



ELSEVIER

Journal of Chromatography A, 760 (1997) 326–332

JOURNAL OF  
CHROMATOGRAPHY A

Short communication

## Determination of anions in rainwater by capillary electrophoresis with conductivity detection

Sara Valsecchi<sup>a,\*</sup>, Gianni Tartari<sup>a</sup>, Stefano Polesello<sup>b</sup>

<sup>a</sup>*C.N.R.-I.R.S.A., via Mornera 25, 20047 Brugherio (MI), Italy*

<sup>b</sup>*C.N.R.-Istituto Italiano di Idrobiologia, L.go Tonolli 50/52, Pellanza (VB), Italy*

Received 12 June 1996; revised 17 September 1996; accepted 23 September 1996

### Abstract

A routine method for the determination of chloride, nitrate and sulfate anions in rainwater by capillary electrophoresis was developed. The system uses an end-column non-suppressed conductivity detector. Linear calibration plots were generated from 0.050 to 20 mg/l, which is the range generally found in wet depositions. Accuracy and precision were evaluated by analyzing certified standards of simulated rainwater and environmental samples, or by comparing CE results with those obtained by IC, the reference technique for anion analysis in wet deposition. The reproducibility of the method was satisfactory except at the lower and upper limits of the analytical range. Sensitivity lay in the range of few  $\mu\text{g/l}$ .

**Keywords:** Water analysis; Environmental analysis; Inorganic anions

### 1. Introduction

Simultaneous determination of anions in fresh-water is generally performed by ion chromatography (IC). In less than ten years since the introduction of commercial instrumentation, approval was granted for an US Environmental Protection Agency (EPA) method utilizing IC (Method 300.0 for anions in drinking water) [1]. Soon after Method 300.0 was instituted, the US EPA gave official designation to chemically suppressed IC for the analysis of anions [2] and cations [3] in wet depositions. Officially sanctioned environmental analysis methods employing IC have rapidly grown all over the world, and this technique is now considered the standard method for ion analysis in environmental matrices [4,5]. IC is an accurate and precise technique, but yet suffers

for some intrinsic limitations, such as low efficiency and selectivity, due to the difficulties in developing gradient elution methods, and the expense of the analytical columns, especially those proposed for specific applications.

The introduction of capillary electrophoresis (CE), with its radically different selectivity mechanism, shows much promise in overcoming some of the inherent selectivity limitations of ion-exchange separations. The use of this technique has been limited by the lack of sensitivity of the universally employed detection mode, i.e., direct and indirect UV absorption, in commercial instrumentation. Typical limits of detection with hydrodynamic injection are between 1 and 3 mg/l for the inorganic anions [6], which are not useful for rainwater analysis. These limits of detection could be improved if preconcentration techniques, such as electromigration [7], which is affected by discrimination effects, or the

\*Corresponding author.

more labor intensive isotachopheresis [8] were employed.

In capillary ion analysis, conductimetric and amperometric detection offers significant enhancement in performance and sensitivity [9]. Since 1979 [10] numerous detector configurations, both on-column and end-column, have been demonstrated in the laboratory, but they have been difficult to reproduce. Suppressed conductivity detection was recently introduced [11], and this technique looks very promising because it gives detection limits in the range of 1–10  $\mu\text{g/l}$  without preconcentration. Nevertheless, no application on real samples has been reported for these detection techniques. The introduction on the market of a new non-suppressed conductivity detector for capillary electrophoresis [12] induced us to verify the suitability of this technique in a monitoring campaign of wet depositions.

The aim of this work is to validate a routine method for the determination of anions in rainwater by capillary electrophoresis with conductivity detection. Dynamic range, accuracy and precision were evaluated using standards, certified samples and environmental samples. The results were compared with those achieved in our laboratories by IC.

## 2. Experimental

### 2.1. Reagents and samples

High purity water, obtained by a Milli-Q System (Millipore, Marlborough, MA, USA), was used to prepare all solutions. Standard solutions were prepared by diluting concentrated stock solutions containing the individual anions, prepared from their dried salts of ACS grade or better.

Osmotic flow modifier (OFM) was 1 mM hexadecyltrimethylammonium bromide (CTAB, Sigma, St. Louis, MO, USA). The running buffer was composed of 100 mM 2-(N-cyclohexamino)ethanesulfonic acid (CHES, Sigma), 40 mM LiOH and 0.02% w/w Triton X-100 (Sigma). All reagent solutions were filtered and degassed prior to analysis.

