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Journal of Chromatography A, 917 (2001) 353–359

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Fast separation of UV absorbing anions using ion-interaction chromatography

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Received 8 December 2000; received in revised form 19 February 2001; accepted 22 February 2001

Abstract

Ion-interaction chromatography on a short (30×4.6 mm) 3 μm ODS column has been investigated with the aim of developing fast chromatographic separations of selected inorganic anions. Tetrabutylammonium chloride (TBA-Cl) was used as the ion-interaction reagent in mobile phases that also contained up to 20% methanol. Separations of simple test mixtures of up to eight UV absorbing anions illustrated how excellent efficiencies (>50 000 plates/m) could be obtained under optimized conditions. The use of an optimised mobile phase containing 20 m*M* TBA-Cl and 20% methanol resulted in the baseline separation of five important anions (iodate, bromate, nitrite, bromide and nitrate) in a separation window of just 28 s, with a shortest total analysis time of 50 s. The method was briefly applied to the rapid analysis of nitrite and nitrate in both a drinking water and a river water sample with a view to future on-line monitoring.

Keywords: Fast ion-interaction chromatography; Inorganic anions

1. Introduction

The principle of using short liquid chromatographic columns packed with small particle size stationary phase materials with the aim of achieving rapid liquid chromatographic separations is not a new one and has been shown in several studies [1–9]. The advantages of such an approach include reduced analysis times and therefore increased sample throughput (for the purposes of this discussion we shall define fast chromatography as encompassing runs of <5 min in total), rapid method development (again due to short run times and faster column equilibration) and substantial economic benefits (both lower column cost and lower reagent consumption).

In the past, fast LC has generally been confined to reversed-phase separations of pharmaceutical compounds [1–4], peptides and proteins [5–8] and other biological macromolecules [9]. For example, Moriyama et al. [5] successfully achieved a separation of six peptides in under 1 min using a 50 mm×4.6 mm I.D. TSKgel Super-ODS column, and Kirkland et al. [9] used a rapid gradient for the separation of a mix of nine proteins in under 2 min on a 75×2.1 mm, 5 μm Poroshell 300 SB-ODS reversed-phase column.

However, the retention times in the above two studies would seem positively long when compared to those shown by Heinig and Henion [1]. Here a

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)00686-0

15×2.1 mm I.D. cartridge packed with 3 μm ODS reversed-phase particles was used to separate five benzodiazepines (previously extracted from urine samples) in under 15 s.

Such reversed-phase separations are possible due to the large range of small particle size (1.5–3 μm) silica based reversed-phase materials commercially available. Short columns packed with these highly efficient stationary phases exhibit improved resolution due to the excellent mass transfer characteristics of the smaller particles, whilst the short columns themselves generate only moderate back pressure and can still be used with conventional flow-rates.

In contrast to the above studies, the rapid chromatographic separation of small inorganic/organic ions using ion-exchange resins has to-date not received much attention. This is due to the lack of commercially available ion-exchange resins that possess the desired properties for rapid chromatographic separations. However, this problem can be overcome by carrying out ion-interaction chromatography using the above reversed-phase materials. Ion-interaction chromatography is a powerful technique that allows conventional reversed-phase columns to be used for the separation of inorganic and organic ions, with comparable efficiency and resolution to that obtained by conventional ion chromatography. A number of reviews have been published on the subject of ion-interaction chromatography of small anions [10,11], including a recent comprehensive review by Gennaro and Angelino [12].

In the following paper, ion-interaction chromatography is carried out on a short 30×4.6 mm I.D. analytical column, packed with a 3 μm particle size, ODS stationary phase, with the aim of developing fast chromatographic separations of UV absorbing inorganic ions. Optimisation of the mobile phase, which contained tetrabutylammonium chloride (TBA-Cl) as the ion-interaction reagent (IIR), was carried out to obtain the best possible resolution of test mixtures of anions in the shortest analysis times. Under optimised conditions near baseline resolution of eight inorganic and organic anions could be achieved in under 4.4 min, and five important inorganic anions could be separated in under 50 s, using only moderate flow-rates, direct UV detection and most importantly, non-specialist HPLC instrumentation. Potential application of the developed methodology is shown with the rapid determination of nitrate and nitrite in both drinking water and river water samples.

