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Short communication

Determination of sorbate and benzoate in beverage samples by capillary electrophoresis—Optimization of the method with inspection of ionic mobilities

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ABSTRACT

The aim of this study was to develop a fast capillary electrophoresis method for the determination of benzoate and sorbate ions in commercial beverages. In the method development the pH and constituents of the background electrolyte were selected using the effective mobility *versus* pH curves. As the high resolution obtained experimentally for sorbate and benzoate in the studies presented in the literature is not in agreement with that expected from the ionic mobility values published, a procedure to determine these values was carried out. The salicylate ion was used as the internal standard. The background electrolyte was composed of 25 mmol L^{-1} tris(hydroxymethyl)aminomethane and 12.5 mmol L^{-1} 2-hydroxyisobutyric acid, at pH 8.1. Separation was conducted in a fused-silica capillary (32 cm total length and 8.5 cm effective length, 50 μm I.D.), with short-end injection configuration and direct UV detection at 200 nm for benzoate and salicylate and 254 nm for sorbate ions. The run time was only 28 s. A few figures of merit of the proposed method include: good linearity ($R^2 > 0.999$), limit of detection of 0.9 and 0.3 mg L^{-1} for benzoate and sorbate, respectively, inter-day precision better than 2.7% ($n = 9$) and recovery in the range 97.9–105%. Beverage samples were prepared by simple dilution with deionized water (1:11, v/v). Concentrations in the range of 197–401 mg L^{-1} for benzoate and 28–144 mg L^{-1} for sorbate were found in soft drinks and tea.

1. Introduction

The quality of commercial beverages is maintained by addition of preservatives with antimicrobial properties aiming at preventing nutritional losses from chemical alterations and preserving the products during their shelf life [1]. Among the substances normally used as preservatives are benzoic and sorbic acids and their sodium, potassium and calcium salts. The maximum acceptable concentrations of preservatives in food and beverages are controlled by legislation. According to Brazilian legislation, the maximum allowed concentration of sorbate and benzoate (acidic form) in beverages is 0.1% (1000 mg kg^{-1}) [2].

Several analytical methods for determination of these preservatives have been reported in the literature, including spectrophotometry [3,4], gas chromatography [5–8], high-performance liquid chromatography [9–12], ion chromatography [13,14], biosensors [15], micellar electrokinetic capillary chromatography [16,17], capillary zone electrophoresis (CZE) [18–21], microemulsion electrokinetic chromatography (MEEKC) [22], capillary electrochromatography [23], etc.

Distinct approaches using electrophoretic methods in the determination of benzoic and sorbic acid and other preservatives have been described in the literature. Huang et al. [22] proposed a method based on microemulsion electrokinetic chromatography (MEEKC) for analysis of seven preservatives which are in commonly use. This separation takes around 15 min and the method was applied to several food products. Han et al. [24] determined benzoic and sorbic acids in food samples using a flow analysis system with an on-line solid-phase extraction (SPE) unit, developed in the laboratory, combined with the CZE method. The analysis takes about 4 min for benzoate, sorbate and the internal standard *p*-hydroxybenzoic acid. Law et al. [25] proposed a method for separation and detection of sorbate, benzoate and vitamin C by conventional CE and microchip electrophoresis with capacitively coupled contactless conductivity detection. A considerable reduction in the analysis time was achieved using microchip electrophoresis, without significant loss in sensitivity under optimal separation conditions. The migration time was around 70 s for microchip separation and 400 s for conventional CE, and the

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methods developed were applied to real samples such as soft drinks and vitamin C tablets. Huang et al. [23] proposed a method for determination of five common preservatives using capillary electrochromatography with a methacrylate ester-based monolithic capillary as the separation column. An optimal separation of preservatives was obtained within 7 min and was applied to real commercial products.

In food analysis, CZE offers attractive advantages over established techniques including low consumption of chemical reagents and samples, good resolution, and reduced residue generation. A useful tool in CZE method development is the inspection of effective mobility *versus* pH curves. Mobility curves assist in the selection of the separation pH, operation mode and the run electrolyte components [26,27]. The use of mobility curves in method development is strongly dependent on accurate values of ionic mobility and pK_a. Deviations due to inaccurate pK_a values can be neglected when pH values at which the species can be considered fully ionized are used. However, ionic mobilities must be known accurately. Ionic mobility values have been compiled in the literature by isotachophoresis (ITP) [28,29], and CZE [30,31] measurements.

