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Short communication

Fast ion chromatography of common inorganic anions on a short ODS column permanently coated with didodecyldimethylammonium bromide

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Abstract

Fast ion-exchange chromatography has been developed and applied to the separation of common inorganic anions. Using a didodecyldimethylammonium bromide (DDAB) coated short (30 mm×4.6 mm) ODS analytical column (3- μ m particle size) and a 5 mM phthalate eluent (pH 7.5) the isocratic separation of nine common anions in 160 s was possible, with the first seven anions, including phosphate, chloride and sulphate, separated within 65 s. Detection was achieved using indirect UV at 279 nm. The high capacity, highly hydrophobic ion-exchange coating demonstrated excellent stability over time, even at elevated temperatures (45 °C) and exhibited unusual selectivity for common anions (retention order=fluoride, carbonate, phosphate, chloride, bromate, nitrite, sulphate, bromide and nitrate). The developed chromatography was successfully applied to the rapid analysis of river water and seawater samples. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

In recent years, an appreciable volume of work has been carried out in the field of so-called “fast chromatography”. What is actually meant by “fast chromatography” is to some extent up for debate, with different modes of chromatography exhibiting differing capabilities. In the case of liquid chromatographic methods, it would also seem reasonable to bracket those separations that are complete in <3 min within the category of “fast chromatography”. The advantages to be gained from reduced chromatographic

run times whilst still maintaining chromatographic efficiency and resolution are clear. These being faster analysis times, faster method development, cheaper columns, lower reagent consumption per analysis and higher sample throughput. Within the field of liquid chromatography most attention has been focused upon the reversed-phase separations of pharmaceuticals [1], biomolecules [2,3] and environmental contaminants using mass spectrometric detection [4].

However, despite the advances made in conventional LC separations, fast separations of anions by anion-exchange chromatography has not yet received much attention. This is despite the fact that the determination of common anions in natural and treated waters is one of the commonest analyses

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routinely carried out by testing laboratories. To shorten retention times, the use of shorter columns (3–5 cm) is an obvious choice. This of course necessitates a requirement for the use of a stationary phase of smaller particle size (3 μm), in order to maintain chromatographic efficiency. Recent work has focused on using ion-interaction chromatography with 3- μm ODS supports for the fast separation of inorganic and organic anions. Previous work by this research group [5] has shown that a rapid isocratic separation of a test group of UV absorbing anions was possible using a 3 cm, 3 μm ODS column and an eluent containing the ion-interaction reagent (IIR) tetrabutylammonium chloride. In this study the baseline separation of iodate, bromate, nitrite, bromide and nitrate was obtained in under 50 s, with the separation being used “on-line” in a later study to determine nitrate and nitrite in tap water, at a sample analysis rate of 60 samples/h [6].

However, the use of direct UV detection in the above studies meant detection of non-UV absorbing common anions was not possible, seriously limiting the application of the above chromatography. Therefore, the present study involved the development of a method using indirect UV detection to allow the detection of a greater range of analytes. To permit the use of indirect detection the IIR was removed from the eluent and instead the column was permanently coated with didodecyldimethylammonium bromide (DDAB). Then using a simple phthalate eluent, the chromatography was specifically developed to rapidly separate and quantify common inorganic anions in a range of water samples, with the separation of nine inorganic anions in a total run time of just 160 s.

2. Experimental

2.1. Equipment

A Dionex DX500 ion chromatograph (Dionex, Sunnyvale, CA, USA), comprising a GP50 gradient pump, LC25 chromatography oven and an AD20 absorbance detector was used. The analytical column used was a Phenomenex Kingsorb, 3- μm particle size, 30 mm \times 4.6 mm I.D. column (Macclesfield, Cheshire, UK). The injection loop used was 25 μl during optimisation studies, and 50 μl for analysis of

real samples. Detection was by indirect UV at 279 nm with data acquisition at a rate of 10 Hz and processing of chromatograms performed using a PeakNet 6.0 chromatography workstation (Dionex).