All samples were filtered through a 0.45- $\mu\text{m}$  membrane filter and were stored in the dark at 4°C

until analyzed. They were allowed to reach ambient temperature prior to analysis.

### 2.2. Electrophoretic separation

A Crystal 310 capillary electrophoresis system (Thermo CE, formerly ATI Unicam, Cambridge, UK) and a Crystal 1000 conductivity detector (Thermo CE) were used for all separations. A fused-silica capillary (Con Cap I, 60 cm $\times$ 50 mm I.D.) with polyimide coated outer surface was employed. The fused-silica capillary had a permanently mounted fiber-optic type connector on the detection side. The detector cell was formed by joining the capillary connector, placed in end-to-end contact with the conductivity sensor, the ConTip with the detection electrodes implanted in a similar fiber-optic type connector. A detector side electrolyte replenishment system automatically rinses the detector cell with fresh electrolytes after each analysis.

New capillaries were washed with 0.1 M NaOH for 10 min, rinsed with water for 10 min and conditioned with the running buffer for 2 h. At the beginning of every working day, the capillary was conditioned with the running buffer for 45 min. Before each analysis the capillary was conditioned with CTAB for 0.4 min, and rinsed with running buffer for 3 min. The applied potential was set to  $-25$  kV giving a resultant current of about  $-13$   $\mu\text{A}$ . The capillary was thermostated at 20°C. Samples were injected in hydrodynamic mode with high pressure filtered air: a pressure of 500 mbar for 0.4 min was applied. Electropherograms were recorded and evaluated on a personal computer using 4880 software for chromatography (Thermo CE).

## 3. Results and discussion

In the present method the electroosmotic flow (EOF) was reversed by derivatizing the surface of the capillary with a CTAB solution before each analysis. This procedure was followed by a rinse with the running buffer to remove the bromide anions. The running buffer was composed of an amphoteric buffer (CHES), an electrolyte (LiOH) which contributes to the ionic strength and adjusts the pH of the buffer solution to 9.3, and a non-ionic

surfactant (Triton X-100) which decreases the surface tension of the coated layer of the flow modifier. The concentration of the running buffer was optimized in order to get baseline resolution of a mixture of eleven anions which could be present in rain and surface waters (Fig. 1).

In this method hydrodynamic injection mode was used. The conditions of the injection were chosen in order to have an useful dynamic range, from 0.05 to 20 mg/l for the chloride, nitrate and sulfate analytes, which is the range normally found in wet depositions. The use of a high pressure injection mode led to a sensitive method without significant loss of efficiency and resolution also at higher concentrations. Besides the presence of a large plug of water in the capillary after the injections, the dispersion was limited and an efficiency of 50 000–100 000 theoretical plates for the analytes could be achieved.

It is well known that migration time reproducibility represents a problem in developing routine methods with CE. Migration time shifts were evaluated by injecting three standards of different concentration during the same days. Results presented in Table 1 show that the dispersion of the migration times is significant and the difference between the

extreme values can range in the order of one minute. This excludes the use of an automated external standard calibration system. The use of an internal standard can efficiently solve this problem, as is shown in Table 1 which gives the corrected migration times, calculated with respect to the chloride.

Nevertheless, the internal standard approach can be effective only if the concentrations of the internal standard and those of the analytes are comparable. In fact, as shown in Fig. 2, the migration times increase with the concentration of the standards. This phenomenon could be explained by the fact that a concentration increase leads to an increase in conductivity in the analyte zone, which causes also a more pronounced fronting of the peaks [13]. Hence the migration time shifts could be attributed to an increase in the peak asymmetry which shifts the peak maxima. This problem is strictly correlated with the technique itself, and thus can only be solved by developing dedicated software.

Weighted linear regression plots were generated for each anion, injecting eight standard mixtures of increasing concentrations in a range from 0.050 to 20 m/l. Regression equations were: chloride,  $y = 48.18(\pm 0.65)x + 0.35(\pm 0.45)$ ; nitrate,  $y =$