A few methods enabling sorbate and benzoate separation using CZE at pH 8.6 [17], pH 8.8 [18] and pH 10 [20] have been proposed in the literature. Interestingly, the high resolution obtained experimentally in the above mentioned studies is not in agreement with what is expected from the ionic mobility database [28], which reports a difference between the sorbate and benzoate mobility of only 0.2 $cm^2\,V^{-1}\,s^{-1}$.

The objective of this study was to develop a fast method for the determination of sorbate and benzoate in beverages samples using effective mobility *versus* pH curves for systematic optimization of the method. The ionic mobility values for sorbate and benzoate reported in the literature were verified through method optimization.

2. Experimental

2.1. Instrumentation

All experiments were performed on an Agilent Technologies HP^{3D}CE apparatus (Palo Alto, CA, U.S.A.), equipped with a diode array detector. Data acquisition and treatment were performed with HP Chemstation software.

Electrophoretic measurements were performed at 25 °C on an uncoated fused-silica capillary (32 cm × 50 μm I.D. × 375 μm O.D.) obtained from Microtube (Araraquara, Brazil). In between runs the capillary was flushed for 0.6 min with background electrolyte (BGE). Standard solutions and samples were introduced from the outlet capillary extremity and injected hydrodynamically at 50 mbar for 3 s (50 mbar = 4996.2 Pa). The applied separation voltage was 30 kV, positive polarity in the injection side.

The ionic mobility determination was performed at 25 °C on an uncoated fused-silica capillary (32 cm × 50 μm I.D. × 375 μm O.D.) obtained from Polymicro Technologies (Phoenix, AZ).

The effective electrophoretic mobilities were measured according to the method by Williams and Vigh [32] as follows: in the first step (4 s, 50 mbar) a mixture of the analyte and acetone was injected as the electroosmotic flow marker, the mixture band was transferred to the thermostatted region of the capillary by applying the injection pressure for 60 s (50 mbar). Then a running voltage of 5 kV was applied and the analyte and acetone were allowed to separate for 2 min, the voltage ramp-up and ramp-down time was 0.2 min. Next, the second acetone band was injected (4 s, 50 mbar) into the capillary, and finally all three bands were mobilized through the detector window by applying the injection pressure (50 mbar).

2.2. Software

For the construction of the effective mobility *versus* pH curves, Microsoft® Excel 2003 worksheets were used.

2.3. Reagents and solutions

All chemicals used in the BGE preparation were of analytical reagent grade. Sodium hydroxide was obtained from Merck (Darmstadt, Germany), tris(hydroxymethyl)aminomethane (Tris), 2-hydroxyisobutyric (HIBA), sorbic, benzoic and salicylic acids were purchased from Aldrich (Milwaukee, WI, U.S.A.). Deionized water (Milli-Q deionizer, Millipore, Bedford, MA, U.S.A.) was used to prepare the solutions. Stock standard solutions (1000 mg L^{-1}) of benzoic, sorbic and salicylic (internal standard) acids were prepared in methanol. All stock solutions were stored under refrigeration at 4 °C.

The optimized electrolyte was composed of 12.5 mmol L^{-1} HIBA and 25 mmol L^{-1} Tris, pH 8.1.

2.4. Samples

Soft drinks, juices and tea samples were purchased from local stores. Samples were prepared by dilution with water (1:11, v/v). Exactly 500 μL of the diluted sample solution were transferred to a vial and spiked with 100 μL of internal standard (salicylic acid) at 120 mg L^{-1} before injection.

3. Results and discussion

3.1. Verification of ionic mobility

The expression for the effective mobility as a function of the pH of the buffer is presented in Eq. (1).

$$\mu_{\mathrm{eff,A^-}} = \frac{\mu_{\mathrm{act,A^-}}}{1 + 10^{\mathrm{p}K_a - \mathrm{pH}}} \quad (1)$$

For a correction of mobility to ionic strength (I), we can apply the Onsanger equation, since a solution of univalent electrolytes at 25 °C is described by Eq. (2) [33].

$$\mu_{\mathrm{act,A^-}} = \mu_{0,\mathrm{A^-}} - [0.23\mu_{0,\mathrm{A^-}} + 31.3 \times 10^{-5}]\frac{\sqrt{I}}{1+\sqrt{I}} \quad (2)$$

