Scientific paper

Development of an Ion Chromatographic Method for Determination of Inorganic Anions in Surface Water by Using Computer-Assisted Gradient Optimization Methodology

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Abstract

This work focuses on computer-assisted gradient elution method development in inorganic anion analysis of surface water using ion chromatography. An integral elution model was applied to model gradient retention behavior based on isocratic experimental information. Applied optimization strategy incorporates in-house developed elimination criteria for optimal condition search routine with gradient retention modeling resulting in baseline separation within satisfactory run time. The reliability of developed method was extensively tested by carrying out a performance characteristics evaluation process. Based on the evaluation results it can be stated that the method developed shows more than satisfactory performance characteristics, proving that the applied computer-assisted method development process is a very useful alternative when surface waters differing in composition significantly have to be analyzed in a limited time frame.

Keywords: ion chromatography, surface water, computer-assisted method development

1. Introduction

Inorganic anions (fluoride, chloride, nitrite, bromide, nitrate and sulfate) are important chemical water quality indicators. They play an important role in biochemical processes and are essential elements for aquatic and terrestrial biota. These ions can be present in rivers naturally as a result of , dissolution of geological deposits, biological degradation of organic matter and seawater intrusion in coastal areas. In addition to natural sources, these ions can enter aquatic ecosystems via sources derived from human activities. These sources include surface runoff generated from urban and agricultural land, excess application of chemical fertilizers, waste discharge, industrial and septic tank effluents, landfill leachates and irrigation drainage.[1–5] Elevated or fluctuating concentrations of inorganic anions in water can be detrimental, especially high levels of fluoride, nitrite and nitrate which have a toxic effect on the physiology of animals and tend to be accumulated in aquatic and terrestrial biota.[6–9] Since degradation of water quality can result in altered species diversity, decrease the overall health of aquatic ecosystem and cause serious harm to human health and the environment, the concentrations of inorganic anions in surface water are regularly monitored.

The determination of inorganic anions in environmental waters is nowadays the most widely used application of ion chromatography (IC).[10,11] IC has several advantages over conventional chemical methods (titration, photometry, turbidimetry and colorimetry)[12] such as simultaneous determination of many ions in a short time, high selectivity and sensitivity, good reproducibility, small sample volume, reliability and different detector options[13]. Many standard organizations (ISO, U.S. EPA, ASTM, AOAC, etc.) have standards or regulatory methods of analysis based upon IC.[14,15]

Public sector laboratories have to constantly provide accurate and reliable results and the methods of analysis used should be evaluated for performance characteristics. The problem arises when samples with significantly different composition and/or concentrations of individual anions have to be analyzed; in this particular case the concentration ratios nitrite : sulfate and bromide : sulfate are in the range 1 : 10 000. This makes the application of standard or accredited methodologies for analysis of river water and simple isocratic elution (20, 25 or 30 mM KOH in eluent) useless due to peak overlapping. Consequently, the method needs to be modified and later on evaluated to prove that it fits the purpose. The development of an IC method can be difficult; it involves examination of the adjustable properties such as concentration of the eluent, temperature or gradient program, where one or more of these factors are varied. The chromatographic property which has a major impact on the separation quality is retention. Retention models can be fitted from either isocratic or gradient experiments,[16–21] i.e., the model parameters are determined from the experiment using least-square techniques. Experiment-based optimizations include two steps: modeling of the system and prediction of the resolution through computer simulation. In the first step, a number of experiments are performed in order to fit equations or train algorithms that will allow the prediction of retention. The aim is to develop systems capable of predicting the separation at any new arbitrary condition. In the second step, large numbers of simulated chromatograms are calculated and the one that is expected to give maximal resolution is selected. Usually, only the resolution is considered, but additional goals, such as shorter analysis time or desirable peak profiles, may be taken into account.

The aim of this work is to rapidly develop the ion chromatographic method for determination of inorganic anions in surface water. Due to the tight schedule it is necessary to rely largely on computer-assisted method development and gradient elution IC. If the isocratic data are available from the literature or from previous user data sets, it is possible to explore whether a gradient separation would give satisfactory results before carrying out any experiment. In this work water samples of the rivers Ilova and Kutinica were analyzed. Gradient retention modeling coupled with resolution prediction and shorter analysis time was used. The gradient model was developed using isocratic experimental data[22] and the performance characteristics of developed method were evaluated.

