE system has been used.

Two different buffers with 25 mM MOPSO (BGE1) and 25 mM TAPS (BGE2) as coions, 50 mM Tris and boric acid as complex-forming agent were tested.

3.3. The effect of pH

Nearly all compounds tested possess structures favourable for complex formation with boric acid. This complex formation is pH-dependent. BGE used for the optimisation of pH contained 15 mM boric acid and the effect of pH was examined in the range 8.0–9.2. The optimum pH values were 8.5 for BGE1 and 8.7 for BGE2 (see Fig. 1A and B). If the pH was changed by more than ±0.5 pH units from the optimum pH value a moderate deterioration of resolution of practically all analytes was observed. In this respect the robustness of the method does not seem to be too high but it is acceptable for practical use.

3.4. Concentration of boric acid

The effect of concentration of boric acid was tested in the range 10–50 mM for BGE1 (pH 8.5) and 20–60 mM for BGE2 (pH 8.7). Optimal results were achieved if the electrolyte contained 15 mM boric acid for BGE1 and 40 mM boric acid for BGE2 (cf. Figs. 1A and 2). At this stage only the quality of separation was monitored, so the peaks were not identified.

3.5. Addition of cyclodextrins

Since no satisfactory separation of the analytes under study was achieved with the pH and concentration of boric acid optimized, the effect of addition of cyclodextrins was examined. It was supposed that selective interactions of the borate complexes with cyclodextrins could contribute to the improvement of their separation. Hence α-(5–15 mM), β-(4–10 mM), γ-(5 mM) and hydroxypropyl-β-(5 mM) cyclodextrins were used for this purpose. Only the addition of 5 mM β-cyclodextrin to either BGE1 and BGE2 bettered the resolution. (Fig. 3A and B). When comparing these two electrolytes more suitable results were obtained with BGE1 (25 mM MOPSO, 50 mM Tris, 15 mM boric acid, 5 mM β-cyclodextrin and pH* 8.5). In this system nearly all the components of the model mixture were well separated to the baseline except of gallic acid caffeic acid and kaempferol quercetin couples that were not resolved. Therefore the BGE1 was used for the analysis of real samples. Since in BGE2 the separation was insufficient the system was not selected for the analysis of real samples and the identification

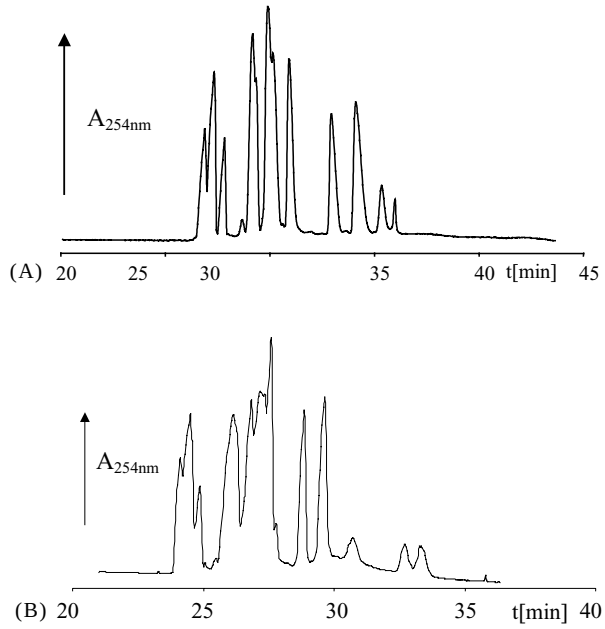

Fig. 1. Electropherograms of a model mixture. Effect of pH (A) BGE1, 25 mM MOPSO, 50 mM Tris, 15 mM H_3BO_3, pH* 8.5; (B) BGE2, 25 mM TAPS, 50 mM Tris, 15 mM H_3BO_3, pH* 8.7.

