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Journal of Chromatography A, 1003 (2003) 133–141
JOURNAL OF CHROMATOGRAPHY A
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Determination of nutrients in the presence of high chloride concentrations by column-switching ion chromatography

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Received 4 February 2003; received in revised form 2 May 2003; accepted 2 May 2003

Abstract

Determination of inorganic anions in waters of high salinity is one of the most difficult task in analytical chemistry. A simple column-switching method, based on an original chromatographic set-up, for the determination of nutrients (nitrate, nitrite and phosphate) in chloride rich aqueous matrices is presented. A pre-separation system (made of two in line pre-columns, Dionex AG9-HC 4 mm) connected to an analytical column (Dionex AS9-HC 4 mm) by a four way pneumatic valve, allows chloride to be eluted off into the waste and nutrients to be separated and detected by a conductimeter and/or a UV spectrophotometer. Neither chemical pre-treatment nor sample dilution are required; sample matrices presenting a large range of chloride concentrations can be investigated. Moreover by using this technology, automation for routine analysis, low analysis time and low costs can be achieved. LODs of 100, 300, 1000 μg/l for nitrate, nitrite and phosphate, respectively, have been obtained by spiking a synthetic sea water sample containing 20 000 mg/l of chloride and 3000 mg/l of sulphate. Analyte calibration curves of analytes are linear ($r>0.99$) in the range between the LODs and 60 mg/l. This method was applied to nutrients determination in sea water samples collected near a river outlet.

Keywords: Nutrients; Water analysis; Sea water; Inorganic anions; Chloride

1. Introduction

Coastal and transition waters are among the ecosystems most exposed to eutrophic hazard. Nutrient survey of these border-line ecosystems can be achieved by monitoring several parameters in many samples with variable salinity. For this type of monitoring, spectrophotometric techniques are usually employed; however, they often need sample pre-treatment and are time consuming because only one parameter at a time can be analysed.

Lower analysis time can be achieved by ion chromatography (IC) which is a common method for freshwater anion determination [1]. IC applied to sea water is difficult for three reasons: (i) chloride concentration can cause column overload; (ii) sometimes analyte concentrations in sea water are very close to IC detection limits [2]; and (iii) a broad chloride peak does not allow accurate determination of nutrients because their peaks are relatively close to chloride.

So far IC methods applied to trace analytes in sea

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00785-4

water have combined chloride removal with pre-concentration techniques [3,4]. Ag^+ cartridges for chloride elimination are commercially available [5–7]. These cartridges can cause analyte adsorption or co-precipitation and/or Ag^+ release which can block the analytical column due to the production of colloidal AgCl [8]. Pre-treatment cartridges are not viable and are expensive for routine analysis [9,10]. An on-column matrix elimination technique [11,12] can be also used in order to minimize matrix-chloride effects. The core of this method is analytical column conditioning by a specific eluent containing the interfering matrix ion. This requires an appropriate eluent for every kind of matrix and use of a non-conductivity detector. Novic et al. [13] demonstrated that, when the matrix ion is chloride and its concentration is over 50 m*M*, this technique cannot eliminate matrix effects.

Another matrix-chloride elimination technology is heart cut column switching. This technology, using a valve system, allows pre-separated chloride to elute off into the waste and switches the target ions to the analytical column. No sample pre-treatments are required [4,14–17].

This work presents a simple column switching method for the determination of nitrites, nitrates and phosphates in high chloride concentration water samples. The great advance of the method proposed is the new chromatographic set-up by which matrix chlorides are separated from nutrients thanks to a two pre-column in-line system connected to the switching valve. The method has been successfully applied to samples having a wide range of salinity.