2. Experimental

2. 1. Sampling Locations

Water samples used in this work were collected from the rivers Ilova and Kutinica in the vicinity of the

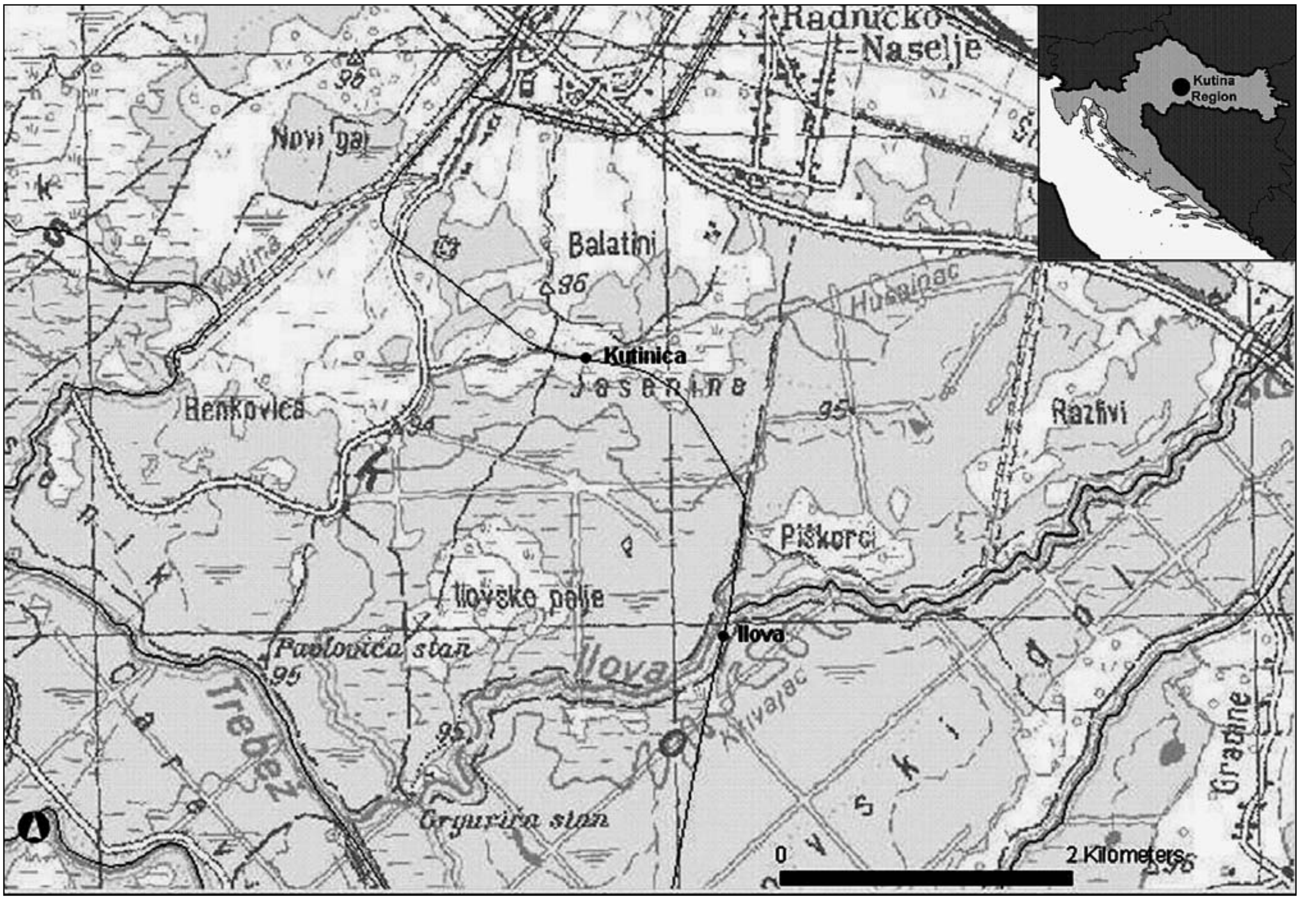

Figure 1. Sampling locations at the rivers Kutinica and Ilova.

town of Kutina. The locations of the two sampling sites are given in Figure 1.