2. Experimental

2.1. Equipment

A Dionex DX500 ion chromatograph (Dionex Corporation, Sunnyvale, CA, USA), comprising of a GP50 gradient pump, LC25 chromatography oven and an AD20 absorbance detector was used. Detection was by direct UV at 225 nm. The analytical column used was a Phenomenex Hypersil, 3 μm particle size, 30 mm×4.6 mm I.D. column (Macclesfield, Cheshire, UK). The injection loop used was approximately 2 μl for the optimisation studies and increased to 50 μl for the analysis of real samples. Data acquisition was at a rate of 10 Hz with processing of chromatograms performed using a PeakNet 6.0 chromatography workstation (Dionex).

2.2. Reagents and chromatographic conditions

For preparation of the mobile phase, water used was obtained from a Millipore MilliQ water purification system (Millipore, Bedford, MA, USA), tetrabutylammonium hydroxide (TBA-OH) was supplied by Aldrich, (Aldrich, Milwaukee, WI, USA) as a 50% (w/v) solution in water, and methanol was obtained from Labscan (Labscan Limited, Stillorgan, Dublin, Ireland). The optimised mobile phase for the separation of eight anions consisted of 50 m*M* TBA-OH in 10% aqueous MeOH, titrated to pH 6.2 using dilute HCl. For the optimised separation of the five early eluting anions the mobile phase consisted of 20 m*M* TBA-OH in 20% aqueous MeOH, titrated to pH 6.2 using dilute HCl. All mobile phases were degassed and filtered using 0.45-μm filters before use. The flow-rates used were 2.5 ml/min and 2.0 ml/min, respectively. Column temperature was set at 45°C for the separation of eight anions and ambient for all other separations.

Stock standard solutions of concentration 1000 mg/l were prepared monthly and working standards prepared from each respective stock solution. Iodate, iodide, nitrite, nitrate, thiosulphate and benzoate

standards were prepared from their respective sodium salts (Aldrich, Milwaukee, WI, USA). Bromide and bromate standards were prepared from their respective potassium salts (Aldrich, Milwaukee, WI, USA). Syringe filters were used for sample pretreatment during tap water and river water analysis. Filters used were 0.45-μm nylon membrane filters from Gelman Laboratories (Michigan, USA).

3. Results and discussion

3.1. Mobile phase optimisation

One of the advantages of fast chromatographic separations is that full method optimisations can be carried out in much shorter times. In this study, two parameters were chosen for optimisation, namely the concentration of the IIR, TBA-Cl, and the concentration of the organic modifier, MeOH. The mobile pH was kept constant in these experiments at 6.2 (for the test mixture of anions investigated here, namely iodate, bromide, nitrite, bromate, nitrate, iodide, thiosulphate and benzoate, pH was unlikely to have a major effect upon selectivity in the region of 3–7). An experimental space incorporating 5–50 m*M* TBA-Cl and 1–20% MeOH was considered reasonable, with 20 mobile phase combinations prepared from within these limits. Injection of the test anion mixture was carried out using each of the 20 mobile phase preparations at a flow-rate of 1 ml/min and the resultant chromatograms were evaluated using the normalised resolution product, *R*, with the aim of selecting the optimum conditions for an even distribution of the peaks over the length of the chromatogram [13].

The independent effects both mobile phase MeOH concentration and IIR concentration have upon the retention of the test mixture of anions could be determined from the above optimisation experiments and these were typical of those expected from an ion-interaction system [14]. The first of these was a decrease in the retention of all test anions when the mobile phase MeOH concentration was increased, due to a decrease in the dynamic capacity of the column. This effect was more pronounced for the less hydrophillic anions, particularly benzoate, as secondary reversed-phase interactions were also reduced. Secondly, by varying the IIR concentration, it was found that a rapid increase in retention for all anions resulted from 0 to 5 m*M* TBA-Cl, as the dynamic ion-exchange capacity of the column increased and the surface adsorption sites become saturated. As the concentration of the IIR was increased above 5 m*M*, there was a clear levelling off in retention, followed by significant reductions in retention, particularly for thiosulphate and benzoate. Thiosulphate was particularly sensitive to TBA-Cl concentration, as selectivity changed from thiosulphate>benzoate>iodide at 2 m*M* TBA-Cl, to benzoate>thiosulphate>iodide at 5–30 m*M* TBA-Cl, to benzoate>iodide>thiosulphate at 50 m*M* TBA-Cl. In general, the trends shown are again typical of an ion-interaction mechanism, with the maximum retention being achieved at the point where the stationary phase surface becomes saturated with the IIR. After this point further addition of the IIR to the mobile phase causes a reduction in retention due to the increased concentration of the IIR counter-ion, in this case chloride.