2. Experimental

2.1. Chemicals

All used reagents were analytical reagent grade (Fluka, Milwaukee, WI, USA). Ultrapure water (resistivity>18.2 MΩ cm) was produced by a Milli-Q system (Millipore, Bedford, MA, USA). All solutions were filtered through a 0.2-μm membrane filter (GTTP 04700, Millipore). All stock solutions (50 and 5 g/l for chloride and sulphate, respectively, and 1 g/l for nitrite, nitrate and phosphate) were prepared by dissolving appropriate amounts of analyte salts in ultrapure water; the solutions were stored in the dark at 4 °C. Standard solutions were prepared daily prior to use.

All used standard solutions are listed in Table 1. Sulphate was added to all standards in order to simulate environmental water samples.

2.2. Instruments

A Dionex DX 500 equipped with two pre-columns (Dionex AG9-HC 4 mm, Sunnyvale, CA, USA) and an analytical column (Dionex AS9-HC 4 mm) was employed. The pumping system consists of two pumps: a Dionex GP 50 gradient pump and a Dionex DXP isocratic pump. Suppression was achieved with a Dionex ASRS-ULTRA micromembrane suppressor. Two detectors were used: a Dionex CD20 conductivity detector and Dionex AD20 UV absorbance detector set at 225 nm. One high-pressure four-way valve was employed for the column switching system. Measurements were performed using a 25-μl injection loop. Eluents flow rate was 1 ml/min.

2.3. Procedure

Most chloride is separated from the nutrients by the pre-column system and eluted into the waste. By switching a four-way valve, residual chloride and all nutrients are eluted in the analytical column.

2.3.1. Chromatographic system configuration

The apparatus configuration (Fig. 1) consists of a gradient pump and an injection valve connected to

Table 1
Standard solutions used

Standard solution	Ion concentration (mg/l)				
	Target ions			Matrix ions	
	Nitrite (NO_2^-)	Nitrate (NO_3^-)	Phosphate (PO_4^{3-})	Sulphate (SO_4^{2-})	Chloride (Cl^-)
A	60	60	60	500	1000
B	15	15	15	2000	10 000
C	6	60	20	500	2000
D	30	30	30	2000	4000
E	3	3	3	2000	4000
F	3	3	3	3000	20 000

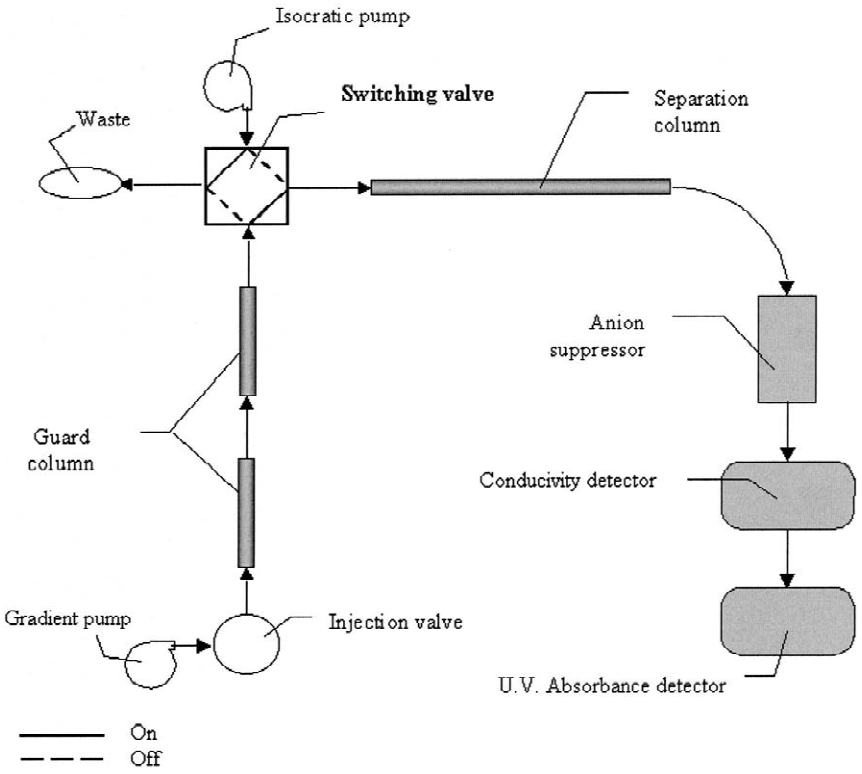

Fig. 1. Chromatographic set-up.