The small river Kutinica is used by the Petrokemija, Kutina fertilizer factory for wastewater disposal. Via the Kutinica-Ilova channel the river Kutinica flows into the river Ilova which is a medium lowland river (97 km) that rises to 200 m above sea level in the northern part of Croatia. Downstream from the confluence of the Kutinica, the river Ilova runs through the Lonjsko Polje and flows into the river Sava. The Lonjsko Polje Nature Park has been included in the Ramsar List of Wetlands of International Importance, on the list of internationally Important Bird Areas and in the ecological network in its entirety as an area important for the conservation of species and habitats. The water quality of the rivers Ilova and Kutinica is monitored regularly in accordance with an operational monitoring program. The previous monitoring results showed different occurrences of certain water quality parameters that appear randomly and mostly depend on weather conditions and different production conditions of the fertilizer factory. These circumstances make the rapid method development process of great significance for the region.

2. 2. Chemicals and Solutions

Standard solutions of fluoride (700 mg/L), chloride (2600 mg/L), nitrite (87 mgN/L), bromide (270 mg/L), nitrate (330 mgN/L) and sulfate (7200 mg/L) were prepared from the air-dried (at 105 °C) salts of individual anions of p.a. grade (all Kemika, Zagreb, Croatia, except fluoride – Merck, Darmstadt, Germany). Appropriate amounts of individual salts were weighed into a volumetric flask (1000 mL) and dissolved with Milli-Q water (Millipore, Bedford, MA, USA; 18 MΩ cm). All standard solutions were kept in a plastic flask in a refrigerator when not in use.

A mixed stock standard solution of fluoride (7 mg/L), chloride (260 mgN/L), nitrite (0.87 mgN/L), bromide (0.27 mg/L), nitrate (33 mgN/L) and sulfate (720 mg/L) was prepared by measuring the appropriate volume of standard solutions into a 500 mL volumetric flask, which was later filled to the mark with Milli-Qwater. Working eluent solutions were prepared on-line by using hydroxide cartridge (Dionex) and Milli-Qwater. Water samples were collected in 2L polyethylene containers and stored at 4 °C. Prior to injection samples were filtered through a Whatman 0.45 μm cellulose nitrate membrane filter (Whatman, Springfield Mill, England). The first 100 mL of filtered sample was discarded.

2. 3. Instrumentation and Chromatographic Conditions

The analyses were carried out on a Dionex ICS-3000 reagent free ion chromatography system (Sunnyvale, CA, USA) which comprised of a DP dual isocratic pump module, an EG dual eluent generator module (with one KOH reservoir cartridge in use for this work) and a DC detector/chromatography module (with dual temperature zone configuration). The DC module was configured with a CRD 200 (4 mm) carbonate removal device and conductivity detector cartridge. Suppression was carried out in the auto-recycle mode using a Dionex ASRS Ultra II (4 mm) suppressor. For removal of anionic contaminants from the water used to generate the eluent, a Dionex continuously regenerating anion trap column (CR-ATC II) was employed. A Dionex hydroxide-selective AS18 (4mm × 250 mm) anion separation column and AG18 (4mm × 50 mm) anion guard column were used for all separations. All experimental measurements were performed using constant sample loop volume (100 μL), eluent flow rate (1.0 mL/min) and temperature (40 °C lower column compartment, 30 °C upper detector compartment). The whole system was computer-controlled by Chromeleon 6.80 SP4 Build 2361 (130805) software.

2. 4. Method Development

For development of the isocratic model both surface water samples were measured using isocratic elution conditions at eleven equidistant eluent concentration levels in the range 5 to 80 mM KOH. The measurements were performed in triplicate and the average of three values was used. The delay time between the eluent generator and the injector was determined by noting when the gradient slope was observed in the detector and by subtracting the void time from the delay time. The determined delay time between eluent generator and injector was 0.15 min. The void time of the ion chromatographic system was 3.19 min. Obtained data were used to construct a gradient elution model. The developed gradient model was fully tested and used for gradient elution method development. All calculations were performed in a MATLAB environment (MATLAB 7.8.0. R2009a, MathWorks, Sherborn, MA, USA).

The developed gradient elution method performance characteristics were evaluated as follows. To reliably test the calibration model all calibration standards were prepared and analyzed independently since a representative estimate of the measurement variance is required. Care was also taken to analyze all standards randomly and to perform the chromatographic analysis in the shortest time possible. Calibration was performed at one sequence out of all experiments. Five concentration levels covering the whole range were applied. Three independent replicates of sample preparation per level and three independent replicates of chromatographic analysis per sample preparation were performed. The experiment for accuracy-related calculation was performed at five concentration levels. Three independent replicates of sample preparation per level and three independent replicates of chromatographic analysis per sample preparation were performed.