From the above optimisation experiments, the optimum separation conditions found were 50 m*M* TBA-Cl in 10% MeOH. These conditions were found to provide the best resolution of the test mixture of eight anions in the shortest total run time of 11 min (flow-rate = 1 ml/min).

However, it was clear from the results obtained that there was an obvious difference in selectivities shown between what can be termed the more hydrophilic anions, iodate, bromate, nitrite, bromide and nitrate, and the less hydrophilic anions, thiosulphate, iodide and benzoate. Therefore, a further calculation of the normalised resolution product, *R*, for the weakly retained anions was made to determine the optimum mobile phase for their separation. Excellent resolution of the weakly retained subgroup of anions was possible when the IIR was present at 20 m*M* TBA-Cl in 20% MeOH. This resulted in the baseline resolution of iodate, bromate, nitrite, bromide and nitrate in a shortest total run time of 2 min (flow-rate = 1 ml/min).

3.2. Column temperature

The effect of column temperature was determined by varying the column oven compartment tempera-

ture from ambient (22°C) to 45°C. Once again the two subgroups of anions behaved somewhat differently, with temperature having little effect upon the retention of the weakly retained anions (including thiosulphate), but considerably shortening the retention times of the less hydrophillic anions, iodide and benzoate. This was as expected as the retention mechanism for these late eluting anions is part electrostatic interaction and part hydrophobic interaction with the ODS stationary phase (particularly benzoate). In the case of such interactions, an increase in temperature shifts the equilibrium to the mobile phase, thus reducing retention and improving efficiency. Therefore, the optimum column temperature for separation of all eight anions was chosen as 45°C. However, since no significant improvements in either resolution or analysis time were noted for the weakly retained anions, further work looking at just this subgroup was carried out at ambient temperature.

3.3. Effect of flow-rate

The effect of mobile phase flow-rate upon both efficiency and normalised resolution product, *R*, was determined. For the first five eluting anions (iodate, bromate, nitrite, bromide, nitrate), peak efficiency in terms of theoretical plate number, *N*, was calculated at 1.0, 1.5 and 2.0 ml/min using the EU standard formula, $N = 5.54\ (T_R/W_{1/2})^2$. The average number of theoretical plates for the first five anions was found to be 1848 (61 600 plates/m) at 1.0 ml/min, 2154 (71 800 plates/m) at 1.5 ml/min, and 1894 (63 133 plates/m) at 2.0 ml/min. The effect of flow-rate upon resolution of these five anions was found to be insignificant. Therefore, as efficiency was only marginally affected and resolution remained the same, a flow-rate of 2.0 ml/min was concluded optimum. Fig. 1 shows the chromatogram obtained from the injection of iodate, bromate, nitrite, bromide and nitrate, under the optimised mobile phase conditions developed earlier, at a flow-rate of 2.0 ml/min. As can be seen from Fig. 1, all five anions can be separated in a total run time of just 50 s, with the actual separation window for the five anions being only 28 s.