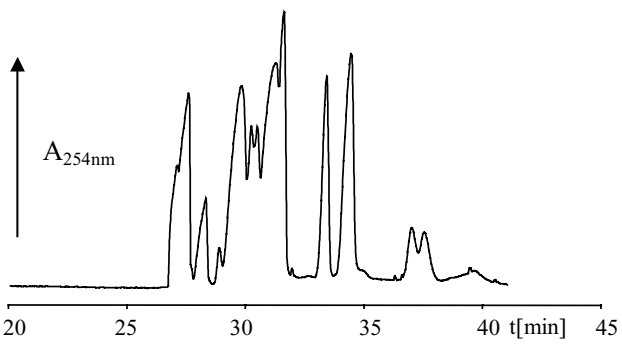

Fig. 2. Electropherograms of a model mixture. Effect of concentration of boric acid. BGE2, 25 mM TAPS, 50 mM Tris, 40 mM H_3BO_3, pH* 8.7.

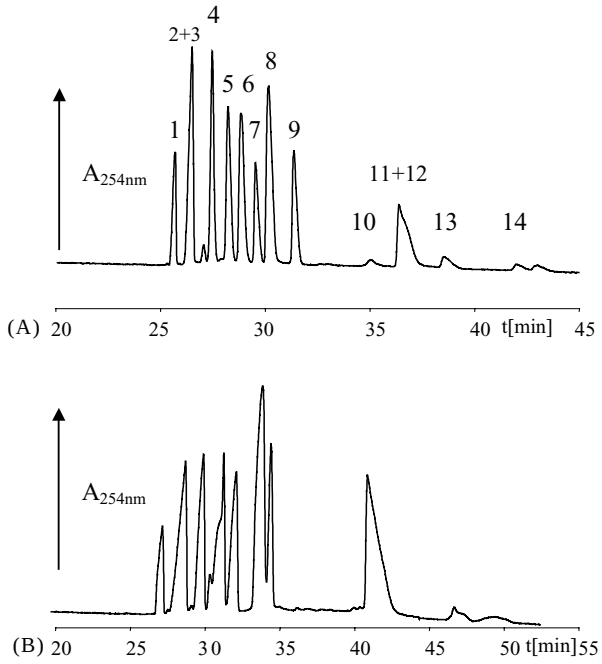

Fig. 3. Electropherograms of a model mixture. Effect of cyclodextrin on the CZE separation: (A) BGE1, (B) BGE2 (see Table 3). 1 = Protocatechuic acid, 2 = gallic acid, 3 = caffeic acid, 4 = vanillic acid, 5 = syringic acid, 6 = ferulic acid, 7 = *p*-coumaric acid, 8 = quercitrin, 9 = rutin, 10 = myricetin, 11 = kaempferol, 12 = quercetin, 13 = epicatechin, 14 = catechin. For the identification of analytes see text.

of the peaks was omitted. Table 2 shows the analytical parameters of the proposed method. To improve the linearity of the method apigenin was employed as internal standard. The calibration was performed in the concentration range 0.125–2.5 μg ml^{-1} for phenolic acids, quercitrin and rutin and 0.25–5 μg ml^{-1} for kaempferol, quercetin, epicatechin and catechin. The regression equations and correlation coefficients determined are shown in Table 2. High values of the correlation coefficients are positive signs of reliability of the method. The *t*-test did not confirm statistically significant difference of the intercepts *b* from zero.

The repeatability of migration times is fairly high; the R.S.D. values do not exceed 1.9%. The repeatability of the peak areas is worse; the R.S.D. values for different analytes range between 0.1 and 11%. This might be associated with limited solubility of some compounds (e.g., quercetin) in aqueous 20% methanol. As for myricetin, it has been reported in earlier literature that there are some problems with its quantification because of its instability [26]. During our work we faced similar problem and therefore no analytical parameters for myricetin are given. The limits of detection (LODs) (estimated as three times signal-to-noise ratio) were 30 ng ml^{-1} for phenolic acids, quercitrin and rutin; 100 ng ml^{-1} for quercetin, kaempferol and epicatechin and 250 ng ml^{-1} for catechin.