The ionic mobility data for benzoic and sorbic acids were verified by carrying out an electrophoretic run of benzoic (pK_a 4.203) and sorbic (pK_a 4.77) acids in a pH 8.1 Tris/HIBA buffer at 10 mmol L^{-1} of ionic strength. This pH value was considered sufficiently high so that solutes are fully ionized so $\mu_{\mathrm{act,A^-}} = \mu_{\mathrm{eff,A^-}}$. The $\mu_{\mathrm{eff,A^-}}$ was determined individually by Williams and Vigh's [32] procedure using acetone as the neutral marker. The $\mu_{0,\mathrm{A^-}}$ was obtained from $\mu_{\mathrm{act,A^-}}$ using Eq. (2). In Table 1, the calculated μ_0 values are compared with those given in the literature, confirming once again that sorbate mobility is in fact much lower and that the baseline resolution of sorbate and benzoate ions is possible, as reported by previous methodologies [17,18,20].

Table 1
The μ_0 values calculated from the regression parameters contrasted with values from literature

Acid	This study ($\times 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$)	μ_0[a] ($\times 10^{-5}$ $cm^2\,V^{-1}\,s^{-1}$)	%
Sorbic	−30.9 ± 0.3	−33.4	92.7
Benzoic	−33.8 ± 0.2	−33.6	100.8

[a] Ref. [28].

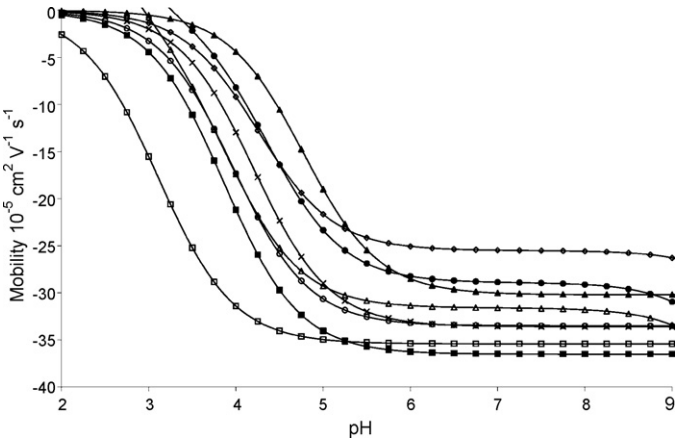

Fig. 1. Effective mobility *versus* pH curves for benzoic (×), sorbic (▲), salicylic (□), 2-hydroxyisobutyric (○), ascorbic (◊), glutamic (●), aspartic (△), lactic (■).

3.2. Choice of background electrolyte constituents

Using the μ_0 and pK_a values from the literature [28] and -30.9×10^{-5} cm^2 V^{-1} s^{-1} as the μ_0 value for the sorbate ion (Table 1), the effective mobility *versus* pH curves were constructed as shown in Fig. 1. As observed, separation between benzoate and sorbate may be approached at any pH between 3 and 12. It is of interest to use a pH higher than 7 because all solutes are fully dissociated, making the method more robust, since the resolution will not be affected by the small pH changes. HIBA was selected as the BGE co-ion since it has an ionic mobility very close to those of the analytes (Fig. 1), contributing to minimizing the electromigration dispersion. However, HIBA does not exhibit good buffering capacity at pH higher than 7. Therefore, Tris (pK_a 8.15) was selected as counter-ion because it supplies buffering capacity to the BGE and for this reason the separation pH was set at around 8. Finally, salicylic acid was chosen as the internal standard due to its intermediate ionic mobility (Fig. 1).

Furthermore, a fast analysis time was achieved by applying the short-end injection mode (L_{det} 8.5 cm) and a high electrical field (937.5 V cm^{-1}).

Fig. 2A shows an electropherogram of a mixture of standards with a BGE comprised of 25 mmol L^{-1} Tris and 12.5 mmol L^{-1} HIBA. A 2:1 Tris/HIBA ratio was used in order to maximize the buffer capacity. Analysis time was only 28 s.

Beverage samples could contain other organic anions and amino acids which can migrate close to the analyte peaks. Fig. 1 shows the effective mobility curves for these potential interferents. It is possible to observe the separation of these substances under the conditions studied. Acetate, and di and tricarboxylic acids, commonly found in some beverages, do not interfere in the method due to their high effective mobility values at the pH of the BGE. In addition, with the exception of ascorbic acid, these substances have low molar absorptivity at the wavelengths used in the method.