two pre-columns in line. The employment of two pre-columns, rather than one, allows the necessary separation between almost all matrix chlorides and target nutrients to be achieved, so that the right switching time window can be easily determined. Moreover, using two pre-columns rather than an additional separation column reduces the risk of loss of efficiency before analysis in the main column and shortens the time for this analysis. This configuration allows fast washing of impurities or chlorides eventually still adsorbed on the stationary phase. The choice of two IonPac AG9-HC columns is due to their high capacity (in comparison with other conventional guard columns such as the AG4) so preventing excess chloride from overloading them. A four-way valve is placed between the last pre-column and the analytical column. The valve is also connected to an isocratic pump which uses 9 m*M* carbonate (EB) eluent to equilibrate the analytical column. When the commutation valve is in the off position, 14 m*M* carbonate–3 m*M* hydrogene carbonate (EA) eluent flows into the two pre-columns and is discharged to waste, while the isocratic pump supplies the analytical column with eluent EB. When the valve is in the on position, the gradient pump passes to eluent EB and the anions eluted by the pre-columns are switched to the analytical column.

The detection is both conductimetric and spectrophotometric. The latter mode, set at the most suitable absorption wavelength (225 nm), allows better detection of nitrite and nitrate. Moreover the spectrophotometer permits nitrite detection in the presence of high cutting-residual chloride concentrations, while the conductimeter (which is non-specific) shows only the large chloride peak under which the nitrite signal is found.

2.3.2. Determination of cutting time and optimisation of elution conditions

In order to optimise the cutting time-window, the

pre-columns were directly connected to detectors. Chromatograms of standard solutions with progressively higher chloride concentrations (up to 20 000 mg/l) were acquired. Carbonate/hydrogencarbonate eluents of different ion strength and pH were tested. Eluent EA provided the best compromise regarding: (i) the required separation between chloride and nutrients, (ii) restraining nutrient chromatographic band width, and (iii) preserving the conductivity detector performance.

In Fig. 2 the conductimetric and spectrophotometric detector signals of two standard solutions, A and B, of Table 1 obtained with this simplified chromatographic method are reported. The chromatograms show substantial chloride elution within a defined time window (0.6–0.9 min) while target ions are not significantly eluted before 0.9 min. Taking into account these results, the most suitable cutting time in the analytical setting (Fig. 1) can be easily obtained by a trial and error procedure.

2.3.3. Experimental settings

Optimised analysis elution steps are shown in Table 2. Pre-elution is best achieved using buffer EA