3.6. Time and current settings

Although the terminating electrolyte contained barium hydroxide, the absorption of CO_2 during working hours lead to the appearance of carbonate step on isotachophoregrams; its length increased in time and consequently the analysis was prolonged. Due to this fact exact timing of current switching was impossible. TE was prepared fresh daily and the replacement of TE in the electrode chamber for BGE was carried out manually after every ITP preparation step. Thus each new analysis began with a replaced TE (BGE-S-BGE system).

The initial driving current was 200 μA for ∼10 min during the pre-separation step; then it was changed and maintained at 100 μA till the transfer of the stacked analyte zones from ITP to CZE capillary. For exact timing of the transfer of isotachophoretically stacked analyte zones into the CZE column (minimising introduction of the residual amounts of leading and terminating ITP electrolytes into the CZE column) picric acid was used as coloured marker. The fastest zone of picric acid migrated in front of the stack; 5 s before the coloured zone of picric acid reached the bifurcation point the current was switched, the ITP zones were transferred into CZE capillary and the current was maintained

Table 2
Parameters of the calibration of phenolic compounds analyzed by ITP–CZE

Compound	Equation	*R*	R.S.D. (%)		Concentration range (μg ml^{-1})
			Time ($n = 6$)	Area ($n = 6$)	
Protocatechuic acid	$y = 0.487x - 0.052$	0.9977	0.50	1.55	2.5–0.125
Gallic and caffeic acid	$y = 0.846x - 0.037$	0.9995	0.44	1.35	2.5–0.125
Vanillic acid	$y = 0.766x - 0.050$	0.9985	0.47	1.25	2.5–0.125
Syringic acid	$y = 0.459x + 0.029$	0.9984	0.42	1.59	2.5–0.125
Ferulic acid	$y = 0.3866x + 0.036$	0.9988	0.38	8.02	2.5–0.125
p-Coumaric acid	$y = 0.533x - 0.026$	0.9989	0.35	1.07	2.5–0.125
Quercitrin	$y = 0.959x + 0.005$	0.9948	0.34	10.98	2.5–0.125
Rutin	$y = 0.412x - 0.0176$	0.9992	0.41	0.007	2.5–0.125
Kaempferol and quercetin	$y = 0.816x + 0.260$	0.9971	0.85	0.55	5–0.25
Epicatechin	$y = 0.0857x + 0.102$	0.9958	1.16	0.51	5–0.25
Catechin	$y = 0.0475x + 0.0514$	0.9973	1.93	1.12	5–0.25

x: analyte concentration (μg ml^{-1}); *y*: peak area ratio (analyte/IS).

at 50 μA for 3.5 min. Thereafter the TE in the system was replaced by BGE and the separation continued in the CZE mode at the current of 200 μA (voltage ∼ 2700 V).

Permanent human supervision was necessary in all experiments either with or without the coloured marker.

3.7. Determination of phenolic compounds in wine samples

The method developed has been applied to the determination of phenolic compounds in wine. Two red wines from different geographic regions were analysed. The samples were of Czech and Portuguese origin both commercially available on the Czech market. The samples of wines were just diluted before the analysis. The dilution was 10-fold for Cabernet Sauvignon wine and 30-fold for Taylor's Port (higher dilution of the latter wine was necessary since it contained considerable amount of matrix components that prolonged the ITP step and caused low-quality separation in the CZE step if analysed only 10-fold diluted). A 30 μl volume of diluted red wine sample was directly subjected to ITP–CZE analysis. During the pre-separation stage a number of accompanying anionic compounds could be seen on the isotachopherograms (Fig. 4). Interfering compounds that migrate faster than compounds under study are eliminated from the system by proper current switching; "slower" compounds are flushed away from the capillary when the TE is replaced by BGE. Peaks appearing in the electropherograms (Fig. 5) were identified by spiking the sample with a small amount of a standard solution which resulted in an increase of the height of the respective peak. To verify these results the electropherograms of red wine were compared with those of standard solutions. Quantification was conducted with use of apigenin (0.5 μg ml^{-1}) as internal standard (Fig. 5A) that permitted an improvement in linearity and repeatability of this method. Either wine sample assayed contained the phenolic acids and quercitrin. Rutin and kaempferol with quercetin were detected only in Taylor's Port wine. Epicatechin and catechin were not present in the wines analysed. Their absence in these two wines was proved by spiking the