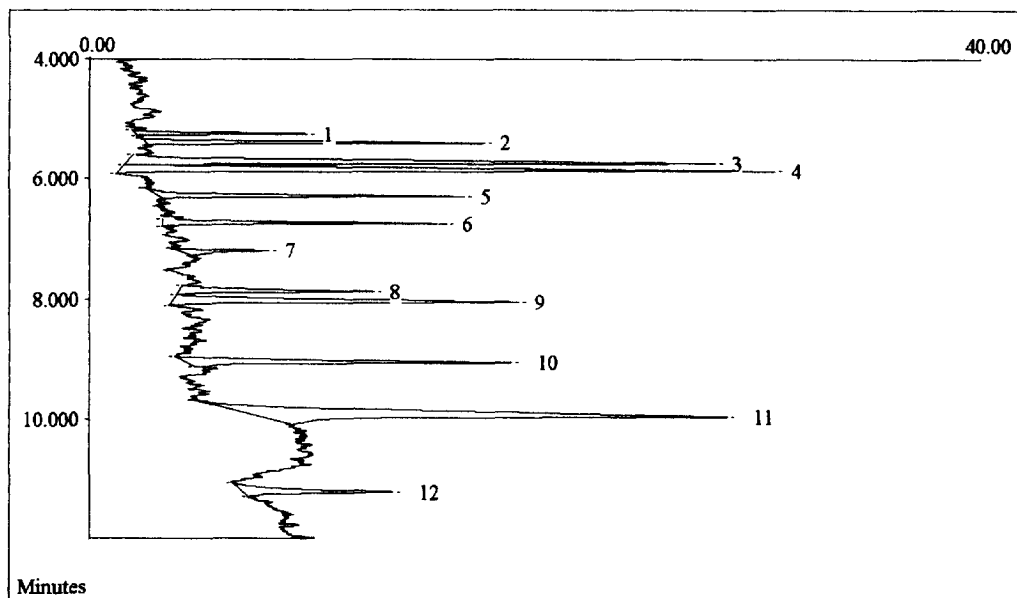


Fig. 1. Electropherogram of a mixture of anion standards (1 mg/l each): 1, bromide; 2, chloride; 3, nitrite; 4, nitrate; 5, sulfate; 6, oxalate; 7, sulfite; 8, formiate; 9, fluoride; 10, phosphate; 11, carbonate; 12, acetate (injection condition: 200 mbar for 0.2 min).

Table 1

Average migration times ( $t_R$ ), migration times corrected with respect to chloride ( $t'_R = t_{R \text{ analyte}} - t_{R \text{ chloride}}$ ) and average peak areas of chloride, nitrate and sulfate, evaluated at three different concentrations ( $n=6$ )

	Concentration (mg/l)	$t_R$ (min)	$t'_R$ (min)	Peak area
Chloride	0.1	7.1±0.3 (4.2)		5.8±0.9 (16.1)
	1.0	7.2±0.2 (2.3)		50.2±0.8 (1.5)
	5.0	7.3±0.1 (1.6)		246±13 (5.1)
	Mean	7.2±0.2 (3.3)		
Nitrate	0.1	7.5±0.3 (3.9)	0.39±0.006 (1.5)	2.6±0.2 (6.0)
	1.0	7.5±0.2 (2.2)	0.38±0.002 (0.5)	27.7±0.6 (2.2)
	5.0	7.7±0.1 (1.5)	0.38±0.003 (0.8)	149±4 (2.5)
	Mean	7.6±0.2 (3.1)	0.38±0.005 (1.4)	
Sulfate	0.1	7.9±0.3 (4.0)	0.86±0.015 (1.7)	4±1 (26.1)
	1.0	8.0±0.2 (2.0)	0.86±0.009 (1.0)	35.1±0.8 (2.4)
	5.0	8.2±0.1 (1.3)	0.88±0.009 (1.0)	182±3 (1.7)
	Mean	8.0±0.3 (3.1)	0.87±0.013 (1.5)	

%R.S.D. in parentheses.

$27.42(\pm 0.31)x - 0.09(\pm 0.09)$ ; sulfate,  $y = 35.97(\pm 0.41)x - 0.16(\pm 0.20)$ . In the examined range good linearity was observed ( $r^2 > 0.999$  for chloride and sulfate and 0.997 for nitrate), and the intercepts were not statistically different from zero.

In the final method procedure a tighter range of five standards was used. The calibration was repeated each working day injecting five standard solutions which contained the three analytes in a fixed ratio (chloride: 1; nitrate: 1; sulfate: 10). This