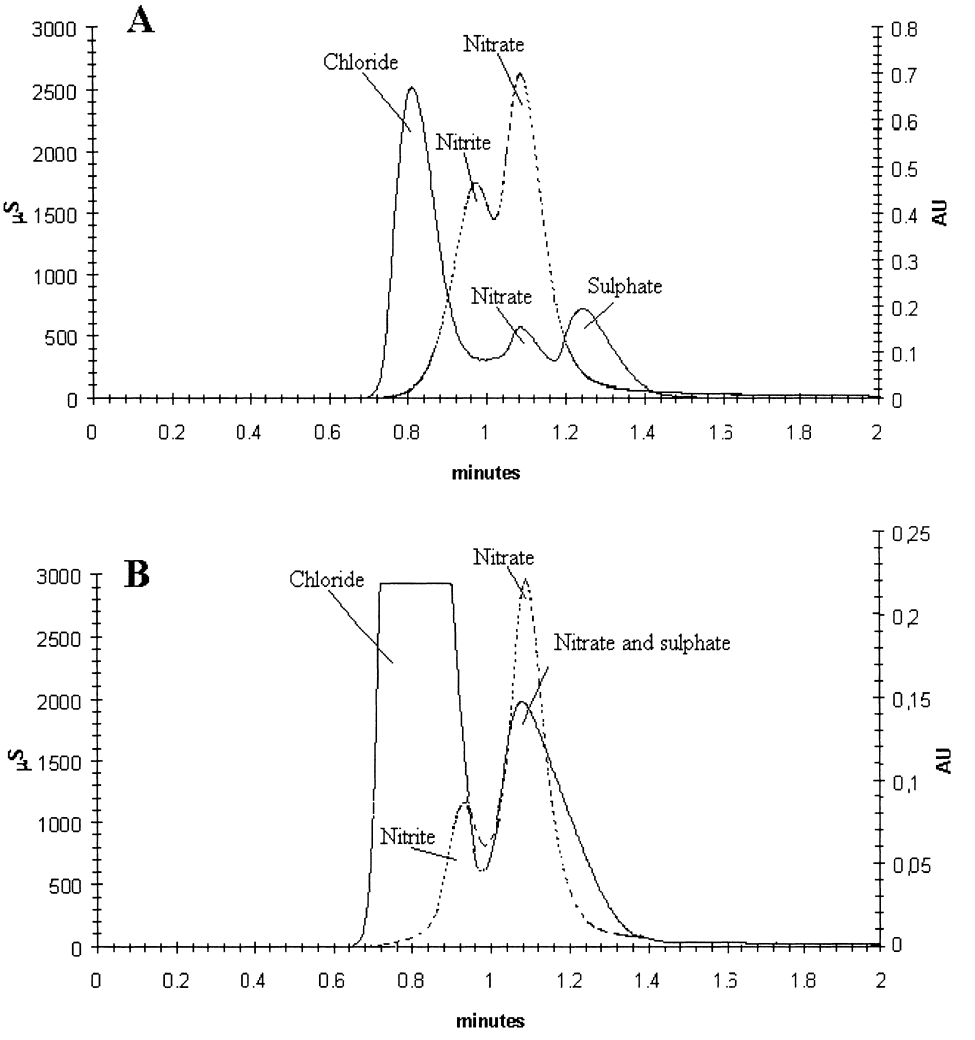

Fig. 2. Acquired chromatograms using chromatographic set-up and eluent EA. Continuous plot: conductivity detector signal (left scale). Dotted plot: UV absorbance detector signal, 225 nm (right scale). (A) Standard solution A plots; (B) standard solution B plots.

Table 2
Elution steps and experimental conditions

Time (min)	Events						
	Chromatographic device	Valve	Cutting	Guard columns	Analytical column	Gradient pump	Isocratic pump
0.0	Start acquisition data	OFF			Equilibration with EB from the isocratic pump	EA to guard columns	EB to analytical column
0.1	Sample injection	OFF	Start chloride cutting step	Pre-separation with EA	Equilibration with EB from the isocratic pump	EA to guard columns	EB to analytical column
0.6	Eluent change step: from EA to EB	OFF		Start conditioning with EB	Equilibration with EB from the isocratic pump	Instantaneous eluent change from 100% EA to 100% EB	EB to analytical column
0.9	Switch valve ON	ON	Start nutrient analysis	Target ions' elution to analytical column	Analytical run of target ions; EB from the gradient pump	EB to guard columns and analytical column	EB to waste
25.0	Start linear gradient from 100% EB to 100% EA	ON				Linear eluent change from 100% EB to 100% EA	EB to waste
30.0	Switching valve OFF; end acquisition data; ready for next run	OFF		Start re-conditioning with EA	Re-equilibration with EB from the isocratic pump		EB to analytical column

while, for analytical column elution, buffer EB is used (as Dionex recommends for AS9-HC columns). The optimised cutting time is 0.9 min. A chromatogram (solution C of Table 1), obtained with the optimised conditions, is shown in Fig. 3. The residual chloride signal is relatively low, thus confirm-