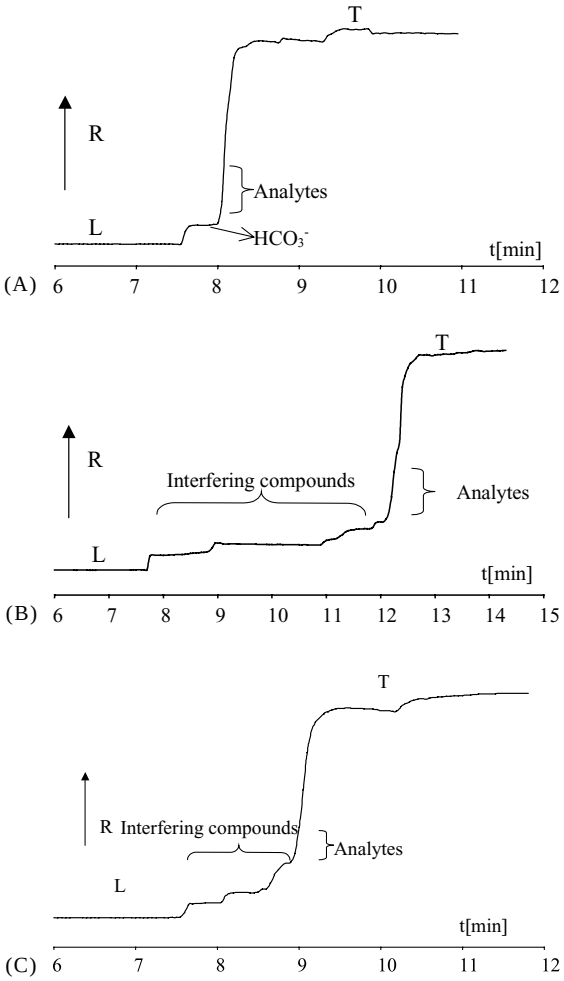

Fig. 4. Isotachopherograms of standards (A), Taylor's Port Special Ruby (B) and Cabernet Sauvignon (C).