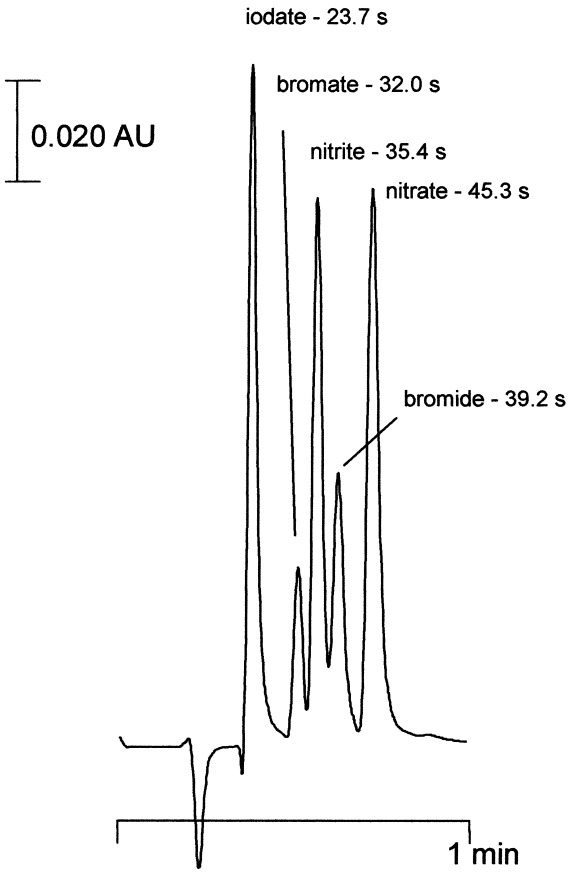

Fig. 1. Optimised separation of five inorganic UV absorbing anions, iodate (25 mg/l), bromate (100 mg/l), nitrite (25 mg/l), bromide (150 mg/l) and nitrate (25 mg/l). Mobile phase: 20 m*M* TBA-Cl; 20% MeOH, pH 6.2; flow rate: 2.0 ml/min; injection volume: 2 μl.

For the separation of the complete group of test anions a higher flow-rate of 2.5 ml/min was possible, without excessive back pressure (approx. 1500 p.s.i.), due to the increased column temperature, set at 45°C. This flow-rate once more had very little effect upon the overall efficiency or resolution of the eight anions, and actually improved the peak symmetry of iodide and benzoate. Fig. 2 shows the chromatogram obtained from the injection of iodate, bromate, nitrite, bromide, nitrate, thiosulphate, iodide and benzoate, under optimised mobile phase conditions, at a flow-rate of 2.5 ml/min. As can be seen, separation and baseline resolution of all eight anions