The precision-related calculations were performed at one level. Six independent replicates of sample preparation followed by three independent replicates of chromatographic analysis per sample preparation were performed.

3. Results and Discussion

The results of the isocratic measurements are given in Figure 2. Those results were used to fit the regression coefficients a_i of the polynomial relationship between the logarithm of the retention factor, k, and the concentration of the eluent c. The parabolic relationship:[22,23]

$$\log k = a_0 + a_1 \log c + a_2 \left(\log c\right)^2 \tag{1}$$

was used.

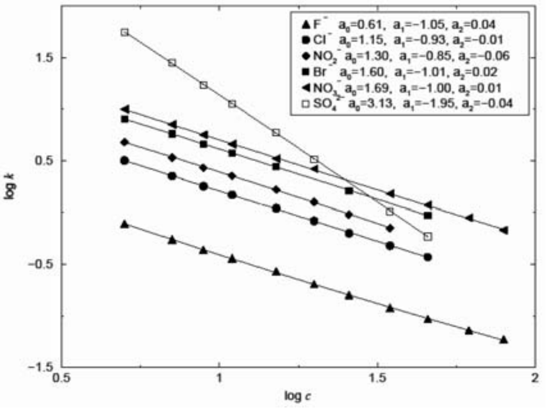

Figure 2. Results of isocratic measurements; lines are representing the quadratic fit of Eq. (1).

The optimal values of coefficients (see Figure 2) were found by applying the least square method to the experimental data of both surface water samples (Ilova and Kutinica) simultaneously.

The isocratic retention model $k(c)$ obtained was then used for calculation of the gradient retention model. The integral equation:

$$t_0 = \int_0^{t_g - t_0} \frac{dt}{k\left(c\left(t\right)\right)} \tag{2}$$

where t_0 is the void time, t_g is the retention time of the solute under gradient conditions and $c(t)$ is the variation of the eluent concentration with time (i.e. the gradient program), was solved numerically[23] for various anions and various gradient programs. To shorten the calculation time only linear gradients were considered in combination with several elimination criteria. The calculation domain consisted of 6 linear segments and 8 concentration levels giving a total of 262144 possible gradient profiles. Out of these 262144 profiles the optimal profile was selected by using four elimination criteria. The first criterion compares resolution values of two neighbour isocratic conditions, selects conditions with $R_s > 2$ and then compares their resolution product. The condition with the smallest resolution product is selected while the other one is discarded, as well as all gradient profiles that lie between them. The second criterion discards all conditions which have equal segments of the already optimized part of the gradient profile, while the third criterion discards all profiles that result with resolution lower than 2. The fourth criterion selects the shortest chromatography run if more than one optimal profile remained after processing of the first three criteria.

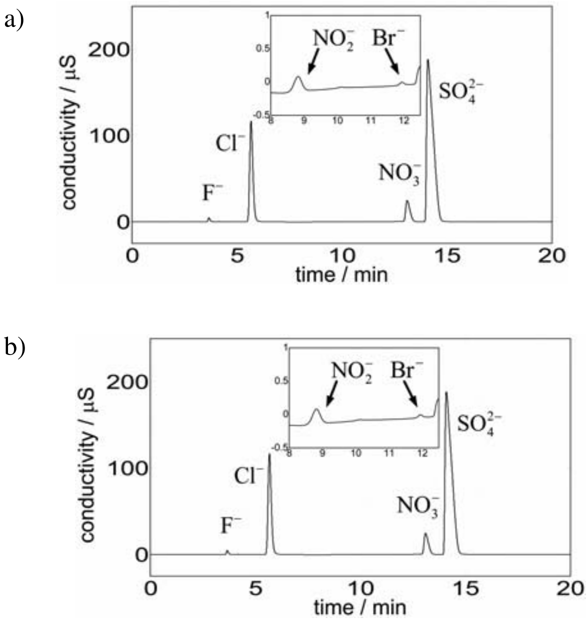

Figure 3. Anion chromatograms obtained by the developed method: A) river Ilova, and B) river Kutinica. Gradient conditions: 30 mM KOH at 0 min; 5.0 mM KOH at 5 min; step to 30 mM KOH at 10 min; 30 mM KOH 10–15 min.