3.3. Figures of merit

Before demonstrating the applicability of the proposed method for sorbate and benzoate determination in beverage samples, a few validation parameters such as linearity, recovery, repeatability, precision, limit of detection (LOD) and limit of quantification (LOQ) were evaluated. In all cases 20 μg mL^{-1} of the internal standard was used and peak area ratios (benzoate or sorbate/salicylate ion) were considered. Analytical performance data and a few figures of merit of the proposed method are compiled in Table 2.

3.3.1. Linearity range

Under the optimized analysis conditions, linearity was studied in the concentration range of 4–45 μg mL^{-1} for benzoate and 2–20 μg mL^{-1} for sorbate with triplicate injection at each concentration level. Acceptable regression coefficients were obtained, better than 0.9992 and 0.9994 for benzoic and sorbic acid, respectively. The linear range was considered satisfactory to quantify several real samples. When the samples analyzed have values above the upper limit of the calibration curve after (1:11, v/v) dilution, another greater dilution must be applied.

3.3.2. Precision

The precision of the proposed method is expressed in terms of relative standard deviations (RSD), and the results are compiled in Table 2. The concentrations of the standard solutions for the

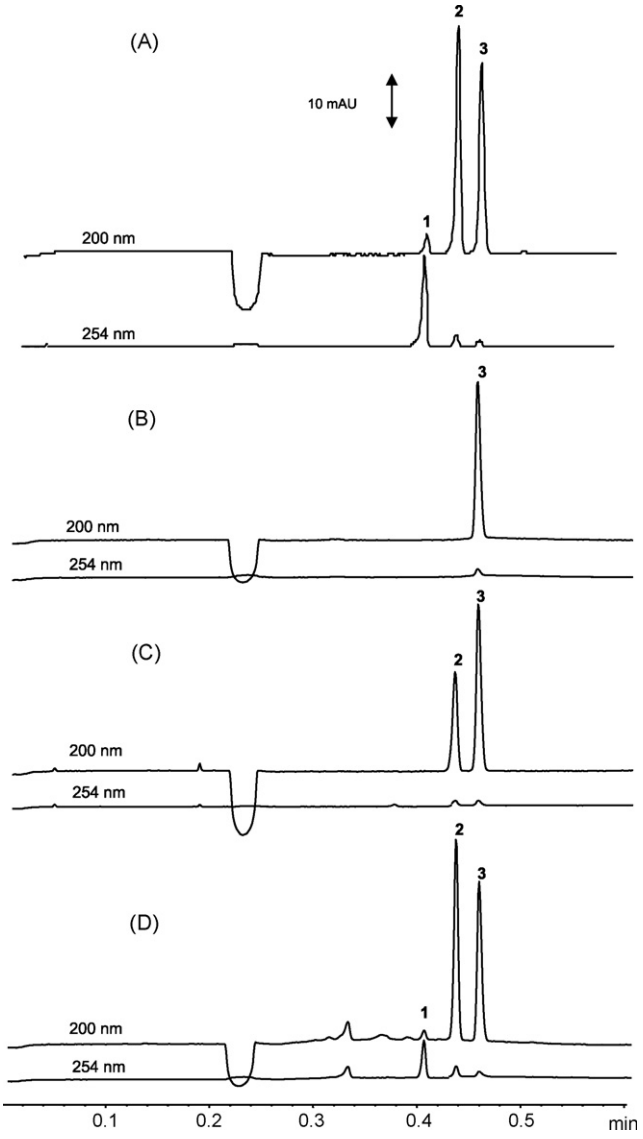

Fig. 2. Fast determination of sorbate and benzoate in beverage samples. Electropherogram of a standard mixture at 10 mg L^{-1} for sorbate and 20 mg L^{-1} for benzoate and internal standard salicylate (A); soft drink without preservatives (B); soft drink (C); and tea samples (D). Electrolyte system composed of 25 mmol L^{-1} Tris and 12.5 mmol L^{-1} HIBA (pH 8.1). Other conditions: short-end capillary hydrodynamic injection (−50 mbar, 3 s), 30 kV applied voltage; 25 °C; direct detection at 200 nm for benzoate and salicylate detection, and 254 nm for sorbate detection. Peak identification: 1 – sorbate, 2 – benzoate, 3 – salicylate.