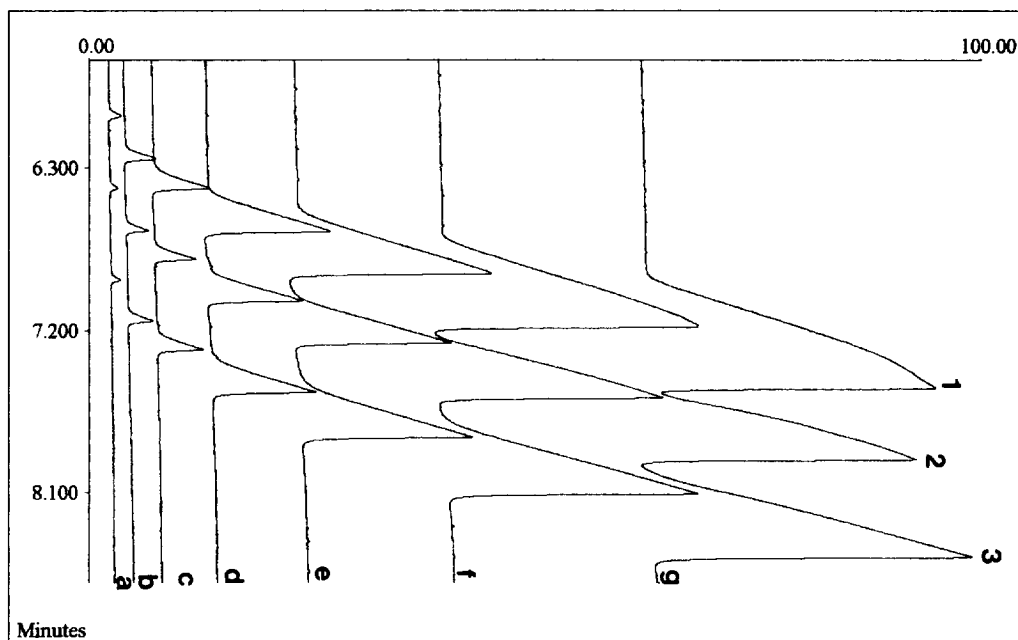


Fig. 2. Migration time shifts of (1) chloride, (2) nitrate, (3) sulfate with respect to increasing concentrations: (a) 0.05; (b) 0.2; (c) 0.5; (d) 2.0; (e) 5.0; (f) 10.0; (g) 15.0 mg/l.

ratio was chosen in order to simulate the natural one. No significant difference was observed in the response factors if compared to those found in the linear regression plots, or those calculated in different working days.

Repeatability was evaluated by six consecutive injections of three standard mixtures of different

concentrations. Table 1 shows the results for 0.1, 1 and 5 mg/l standards: at low concentrations R.S.D. is more than 10%, and this could be attributed to errors in the integration of this small amount. The R.S.D.s for the two other concentrations are between 2 and 5%. These values contain the contributions to the total error from sample preparation, injection,