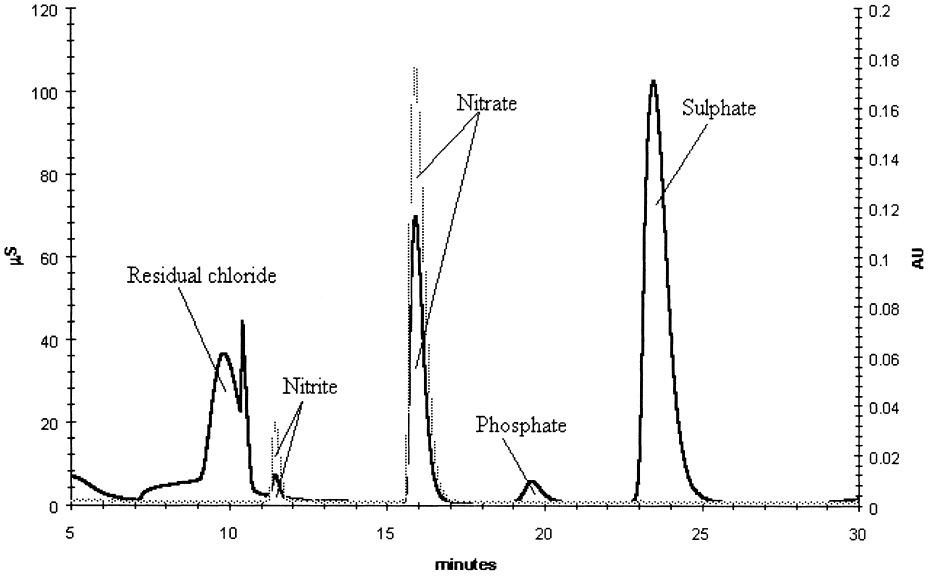

Fig. 3. Acquired chromatograms of standard solution C using chromatographic set-up shown in Fig. 1 and Table 3 settings. Black plot: conductivity detector signal (left scale). Grey plot: UV absorbance detector signal, 225 nm (right scale).

ing that it is possible to discharge most chloride to waste without compromising target ion analysis.

3. Results and discussion

3.1. Method testing on surrogate standard solutions

The described method was tested on high chloride content standard solutions (4000–20 000 mg/l); concentration of ion analytes ranged from 0.1 to 60 mg/l. Each sample was injected three times.

Fig. 4A–C shows the conductimetric and spectrophotometric signals of standard solutions D–F, respectively. Conductimetric chromatograms show asymmetric peaks due to residual chlorides and to valve switching. A linear correlation can be found between the residual chloride peak height and the sample chloride content. Moreover conductivity measurements show that nitrate and phosphate peaks are not affected by increasing chloride concentrations, while the nitrite peak is partially or entirely hidden by the chloride peak. Spectrophotometric peaks of nitrite and nitrate are well defined as shown in Fig. 4.

3.2. Relationship between sensitivity and salinity

Analyte calibration curves are linear ($r>0.99$) in the investigated range (LODs to 60 mg/l). The calibration curve slope decreases with chloride concentration (and therefore with salinity) as shown in Table 3, owing to a loss of efficiency during the pre-separation step through the pre-columns, before chloride cutting. Phosphate detection is particularly affected by high chloride concentration; this is probably due to the weak acid nature of phosphate. In fact phosphate forms a strong ion couple with hydronium ions, exchanged in the suppressor with sodium ions, thus decreasing its conductivity [4,18].

In order to reproduce real-sample salinity, each nutrient calibration was performed at four chloride concentrations: 4000, 7000, 10 000 and 20 000 mg/l. For real sample analysis, a conductivity measurement is required for determining which calibration curve has to be used. Sample salinity can also be inferred by residual chloride peak height in the chromatogram. LODs were determined for the samples of higher chloride content (20 000 mg/l).

4. Application to real matrices

The chromatogram of an analysed sea water sample is shown in Fig. 5. The sample was collected in July 2000, near the Basento river outlet (Ionian Sea, Southern Italy). The outlet is polluted by nutrients, particularly in summer due to both tourist overpopulation and hydrodynamic stagnation.