Table 2
Analytical performance data and a few figures of merit

Parameter	Sorbate	Benzoate
Number of plates (*N*/meter)	23590	20831
Peak asymmetry	0.61	0.74
Resolution (peaks 1–2; 2–3)	1.48	1.11
Instrumental precision ($n = 8$; RSD %); peak area ratio	1.21	1.10
Instrumental precision ($n = 8$; RSD %); migration time	0.21	0.19
Intra-day precision ($n = 15$; RSD %); migration time	0.98	0.95
Intra-day precision ($n = 15$; RSD %); peak area ratio	2.12	1.95
Inter-day precision ($n = 14$; RSD %); migration time	0.95	0.91
Inter-day precision ($n = 14$; RSD %); peak area ratio	2.84	2.57
Linearity – slope[a]	0.0312	0.0328
Slope standard deviation	0.0003	0.0004
Linearity – intercept	0.0135	0.0761
Intercept standard deviation	0.0033	0.0004
Linearity – regression coefficient	0.9994	0.9992
F	2330	1688
Accuracy (% recovery); (add 60 and 170 mg L^{-1})	98.2	97.9
Accuracy (% recovery); (add 80 and 210 mg L^{-1})	98.6	103.3
Accuracy (% recovery); (add 100 and 280 mg L^{-1})	103.7	102.8
Accuracy (% recovery); (add 120 and 300 mg L^{-1})	105.0	100.3
Accuracy (% recovery); (add 140 and 350 mg L^{-1})	104.4	104.6
LOQ (mg L^{-1})	1.1	3.1
LOD (mg L^{-1})	0.3	0.9

In all cases, 20 μg mL^{-1} of the internal standard was used and peak area ratios benzoate of sorbate/salicylate ions were considered.

[a] Linearity was studied in the concentration range of 4–45 μg mL^{-1} for benzoate and 2–20 μg mL^{-1} for sorbate with triplicate injection at each concentration level. In the precision experiments were used 10 and 20 mg L^{-1} for sorbate and benzoate, respectively.

measurements and calculations were 10 and 20 mg L^{-1} for sorbate and benzoate, respectively. Instrumental precision was established through eight consecutive injections of a standard solution. Repeatability values for the migration time and corrected peak area were better than 0.21% and 1.21% for sorbate and 0.19% and 1.10% for benzoate, respectively. Repeatability (intra-day precision) was established through five independent sample preparations and triplicate injections. Repeatability values for migration time and peak area ratio were better than 0.95% and 1.95% for benzoate and 0.98% and 2.12% for sorbate, respectively. Intermediate precision (inter-day precision) was established through 14 injections of a standard solution, on three different days. Repeatability values for migration time and peak area ratio were better than 0.91% and 2.57% for benzoate and 0.95% and 2.84% for sorbate, respectively.

3.3.3. LOQ and LOD

Signal to noise ratios (S/N) of 3 and 10 were considered to estimate LOD and LOQ, respectively. For benzoate and sorbate, LOD values were 0.9 and 0.3 μg mL^{-1}, respectively and the LOQ values were 3.1 and 1.1 μg mL^{-1}, respectively.

3.3.4. Recovery

Recovery test were performed at five concentration levels and good results were achieved, as can be observed in Table 2. Recoveries were calculated with the peak area ratio and the results ranged from 97.9% to 105.0%, as can be observed in Table 2.

3.4. Applicability

To demonstrate the applicability of the developed electrophoretic method, several commercially available beverage samples were analyzed. Fig. 2B and C show the electropherograms for soft drink samples and Fig. 2D shows an electropherogram of tea. Samples were prepared in duplicate and injected in triplicate. Sample concentrations of sorbate and benzoate are compiled in Table 3. According to the Brazilian regulation described by Anvisa [2], all samples concentrations were below the specified values for these compounds.

Table 3
Sample concentrations of benzoate and sorbate in commercial beverages

Samples	Benzoate (mg L^{-1})	Sorbate (mg L^{-1})
Tonic water (apple)	273.5 ± 2.7	<LOD
Tonic water (lemon)	197.2 ± 5.0	<LOD
Tea	392.6 ± 11.0	73.2 ± 1.0
Soft drink A	286.7 ± 15.6	65.1 ± 5.2
Soft drink B	<LOD	<LOD
Soft drink C	323.9 ± 3.4	60.0 ± 2.4
Soft drink D	401.2 ± 2.6	<LOD
Juice A	<LOD	<LOD
Juice B	<LOD	<LOD
Juice C	<LOD	<LOD
Ice tea A	219.7 ± 7.2	27.5 ± 2.8
Ice tea B	218.2 ± 1.8	143.6 ± 3.8
Ice tea C	274.3 ± 2.1	40.7 ± 0.2

4. Conclusions

In this study a simple, fast and reliable method for determination of sorbate and benzoate preservatives in beverages was developed, optimized and validated. The analytical performance of the method, particularly the very short analysis time, low cost and simple sample pretreatment, verifies its potential applicability for routine and automated analysis of these preservatives in the quality control of beverages.