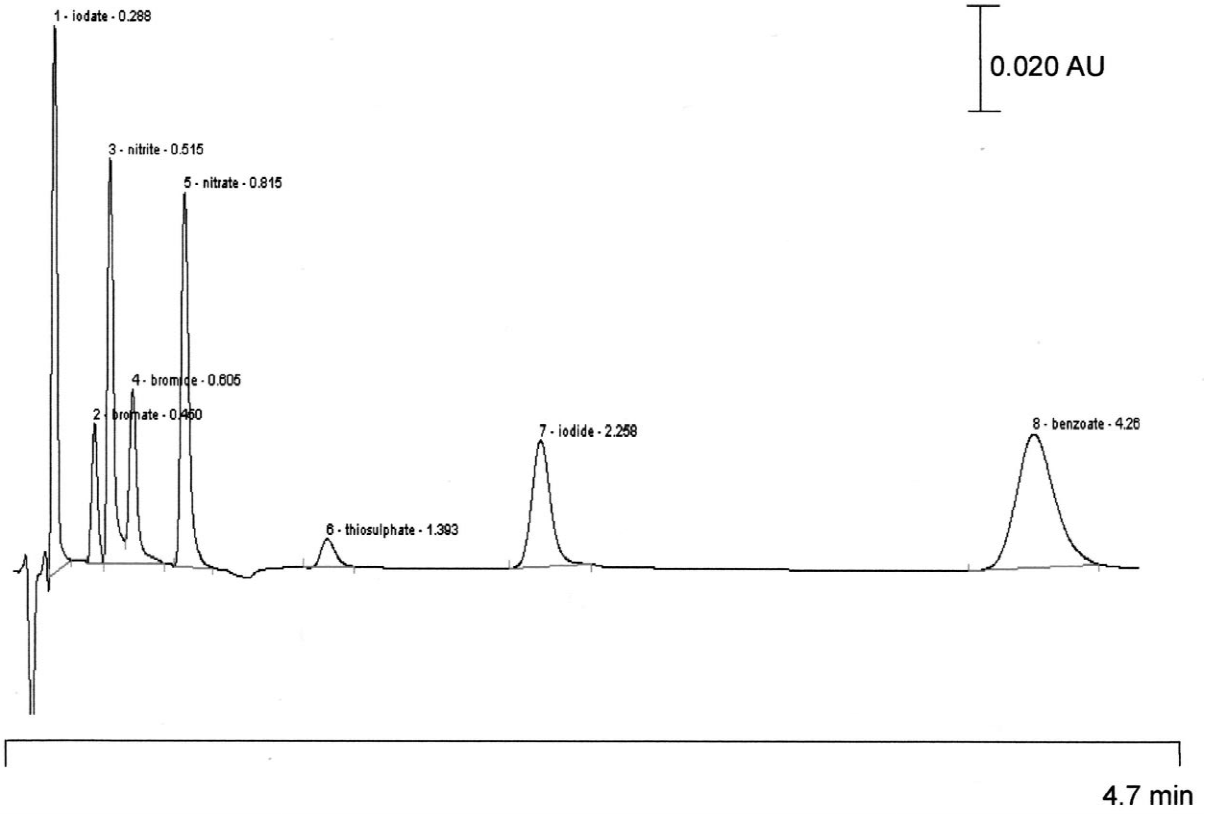

Fig. 2. Optimised separation of eight UV absorbing anions, iodate (25 mg/l), bromate (100 mg/l), nitrite (25 mg/l), bromide (150 mg/l), nitrate (25 mg/l), thiosulphate (75 mg/l), iodide (25 mg/l) and benzoate (100 mg/l). Mobile phase: 50 m*M* TBA-Cl; 10% MeOH, pH 6.2; 45°C; flow rate: 2.5 ml/min; injection volume: 2 μl.

was possible in a shortest total run time of just 4.4 min.

3.4. Speciation studies

Simple inorganic speciation methods are particularly important in the monitoring of environmental processes and also in the analysis of natural and treated waters. It was clear from the earlier experiments that the rapid separation of a number of inorganic anion pairs was possible using the developed ion-interaction method. Therefore, the conditions required for the rapid separation of nitrite and nitrate, bromide and bromate and iodide and iodate were determined. For nitrite and nitrate, and bromide and bromate, the conditions used in Fig. 1 were again considered optimum. These resulted in the separation of bromate and bromide in total run times of 42 s and the separation of nitrite and nitrate within 50 s.

A rapid separation of iodate and iodide was possible by reducing the concentration of IIR added to the mobile phase. It is clear from the chromatogram shown in Fig. 2, that iodate belonged to the subgroup of weakly retained hydrophillic anions, whereas the selectivity of the method for iodide was considerably greater. From the mobile phase studies it was known that the retention of iodide decreased sharply with a decrease in concentration of TBA-Cl in the mobile phase. Therefore, a mobile phase

containing 0.5 m*M* TBA-Cl in 20% MeOH was used to reduce the retention time of iodide, with the retention of iodate remaining unaffected. Using these conditions the separation of iodate and iodide was possible in a total run time of 40 s.

3.5. Determination of nitrate/nitrite in water samples

To illustrate how the methodology developed here has real application potential, the method was briefly applied to the determination of nitrite and nitrate in both drinking water and a local freshwater river sample. To increase method sensitivity the injection volume was increased to 50 μl. Despite this rather large injection volume, particularly for such a small column, neither peaks shapes or resolution were noticeably affected. The method was checked for linearity and found to be linear over the concentration range of interest for both nitrite and nitrate (0.5–25.0 mg/l, $R^2 > 0.999$ for both nitrite and nitrate, $N = 4$). The method also proved highly reproducible for both peak area (0.53% RSD for peak area, calculated from 30 consecutive injections of a tap water sample containing 3.9 mg/l nitrate) and retention time (0.42% RSD, calculated from same 30 injections of above sample). Detection limits were calculated using peak height equivalent to three times the baseline noise and found to be approx. 3 μg/l for nitrite and 7 μg/l for nitrate (nitrate was slightly higher due to the present of a small system peak which partially co-eluted with nitrate and made intergration difficult at concentrations <10 μg/l). However, detection limits were low enough for the method to be useful for the monitoring of drinking water and freshwater samples. Therefore, two samples, one of tap water and one from a freshwater river, were obtained, treated only to filtration (0.45-μm), and then analysed using the developed method. The resultant chromatograms from the injection of a standard and blank solution, and the two samples, both spiked and unspiked, are shown in Fig. 3. As can be seen from the figure, neither the tap water or freshwater sample matrix caused any interference with the separation of nitrite and nitrate and both anions could be readily quantified in the two samples. The tap water sample was shown to contain 3.4 mg/l nitrate, with no nitrite peak being detectable. In the freshwater river sample nitrate was present at a lower concentration of just 0.6 mg/l, but a peak for nitrite was clearly present indicating a concentration of 0.07 mg/l.