Figure 3 shows the chromatograms of the Kutinica and Ilova river water samples obtained using the optimal conditions: 30 mM KOH at 0 min; 5.0 mM KOH at 5 min; step to 30 mM KOH at 10 min; 30 mM KOH 10–15 min. One can observe the application of a negative gradient step at the beginning of the separation. This particular part of the gradient profile ensures good separation of anions eluting between 5 and 10 minutes by making the eluent concentration low enough (5 mM KOH in the 5th minute) to enable baseline separation. On the other hand, a higher KOH concentration in the eluent at the beginning of the chromatographic run (30 mM KOH) speeds up analysis by saving time before the 5th minute where only the fluoride peak (and void peak) elute. After the 10th minute isocratic separation using 30 mM KOH proves to be most

Table 1. Calibration curve parameters calculated for ion chromatographic determination of inorganic anions in surface water together with lack of fit calculations. The critical *F*-value for $\alpha = 0.05$ is $F_{\text{crit}} = 2.8388$.

Ion	**Range**	R^2	**Slope µS/mgL^{-1}**		**Intercept µS**		**Lack of fit test**		
			Value	**± Standard error**	**Value**	**± Standard error**	MS_{PE}	MS_{LOF}	F
Fluoride	0.35–1.05	0.9996	2.037	0.006	–0.007	0.004	0.0001	0.0002	2.5403
Chloride	13.00–39.00	0.9999	1.266	0.001	0.13	0.04	0.0077	0.0067	0.8691
Nitrite	0.04–0.13	0.9999	2.987	0.005	–0.0345	0.0005	1.15×10^{-6}	1.76×10^{-6}	1.5264
Bromide	0.01–0.04	0.9991	0.358	0.002	–0.00095	0.00005	9.94×10^{-9}	1.71×10^{-8}	1.7281
Nitrate	1.65–4.95	0.9999	3.175	0.004	–0.02	0.01	0.0009	0.0020	2.1797
Sulfate	36.00–108.00	1.0000	0.911	0.001	0.85	0.07	0.0208	0.0426	2.0488

suitable for the two reasons: firstly it enables good separation without significant prolongation of analysis time and secondly it can simultaneously serve as an equilibration part of the chromatographic sequence since the gradient straining concentration of the next run in the sequence is the same. This certainly indicates yet another advantage of the developed methodology: the time of the overall chromatographic sequence is significantly reduced. However, all components of interest are detected and baseline separated proving that the selectivity of the proposed method is satisfactory and further evaluation of the performance characteristic can proceed.

The calibration functions for each ion were established by analyzing five multi-ion (working) standard solutions at five concentration levels. Results of linear regression between peak area and concentration are given in Table 1.

Inspecting the table it can be seen that all correlation coefficients have satisfactory values ($R^2 > 0.999$) indicating the existence of strong linear relationships between peak area and concentration for all anions in the given concentration ranges. To further examine the adequacy of the linear model, a so-called lack-of-fit test was performed. It consists of the analysis of variance (ANOVA), i.e., braking up the total variance into components due to different sources of variation. The pure experimental uncertainty (MS_{PE}) is compared with the differences of local averages from fitted values (MS_{LOF}) and if the values are comparable, the model is justified. Formally, the ratio $F = MS_{\text{LOF}}/MS_{\text{PE}}$ was compared with the corresponding *F*-distribution at the 95% significance level, and it was concluded that the linear model is adequate to describe the relationship between peak area and concentration for all investigated anions in the given range of concentrations.

The slopes of calibration curves present the sensitivity of the proposed method; a larger slope means a higher sensitivity. Values given in Table 1 prove the good sensitivity of the method. Standard errors s_x given in the table indicate the boundaries for the expected values. The 95% confidence limit is located at approximately ±2 s_x from the mean value. The exact value of the $(1\text{-}\alpha) \cdot 00\%$ confidence level is given by the product of standard error and the appropriate value of the *t*-distribution $t_{\alpha/2,\, n-2}$ for n–2 degrees of freedom.

The same measurements made for calibration were used to test the accuracy of the proposed method. The recovery rates and relative standard deviations (*RSD*) are given in Table 2.