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References

[1] http://www.anfarmag.org.br/home.php.
[2] http://www.anvisa.gov.br/e-legis/.
[3] V.A. Lozano, J.M. Camiña, M.S. Boeris, E.J. Marchevsky, Talanta 73 (2007) 282.
[4] N.R. Marsili, A. Lista, B.S.F. Band, H.C. Goicoechea, A.C. Olivieri, J. Agric. Food Chem. 52 (2004) 2479.
[5] C. Dong, W. Wang, Anal. Chim. Acta 562 (2006) 23.
[6] M. Gonzalez, M. Gallego, M. Valcarcel, J. Chromatogr. A 823 (1998) 321.
[7] M. Kakemoto, J. Chromatogr. 594 (1992) 253.
[8] Z. Pan, L. Wang, W. Mo, C. Wang, W. Hu, J. Zhang, Anal. Chim. Acta 545 (2005) 218.
[9] I.R. Techakriengkrai, J. Surakarnkul, Food Compos. Anal. 20 (2007) 220.
[10] B. Saad, M.F. Bari, M.I. Saleh, K. Ahmad, M.M.K. Talib, J. Chromatogr. A 1073 (2005) 393.
[11] F.J.M. Mota, I.M.P.L.V.O. Ferreira, S.C. Cunha, M.B.P.P. Oliveira, Food Chem. 82 (2003) 469.
[12] S.A.V. Tfouni, M.C.F. Toledo, Food Control 13 (2002) 117.
[13] Q.-C. Chen, J. Wang, J. Chromatogr. A 937 (2001) 57.
[14] Y.-T. Xie, P. Chen, W.-Z. Wei, Microchem. J. 61 (1999) 58.
[15] M.D. Morales, S. Morante, A. Escarpa, M.C. González, A.J. Reviejo, J.M. Pingarrón, Talanta 57 (2002) 1189.
[16] I. Pant, V.C. Trenerry, Food Chem. 53 (1995) 219.
[17] C.O. Thompson, V.C. Trenerry, J. Chromatogr. A 694 (1995) 507.
[18] Y. Tang, W. Mingjia, Food Chem. 103 (2007) 243.
[19] J.C. Walker, S.E. Zaugg, E.B. Walker, J. Chromatogr. A 781 (1997) 481.
[20] K.-L. Kuo, Y.-Z. Hsieh, J. Chromatogr. A 768 (1997) 334.
[21] I. Mato, J.F. Hidobro, J. Simal-Lozano, M.T. Sancho, Anal. Chim. Acta 565 (2006) 190.
[22] H.-Y. Huang, C.-L. Chuang, C.-W. Chiu, J.-M. Yeh, Food Chem. 89 (2005) 315.
[23] H.-Y. Huang, C.-W. Chiu, I.-Y. Huang, J.-M. Yeh, Electrophoresis 25 (2004) 3237.
[24] F. Han, Y.-Z. He, L. Li, G.-N. Fu, H.-Y. Xie, W.-E. Gan, Anal. Chim. Acta 618 (2008) 79.
[25] W.S. Law, P. Kubán, J.H. Zhao, S.F.Y. Li, P.C. Hauser, Electrophoresis 26 (2005) 4648.

[26] L.C. Goncalves, G.A. Micke, J. Chromatogr. A 1154 (2007) 477.
[27] A.C.O. Costa, J.L. da Costa, F.G. Tonin, M.F.M. Tavares, G.A. Micke, J. Chromatogr. A 1171 (2007) 140.
[28] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, J. Chromatogr. 271 (1983) D1.
[29] M. Polášek, B. Gaš, T. Hirokawa, J. Vacík, J. Chromatogr. 596 (1992) 265.
[30] J.L. Beckers, F.M. Everaerts, M.T. Ackermans, J. Chromatogr. 537 (1991) 407.
[31] K. Včeláková, I. Zusková, E. Kenndler, B. Gaš, Electrophoresis 25 (2004) 309.
[32] B.A. Williams, G. Vigh, Anal. Chem. 68 (1996) 1174.
[33] P. Boček, M. Deml, P. Gebauer, V. Dolník, B.J. Radola (Eds.), Analytical Isotachophoresis, first ed., VCH, New York, 1988.