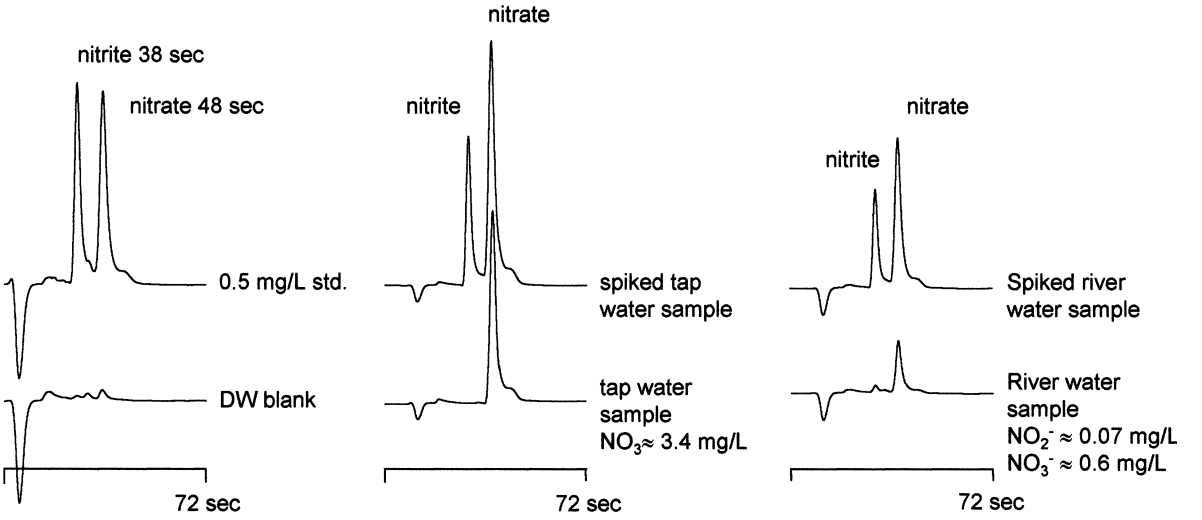

Fig. 3. Chromatograms showing the fast separation of nitrate and nitrite in water samples; de-ionised water, tap water sample and river water sample. Mobile phase: as Fig. 1; flow rate: 2.0 ml/min; injection volume: 50 μl.

4. Conclusions

In this paper, fast-chromatography, previously predominantly confined to reversed-phase separations has successfully been applied to anion analysis using ion-interaction chromatography. The separation efficiencies and resolution achieved, compare very favourably with similar separations performed on columns of more traditional length and analysis times have been significantly reduced. Possible applications include rapid screening of samples for particular anions (for example, nitrate and nitrite in drinking water) without interference from other common matrix anions. This would allow rapid on-line monitoring of flowing systems, a possibility that will be investigated in future work.

Acknowledgements

The authors would like to thank Swords Laboratories Ltd. for their financial contribution towards this project.

References

[1] K. Heinig, J. Henion, J. Chromatogr. B 732 (1999) 445.
[2] P.K. Bennett, Y.T. Li, R. Edom, J. Henion, J. Mass Spectrometr. 32 (1997) 739.
[3] T.H. Eichold, L.J. Greenfield, S.H. Hoke, K.R. Wehmeyer, J. Mass Spectrometr. 32 (1997) 1205.
[4] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[5] H. Moriyama, M. Anegayama, Y. Kato, J. Chromatogr. A 729 (1996) 81.
[6] H.M.H. Van Eijk, D.R. Rooyakkers, N.E.P. Deutz, J. Chromatogr. 620 (1993) 143.
[7] M.W. Dong, A.D. Tran, J. Chromatogr. 499 (1990) 125.
[8] N.D. Danielson, J.J. Kirkland, Anal. Chem. 59 (1987) 2501.
[9] J.J. Kirkland, F.A. Truszkowski, C.H. Dilks Jr., G.S. Engel, J. Chromatogr. A 890 (2000) 3.
[10] P.R. Haddad, A.L. Heckenberg, J. Chromatogr. 300 (1984) 357.
[11] M.L. Marina, J.C. Diez-Masa, M.V. Dabrio, J. Liq. Chromatogr. 12 (1989) 1973.
[12] M.C. Gennaro, S. Angelino, J. Chromatogr. A 789 (1997) 181.
[13] K.L. Ng, B. Paull, P.R. Haddad, K. Tanaka, J. Chromatogr. A 850 (1999) 17.
[14] P.R. Haddad, P.E. Jackson, Ion Chromatography, Principles and Applications, Elsevier Science Publications, The Netherlands, 1990.