Using the calibration curves corresponding to sample salinity, 5 mg/l of nitrate were detected by the standard addition method, while nitrite and phosphate concentrations were under their LODs. Nitrite and phosphate were added to the same sample in order to reach their relative LODs (300 μg/l nitrites and 1000 μg/l phosphates). The calculated values were 342 and 852 μg/l, respectively, confirming method accuracy for a real matrix. Calibration curves obtained using this sea water sample for nitrite and phosphate were not significantly different from those obtained using surrogate samples.

5. Conclusions

The discussed method widens the ion chromatography application field, allowing nutrient analysis in high salinity matrices with simple, fast and simultaneous determination. An important goal is achieved by removing interfering matrix ions without any pre-treatment that could cause pollution risks or sample dilution. The proposed method presents a short analysis time and low cost and can be easily adapted to automatic monitoring of eutrophic sites.

Nutrient concentrations in non-eutrophic sites are usually lower than conventional IC LODs. Lower detection limits could be achieved with a better suppression system such as the new generation electrolytic suppressors which have recently become available. Furthermore, in order to increase sensitivity, a pre-concentration system could be placed beyond the cut system.

At present the main applications for this method are: surveying of eutrophic sites, monitoring of aquaculture activities and for multiple analysis of

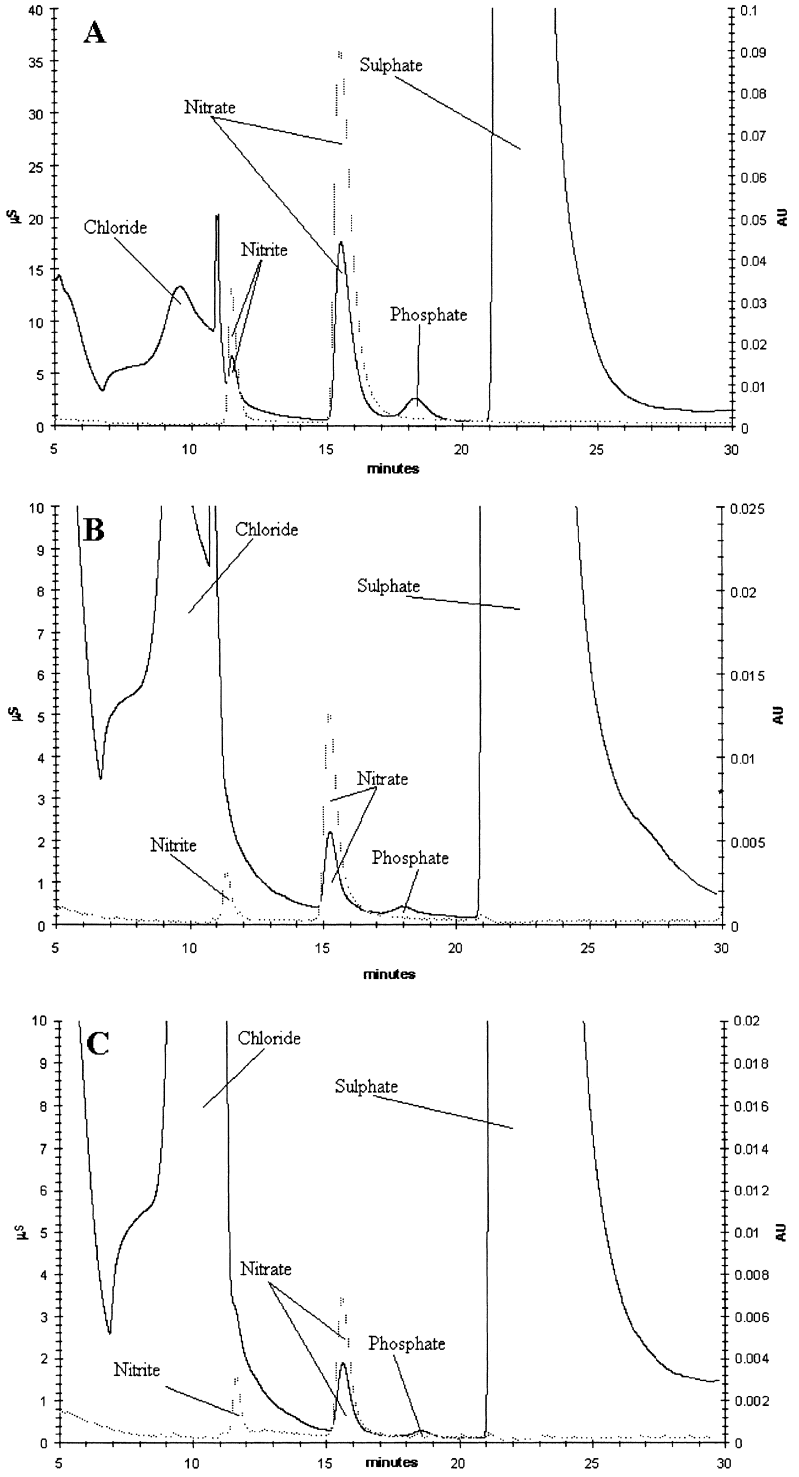

Fig. 4. Acquired chromatograms using chromatographic set-up shown in Fig. 1 and Table 3 settings. Black plot: conductivity detector signal (left scales). Grey plot: UV absorbance detector signal, 225 nm (right scales). (A) Standard solution D plots; (B) standard solution E plots; (C) standard solution F plots.

Table 3
Calibration curve slopes and correlation coefficients

Chloride content (mg/l)	Nitrite (spectrophotometric detector)		Nitrate (spectrophotometric detector)		Nitrate (conductivity detector)		Phosphate (conductivity detector)	
	Slope	*R*	Slope	*R*	Slope	*R*	Slope	*R*
4000	22 977	0.9913	141 950	0.9998	256 623	0.9953	22 977	0.9913
7000	18 890	0.9985	132 438	0.9979	218 023	0.9988	18 890	0.9985
10 000	15 992	0.9977	99 048	0.9983	168 890	0.9988	15 992	0.9977
20 000	16 799	0.9975	99 048	0.9983	98 860	0.9977	16 799	0.9975