All recovery factors are in the range 99.99–100.02 which proves excellent recovery of the method. Also, the regression technique was applied and the relationship between the amount found and the amount added was analyzed. If there were no measurement errors and no bias, the relationship should be an identity function. Because at least random errors are made, the determined relationship differs from identity. The linear relationship between the amount found and the amount added was considered and those results are also presented in Table 2. High values of correlation coefficients ($R^2 > 0.999$) indicate strong linear relationship, while the slope and intercept values do not

Table 2. Recovery factors of ion chromatographic determination of inorganic anions in surface water together with results of regression of the amount found against the amount added.

Ion	**Recovery**		R^2	**Slope**		**Intercept**	
	Value	***RSD***		**Value**	**± Standard error**	**Value**	**± Standard error**
Fluoride	99.99	0.33	0.9996	1.000	0.003	0.000	0.002
Chloride	100.01	0.09	0.9999	1.000	0.001	0.00	0.03
Nitrite	99.99	0.16	0.9999	1.000	0.002	0.0000	0.0002
Bromide	100.02	0.55	0.9991	1.000	0.004	0.0000	0.0001
Nitrate	100.01	0.13	0.9999	1.000	0.001	0.000	0.004
Sulfate	100.00	0.09	1.0000	1.000	0.001	0.00	0.07

differ significantly from 1 and 0, respectively (the 1 and 0 values are within the interval $\pm 2s_x$) at 95% confidence level. Consequently it was concluded that the relationship between the amounts found and the amounts added is linear and that there is no systematic error present in the analytical system.

To test the precision of the obtained method both surface water samples were measured 6 times. The *RSD* values for those measurements are presented in Table 3.

Table 3. Precision of ion chromatographic determination of inorganic anions in surface water.

Precision	*RSD*/%
Fluoride	0.52
Chloride	0.16
Nitrite	0.46
Bromide	1.77
Nitrate	0.20
Sulfate	0.20

With the exception of bromide, the *RSD* values are smaller than 0.53% what proves that the precision of ion chromatographic determination of fluoride, chloride, nitrite, nitrate and sulphate in surface water is satisfactory. The value of 1.78% obtained for bromide is due to low concentration.

The sample preparation procedure consisted of sample dilution. The influence of the dilution procedure on the final analytical result quality (accuracy) was tested by using one-way ANOVA (Table 4).

Table 4. Calculation-related sample preparation influence on accuracy of ion chromatographic determination of inorganic anions in surface water

Ion	Sample preparation Influence on accuracy		
	MS between sample preparation	MS between injections	*p* value
Fluoride	0.45	0.40	0.39
Chloride	0.04	0.04	0.36
Nitrite	0.04	0.26	0.85
Bromide	4.28	1.43	0.12
Nitrate	0.13	0.12	0.40
Sulfate	0.01	0.05	0.90

The mean square errors due to sample preparation are compared to those due to injection. It can be seen from *p* values, which are greater than 0.05 that the mean square errors due to dilution are not significantly different from mean square errors due to injections, by taking into account 95% confidence. That yields the conclusion that the analytical result quality (accuracy) is not significantly affected by using the sample preparation procedure (dilution).

4. Conclusions

In this work the development of an ion chromatographic method for determination of fluoride, chloride, nitrite, bromide, nitrate and sulphate in surface water was presented. The selectivity of the ion chromatographic method plays one of the most important roles in ion chromatographic analysis. Concentrations of eluent competing ions are optimized by using the gradient elution retention model. It is shown that calculated optimal conditions enable very good separation within reasonable analysis time. Optimal conditions for separation are: 30 mM KOH at 0 min; 5.0 mM KOH at 5 min; step to 30 mM KOH at 10 min; 30 mM KOH 10–15 min. All components of interest are detected and baseline separated, indicating that the proposed method is a candidate for routine application. Method performance characteristics evaluation is the last step in method development procedures. Once a candidate method has been obtained, it has to be shown to meet requirements of the user. Method performance characteristics evaluation is carried out to ensure the quality of the method. It is, therefore, an essential part of any quality assurance program in the laboratory. Results show that the proposed method is characterized by superior performance characteristics (linearity $R^2 \geq 0.9991$; recovery = 99.99–100.02%; repeatability *RSD* ≤ 1.78%) and the sample preparation procedure does not influence the analysis result quality. It can be concluded that the developed ion chromatographic method can be recommended and successfully used for routine monitoring of surface water.