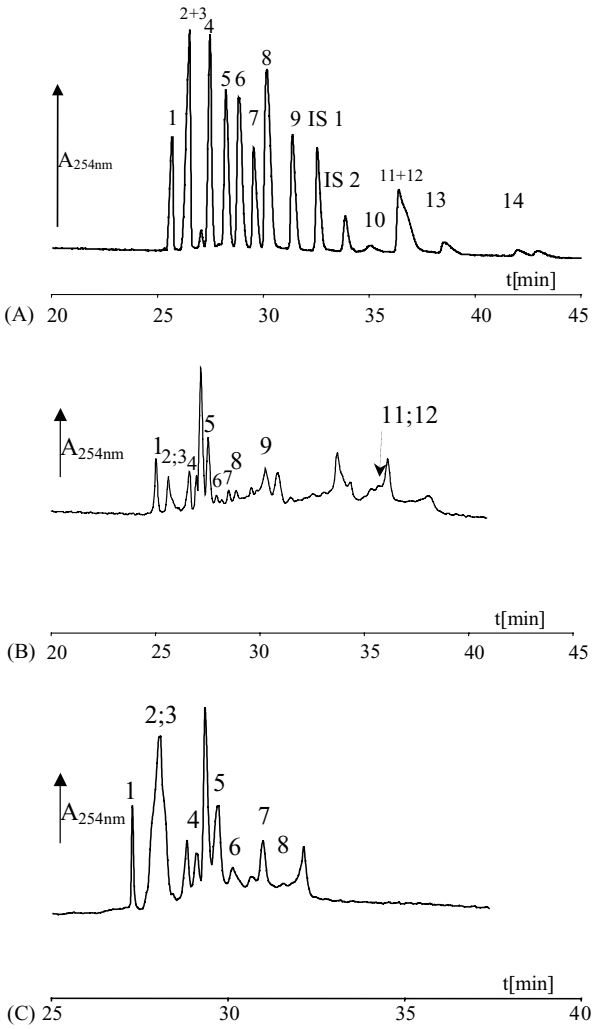

Fig. 5. Electropherograms of standards (A) and wine samples Taylor's Port Special Ruby (B) and Cabernet Sauvignon (C). Samples: see Fig. 3. IS1 = vitexin, IS2 = apigenin.

Table 3
The determination of phenolic compounds in red wine

Compound	Taylor's Port ($\mu g\,ml^{-1}$)	R.S.D. (%) ($n = 6$)	Cabernet sauvignon ($\mu g\,ml^{-1}$)	R.S.D. (%) ($n = 6$)
Protocatechuic acid	3.73	2.80	2.40	3.2
Gallic and caffeic acid	8.17	1.50	22.04	1.80
Vanillic acid	2.01	2.20	0.61	3.26
Syringic acid	10.29	2.45	3.96	2.78
Ferulic acid	1.30	6.35	1.38	7.16
p-Coumaric acid	2.12	3.18	2.12	2.90
Quercitrin	1.75	9.50	0.37	9.20
Rutin	9.97	2.10	NF	–
Kaempferol and quercetin	1.05	3.01	NF	–

NF: not found.

real samples with epicatechin and catechin; two new peaks appeared and their position corresponded to epicatechin and catechin as observed in the electrophoregram of the standard mixture.

The results of the assay of red wine samples for phenolic compounds by the devised ITP–CZE method are shown in Table 3.

Even though gallic + caffeic acid and kaempferol + quercetin gave single peaks in CZE analysis, the sums of these compounds were quantified because they belong to important minor constituents of wines. This approach is quite common in the analysis of natural samples when two or more compounds give a single peak in CZE [27].

4. Conclusion

The on-line capillary isotachophoresis-capillary electrophoresis method for the separation and determination of fourteen phenolic compounds has been devised as an alternative to the commonly used separation methods (HPLC, CE, etc.). The sample pre-treatment involves only its dilution with water. Considering the fact that the ITP–CZE instrument available was equipped with an UV detector operating at fixed wavelength of 254 nm only, the limits of detection achieved were favourable and the concentrating effect of ITP was confirmed. The detection limit values attained are nearly the same as those of an HPLC method with column switching technique [19], lower compared to HPLC where off-line pre-treatment of sample is applied [10] and approximately 10-fold lower compared to FI-CZE [20]. In ref. [12] the LOD is 3 pmol using DAD at 220 and 380 nm but the pretreatment of the plant beverages is complicated and considerably time-consuming (it involves, e.g. Soxhlet extraction for 20 h or supercritical fluid extraction). In ref. [14] the LOD were 0.05 and 0.01 $mg\,l^{-1}$ using fast scanning detector; here the selectivity increased by the detection at 305 nm but only gentisic and *p*-coumaric acids were determined in wines.

In the proposed ITP–CZE method total duration of analysis was 45 min including the time for the pre-separation and separation of 14 analytes in the wine. This run time seems to be by 15 min shorter compared to a reversed-phase HPLC with direct injection of the sample without sample pretreatment [18] that was used for the analysis only four phenolic components in wine.

Considering the facts discussed above the proposed ITP–CZE method is suitable for the analysis of minor components in the presence of large amounts of macrocomponents in complex natural samples. Sufficient sensitivity, low running costs and low consumption of organic solvents are the main merits of this method.