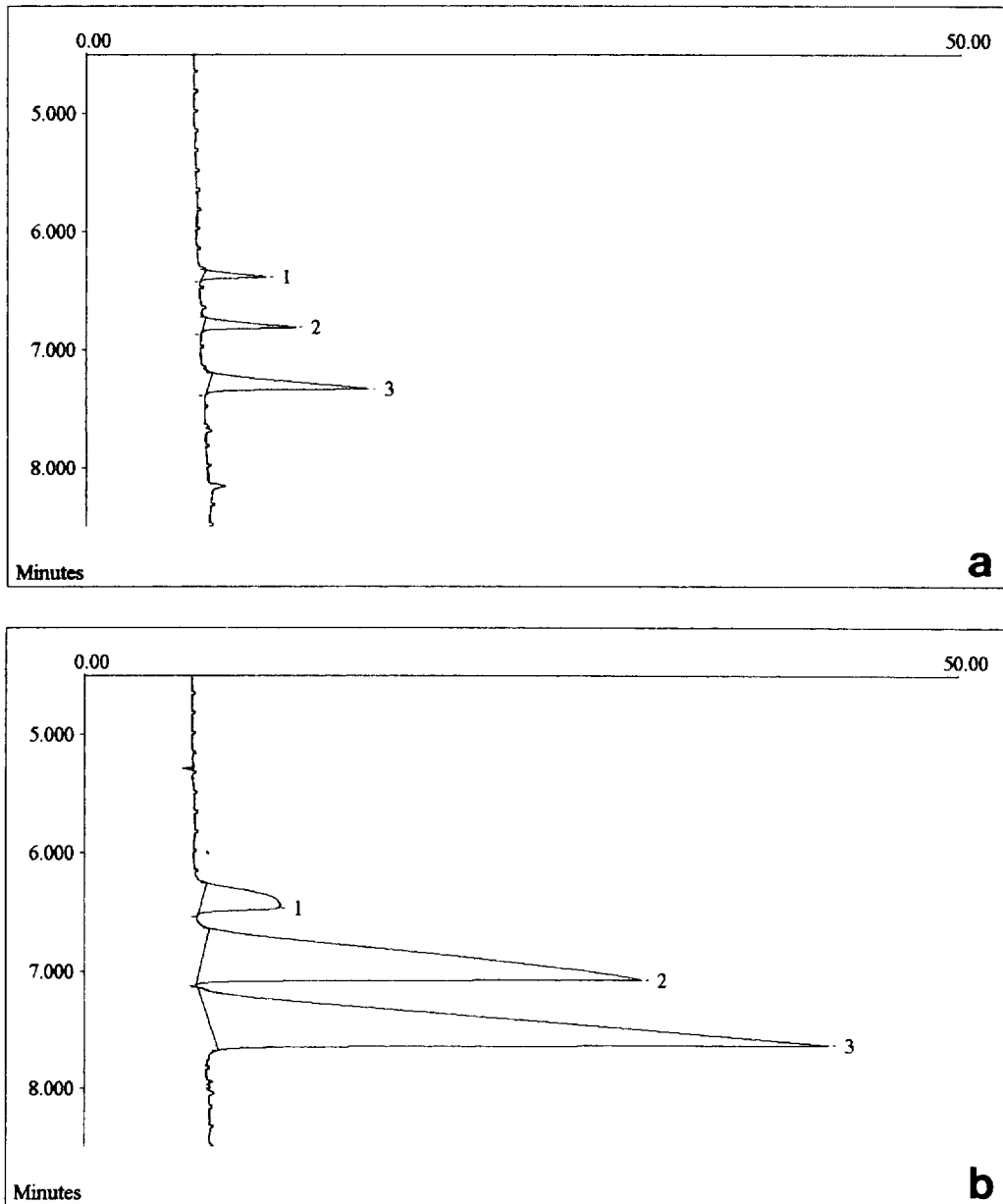


Fig. 3. Electropherograms of (a) sample A and (b) sample B: (1) chloride, (2) nitrate, (3) sulfate.

detection and the automated integration by the software.

Reproducibility was evaluated analyzing two environmental samples (Fig. 3), obtained from monitoring campaigns of acid depositions in Italian Alpine zone (Valtellina). The samples were chosen so that their concentrations lay at the lower and upper limits of the analytical range. Each sample was injected five times a day for five consecutive days. Every day, calibration procedure was repeated. The R.S.D.s, calculated from all data (Table 2), were in the range 3–7%, apart from the lowest concentrations.

Accuracy was established by determining chloride, nitrate and sulfate both on environmental samples, and on certified samples of simulated rainwater (NBS Standard Reference Materials: 2694-I and 2694-II): the results were also compared with those obtained by IC, according to the official methods [2]. The significance of CE results with respect to certified values and IC data were estimated by a double sided Student's *t*-test at the 95% confidence level (Table 2). Accuracy was generally satisfactory, and the two techniques were in good agreement, but IC appeared to be a more precise technique, especially at high concentration [14].

The limits of detection (LOD) and quantification (LOQ) were evaluated, according to IUPAC sug-

Table 3

Calculated limit of detection (LOD) and limit of quantification (LOQ) ( $\mu\text{g/l}$ ) of the CE method

	Chloride	Nitrate	Sulfate
LOD	2	3	2
LOQ	5	8	6

gestions [15], as respectively 3 and 10 times the standard deviation of the background noise. The background noise was evaluated by integrating the baseline for a few minutes in the vicinity of the analyte peaks in different runs on consecutive days. The estimated values were in the range of few  $\mu\text{g/l}$  (Table 3).

#### 4. Conclusions

The suitability of using a new electrophoretic system, with an end-column non-suppressed conductivity detector, in the determination of the main anions (chloride, nitrate and sulfate) in rainwater was explored. A method was developed and validated by analyzing certified samples and by comparison with IC, the reference technique in ion analysis.