5. References

1. B. Malmqvist, S. Rundle, *Environ. Conserv.* **2002**, *29*, 134–153.
2. J. N. Galloway, *Environ. Pollut.* **1998**, *102*, 15–24.
3. B. Mayer, E. W. Boyer, C. L. Goodale, N. A. Jaworski, N. van Breemen, R. W. Howarth, S. Seitzinger, G. Billen, K. Lajtha, K. Nadelhoffer, D. van Dam, L. J. Hetling, M. Nosal, K. Paustian, *Biogeochemistry* **2002**, *57/58*,171–197.
4. M. Meybeck, *Hydrobiologia* **1998**, *373*, 1–20.
5. F. T. Wakida, D.N. Lerner, *Water Res.* **2005**, *39*, 3–16.
6. W. F. Sigler, J. M. Neuhold, *J. Wildlife Dis.* **1972**, *8*, 252–254.
7. J. A. Camargo, *Chemosphere* **2003**, *50*, 251–264.
8. H. Kroupova, J. Machova, Z. Svobodova, *Vet. Med. – Czech* **2005**, *50*, 461–471.
9. S. J. Hecnar, *Environ. Toxicol. Chem.* **1995**, *14*, 2131–2137.
10. P. E. Jackson, C. Weigert, C.A. Pohl, C. Saini, *J. Chromatogr. A* **2000**, *884*, 175–184.

11. J. Weiss, Handbook of Ion Chromatography, 3rd Ed., Wiley-VCH, Weinheim, **2004**, pp. XIII–XIV.
12. M. J. Moorcroft, J. Davis, R. G. Compton, *Talanta* **2001**, *54*, 785–803.
13. R. Michalski, I. Kurzyca, *Pol. J. Environ. Stud.* **2006**, *15*, 5–18.
14. P. E. Jackson, in: R. A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, **2000**, pp. 2779–2801.
15. R. Michalski, *Crit. Rev. Anal. Chem.* **2006**, *36*, 107–127.
16. P. J. Schoenmakers, H. A. H. Billiet, L. de Galan, *J. Chromatogr.* **1981**, *205*, 13–30.
17. G. Vivó-Truyols, J. R. Torres-Lapasió, M. C. García-Álvarez-Coque, *J. Chromatogr. A* **2003**, *1018*, 169–181.
18. V. Drgan, M. Novič, B. Pihlar, M. Novič, *J. Chromatogr. A* **2008**, *1185*, 109–116.
19. R. A. Shellie, B. K. Ng, G. W. Dicinoski, S. D. H. Poynter, J. W. O'Reilly, C. A. Pohl, P. R. Haddad, *Anal. Chem.* **2008**, 80, 2474–2482.
20. P. Zakaria, G. W. Dicinoski, B. K. Ng, R. A. Shellie, M. Hanna-Brown, P. R. Haddad, *J. Chromatogr. A* **2009**, *1216*, 6600–6610.
21. T. Bolanča, Š. Cerjan-Stefanović, M. Luša, Š. Ukić, M. Rogošić, *J. Sep. Sci.* **2008**, *31*, 705–713.
22. T. Bolanča, Š. Cerjan-Stefanović, M. Luša, M. Rogošić, Š. Ukić, *J. Chromatogr. A* **2006**, *1121*, 228–235.
23. P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen, L. de Galan, *J. Chromatogr.* **1978**, *149*, 519–537.

Povzetek

Delo obravnava računalniško podprto ionsko kromatografijo za analizo anorganskih ionov v površinskih vodah. Za modeliranje retencije pri gradientnih pogojih smo uporabili integralni model elucije, ki je osnovan na izokratskih eksperimentalnih podatkih. Uporabljena strategija optimizacije vključuje lastne izločitvene kriterije za rutinsko iskanje optimalnih pogojev na osnovi modeliranja retencije pri gradientnih pogojih. Na ta način smo dobili ločbo vrhov na bazni liniji v zadovoljivem časovnem oknu. Zanesljivost razvite metode smo ekstenzivno testirali s preverjanjem njenih karakteristik. Glede na rezultate ovrednotenja ima razvita metoda več kot zadovoljive karakteristike, kar dokazuje, da je računalniško podprt razvoj metode zelo koristna alternativa, še posebno pri časovno omejeni analizi površinskih vod, ki se signifikantno razlikujejo po sestavi.