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References

[1] M. Netzel, G. Strass, I. Bitsch, R. Kőnitz, M. Christmann, R. Bitsch, J. Food Eng. 56 (2003) 223.
[2] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, J. Nutr. Biochem. 13 (2002) 572.
[3] A.A. De Lorimier, Am. J. Surg. 180 (2000) 360.
[4] F. Buiarelli, G. Cartoni, F. Coccioli, Z. Levetsovitou, J. Chromatogr. A 695 (1995) 229.
[5] J. Burns, P.T. Gardner, J. O'Neil, S. Crawford, I. Morecroft, D.B. McPhail, C. Lister, D. Matthews, M.R. MacLean, M.E.J. Lean, G.G. Duthie, A. Crozier, J. Agric. Food Chem. 48 (2000) 220.
[6] S. Malovaná, F.J. García Montelongo, J.P. Pérez, M.A. Rodríguez-Delgado, Anal. Chim. Acta 428 (2001) 245.
[7] O. Palomino, M.P. Gómez-Serranillos, K. Slowing, E. Carretero, A. Villar, J. Chromatogr. A 870 (2000) 449.
[8] M. Castellari, E. Sartini, A. Fabiani, G. Arfelli, A. Amati, J. Chromatogr. A 973 (2002) 221.
[9] P. Viñas, C. López-Erroz, J.J. Marín-Hernández, M. Hernández-Córdoba, J. Chromatogr. A 871 (2000) 85.
[10] M.A. Rodríguez-Delgado, S. Malovaná, J.P. Pérez, T. Borges, F.J. García Montelongo, J. Chromatogr. A 912 (2001) 249.
[11] G. Cartoni, F. Coccioli, R. Jasionowska, J. Chromatogr. A 709 (1995) 209.
[12] A. Kulomaa, H. Sirén, M.-L. Riekkola, J. Chromatogr. A 781 (1997) 523.

[13] P. Andrade, R. Seabra, M. Ferreira, F. Ferreres, C. García-Viguera, Z. Lebensm. Unters. Forsch. A 206 (1998) 161.
[14] J. Pazourek, G. González, A.L. Revilla, J. Havel, J. Chromatogr. A 874 (2000) 111.
[15] C. García-Viguera, P. Bridle, Food Chem. 54 (1995) 349.
[16] M.A. Rodríguez-Delgado, M.L. Pérez, R. Corbella, G. González, F.J. García Montelongo, J. Chromatogr. A 871 (2000) 427.
[17] S. Pérez-Magariño, I. Revilla, M.L. González-SanJosé, S. Beltrán, J. Chromatogr. A 847 (1999) 75.
[18] M. López, F. Martínez, C. Del Valle, C. Orte, M. Miró, J. Chromatogr. A 922 (2001) 359.
[19] M. Ollanketo, M.-L. Riekkola, J. Liq. Chromatogr. Rel. Technol. 23 (9) (2000) 1339.
[20] L. Arce, M.T. Tena, A. Rios, M. Valcárel, Anal. Chim. Acta 359 (1998) 27.
[21] L. Křivánková, A. Vraná, P. Gebauer, P. Boček, J. Chromatogr. A 772 (1997) 283.
[22] M. Urbánek, L. Blechtová, M. Pospíšilová, M. Polášek, J. Chromatogr. A 958 (2002) 261.
[23] M. Urbánek, M. Pospíšilová, M. Polášek, Electrophoresis 23 (2002) 1045.
[24] Z. Grodzinska-Zachwieja, J. Chromatogr. 241 (1982) 217.
[25] P. Morin, F. Villard, M. Dreux, J. Chromatogr. 628 (1993) 161.
[26] U. Justesen, P. Knuthsen, T. Leth, J. Chromatogr. A 799 (1998) 101.
[27] G. Toker, M. Aslan, E. Yesilada, M. Memisoglu, S. Ito, J. Pharm. Biomed. Anal. 26 (2001) 111.