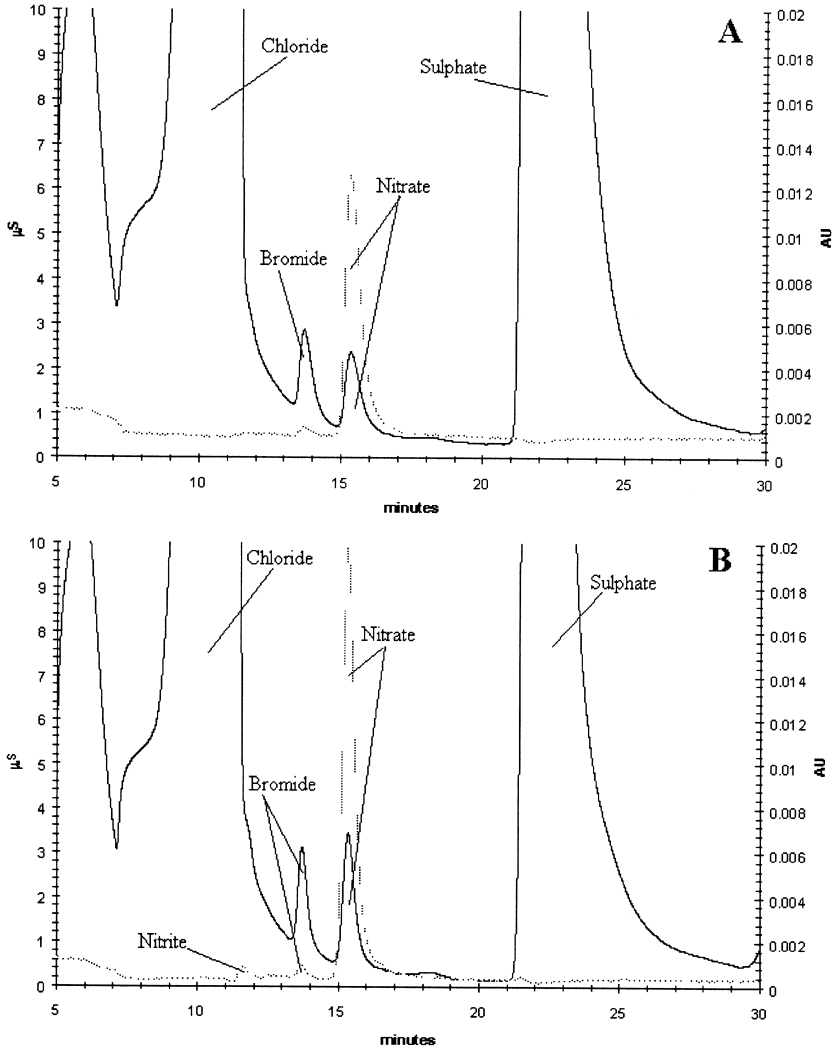

Fig. 5. Sea water sample chromatograms (collected at 30 m in front of Basento river outlet). Left scale for conductimetric black signal; right scale for spectrophotometric dotted signal. (A) Calculated amount of nitrate was 5 mg/l; nitrite and phosphate concentrations were below the LODs. (B) The same sample spiked with a nitrite, nitrate and phosphate standard solution for final concentrations of 300 μg/l, 10 mg/l and 1000 μg/l, respectively.

samples with different salinity, such as estuary or transition waters.

References

[1] H. Small, Ion Chromatography, Plenum, New York, 1982, Chapter 9.
[2] D.P. Hautman, D.J. Mumch, U.S. EPA Method 300.1, Determination of Inorganic Anions in Drinking Water by I.C. Revision 1.0, U.S. EPA, Cincinnati, OH, 1997.
[3] T.B. Hoover, G.D. Yager, Anal. Chem. 56 (1984) 221.
[4] Y. Huang, S. Mou, K. Liu, J.M. Rivielo, J. Chromatogr. A 884 (2000) 53.
[5] F.A. Buytenhuys, J. Chromatogr. 218 (1981) 57.
[6] P. Pastore, I. Lavegnini, A. Boaretto, F. Magno, J. Chromatogr. 475 (1985) 331.
[7] R.W. Slingsby, C.A. Pohl, J. Chromatogr. A 739 (1996) 49.
[8] P.R. Haddad, J. Chromatogr. 482 (1989) 267.
[9] H.S. Weinberg, H. Yamada, Anal. Chem. 70 (1998) 1.
[10] P.R. Haddad, P.E. Jackson, in: Ion Chromatography—Principles and Applications, Elsevier, Amsterdam, 1990, p. 435.
[11] A.C.M. Brandao, W.W. Buchberger, E.C.V. Butler, P.A. Fogar, P.R. Haddad, J. Chromatogr. A 706 (1995) 271.
[12] P.R. Marheni, A.R. Haddad, A.R. McIaggard, J. Chromatogr. 546 (1991) 221.
[13] M. Novic, B. Divjak, B. Pilhar, V. Hudnik, J. Chromatogr. A 739 (1996) 35.
[14] J.H. Killgore, S.R. Villasenor, J. Chromatogr. A 739 (1996) 43.
[15] S. Peldszus, P.M. Huck, S.A. Andrews, J. Chromatogr. A 793 (1998) 198.
[16] S.R. Villasenor, Anal. Chem. 63 (1991) 1362.
[17] J. Dahllof, O. Svensson, C. Torstesson, J. Chromatogr. A 771 (1997) 163.
[18] R.P. Singh, N.M. Abbas, S.A. Smesko, J. Chromatogr. A 733 (1996) 73.