The analytical parameters (linearity, precision, accuracy and sensitivity), measured in the validation

Table 2

Average concentrations (mg/l) determined in two control samples of natural rainwater and two reference materials of simulated rainwater

Sample	Method	Chloride	Nitrate	Sulfate	$t_{0.025}$
Sample A	CE	0.17±0.02 (14.5)	0.55±0.04 (6.6)	1.28±0.07 (5.4)	2.05
	IC	0.10±0.01 (6.3)	0.58±0.01 (1.6)	1.32±0.02 (1.9)	
	$t_{\text{calc CE-IC}}$	8.26	1.80	1.37	
Sample B	CE	0.99±0.07 (6.9)	12.5±0.7 (5.7)	13.0±0.4 (3.0)	2.05
	IC	0.98±0.03 (3.3)	12.4±0.1 (1.2)	12.57±0.02 (0.2)	
	$t_{\text{calc CE-IC}}$	0.34	0.27	0.48	
SRM 2694-I	CE	0.24±0.01 (5.9)	n.d.	2.7±0.2 (8.0)	2.31
	IC	0.25±0.02 (5.9)	n.d.	2.83±0.09 (3.3)	
	c.v.	0.24*	n.d.	2.75±0.05 (1.8)	
	$t_{\text{calc CE-IC}}$	1.06		1.19	
	$t_{\text{calc CE-c.v.}}$	–		0.67	
SRM 2694-II	CE	1.05±0.03 (3.0)	7.2±0.2 (2.6)	10.7±0.3 (2.9)	2.57
	IC	1.05±0.02 (1.6)	7.13±0.08 (1.1)	10.45±0.08 (0.8)	
	c.v.	1.0*	7.06±0.15 (2.1)	10.9±0.2 (1.8)	
	$t_{\text{calc CE-IC}}$	0	0.46	0.62	
	$t_{\text{calc CE-c.v.}}$	–	2.18	1.73	

Statistical comparison was performed by using Student's *t*-test at 95% confidence level.

% R.S.D. in parentheses; n.d.=not detectable; c.v.=certified values; \* indicative values.

procedure, show that the method can be applied to the monitoring of wet depositions. The main limit in the application of this method in the routine analysis is that the migration time is not reproducible. The second problem is the close dynamic range if the method is extended to any kind of surface water.

When the official IC method is compared to our developed CE method, IC appears to be a more reliable technique for anion analysis, especially with respect to the dynamic range and precision, but CE is less expensive and its different selectivity mechanism could allow the analysis of some important anions – such as orthophosphate and those of organic acids – in surface waters with a minimal sample amount and preparation.

### Acknowledgments

We thank Thermo CE and Dr. Mozzati (ATI Unicam Italia), for providing the instrumentation, and Dr. Mebs Surve (Thermo CE, Cambridge, UK) for discussing results.

### References

- [1] Determination of Inorganic Anions in Water by Ion Chromatography, Method 300.0, US EPA, Cincinnati, OH, 1991.
- [2] Chloride, Orthophosphate, Nitrate and Sulfate in Wet Deposition by Chemically Suppressed Ion Chromatography, Method 300.6, US EPA, Cincinnati, OH, 1986.
- [3] Dissolved Sodium, Ammonium, Potassium, Magnesium and Calcium in Wet Deposition by Chemically Suppressed Ion Chromatography, Method 300.7, US EPA, Cincinnati, OH, 1986.
- [4] APHA, AWWA and WEF, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 18th ed., 1992, Method 4110, p. 4–1.
- [5] R.A. Durst, W. Davison, K. Toth, J.E. Rothert, M.E. Peden and B. Griepink, *Pure Appl. Chem.*, 63 (1991) 908.
- [6] M.M. Rhemrev-Boom, *J. Chromatogr. A*, 680 (1994) 675.
- [7] A. Roder and K. Bachmann, *J. Chromatogr. A*, 689 (1995) 305.
- [8] D. Kaniansky, I. Zelensky, A. Hybenova and F.I. Onuska, *Anal. Chem.*, 66 (1994) 4258.
- [9] P. Gebauer, M. Deml, P. Bocek and J. Janak, *J. Chromatogr.*, 267 (1983) 455.
- [10] F.E. Mikkers, F.M. Everaerts and Th. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
- [11] N. Avdalovic, C.A. Pohl, R.D. Rocklin and J.R. Stillian, *Anal. Chem.*, 65 (1993) 1470.
- [12] C. Haber, W.R. Jones, J. Soglia, M.A. Surve, M. McGlynn, A. Caplan, J.R. Reineck and C. Krstanovic, *J. Cap. Elec.*, 3 (1996) 1.
- [13] D.N. Heiger, *High-Performance Capillary Electrophoresis, an Introduction*, Hewlett-Packard, 1992.
- [14] A. Marchetto, R. Mosello, G. A. Tartari, H. Muntau, M. Bianchi, H. Geiss, G. Serrini and G. Serrini Lanza, *J. Chromatogr. A*, 706 (1995) 13.
- [15] J.D. Winefordner and G.L. Long, *Anal. Chem.*, 55 (1983) 712A.