2.2. Reagents and chromatographic conditions

The water used for mobile phase and standard preparation was obtained from a Millipore Milli-Q water purification system (Millipore, Bedford, MA, USA). Before coating of the column, the new column was conditioned with 50% MeOH, supplied by Labscan (Stillorgan, Dublin, Ireland) at 1 ml/min for \sim 1 h, followed by water. The DDAB used for permanent coating of the column, was supplied by Aldrich (Milwaukee, WI, USA). The coating solution of 10 mM DDAB was filtered using 0.45- μm nylon membrane filters from Gelman Laboratories (MI, USA). The flow-rate during column coating was 1 ml/min for 30 min with the eluent absorbance monitored at 190 nm to determine breakthrough. The optimised eluent for all separations was 5 mM phthalate adjusted to pH 7.5 with dilute NaOH. The eluent was prepared from potassium hydrogen phthalate (Aldrich). The mobile phase was degassed and filtered before use using 0.45- μm nylon membrane filters from Gelman Laboratories. The flow-rate used was 2.0 ml/min with column temperature set at 45 $^{\circ}\text{C}$ for all separations.

Stock standard solutions of concentration 1000 mg/l were prepared monthly and working standards prepared daily from each respective stock solution. Phosphate, bromate and bromide standards were prepared from their respective potassium salts (Aldrich). Fluoride, carbonate, chloride, nitrite, sulphate and nitrate standards were all prepared from their respective sodium salts (Aldrich). Seawater samples were collected and stored at 4 $^{\circ}\text{C}$ until use. Seawater samples were diluted with Milli-Q water and syringe filtered prior to injection. River water samples were collected and filtered at source, and analysed within 24 h.

3. Results and discussion

3.1. Coating of column

A 10 mM solution of DDAB was prepared in

Milli-Q water, with a sonic bath used to aid dissolution. The coating solution was pumped through the column at 1 ml/min, with the column effluent directed through the AD20 absorbance detector (190 nm). This facilitated the use of the breakthrough method to determine approximate column capacity. The column was coated for 30 min, with breakthrough reached after approximately 12 min, representing ~46 mg coated DDA⁻. This corresponds to a maximum column capacity of 120 µequiv., which is high when compared to that of a standard ion-exchange column (e.g. Dionex 250×4 mm AS14= 65 µequiv./column). The column was subsequently washed with Milli-Q water to remove weakly adsorbed surfactant from the column, with the remaining coating representing a stable high capacity anion-exchange surface on the 3-µm ODS support.

3.2. Eluent concentration

The group of test anions chosen for this study were those that are typically routinely determined in both river or potable water samples, namely, fluoride, carbonate, phosphate, chloride, bromate, nitrite, sulphate, bromide and nitrate. Phthalate was chosen as the UV absorbing eluent probe. Eluent concentration was varied from 1 to 5 mM with eluents initially adjusted to pH 6.0. Throughout the optimisation of mobile phase, chromatographic conditions were held constant at a flow-rate of 1 ml/min, column temperature at ambient (~22 °C) and loop size of 25 µl. As expected, retention times decreased with an increase in eluent concentration, with 4 mM phthalate initially chosen as optimum, based on a compromise between the resolution of the test anion mix achieved, and the runtime. Elution order was fluoride (0.67 min), carbonate (0.81 min), phosphate (0.87 min), chloride (1.4 min), bromate (1.6 min), nitrite (1.9 min), sulphate (2.9 min), bromide (3.9 min) and nitrate (5.1 min). The unusual elution order observed (relative to a conventional anion-exchange resin of equivalent capacity) was presumably due to the highly hydrophobic nature of the column coating, which resulted in the weak retention of hydrophilic anions such as fluoride, phosphate etc., and the much longer retention of the less hydrophilic anions bromide and nitrate. In fact, this hydrophobic effect was such that at low eluent concentration (<2 mM), sulphate eluted between bromide and nitrate, with

the subsequent increase in eluent concentration causing a change in elution order as both bromide and nitrate were less affected by eluent concentration than sulphate.

3.3. Effect of pH

Due to the nature of the silica stationary phase, extremes of pH were avoided. Therefore, the pH of the eluent (4 mM) was simply varied from pH 6.0 to pH 7.5. At pH 6.0 the elution order of the first four eluting anions was fluoride, carbonate, phosphate, chloride, with carbonate eluting closely to phosphate at 0.9 min. Increasing the eluent pH did not significantly affect the retention of fluoride, carbonate and chloride (or indeed the later eluting anions) but phosphate could be readily removed from carbonate by an increase in eluent pH to 6.5 or above. An eluent pH of 7.5 was found to produce the best resolution of the above anions, with phosphate resolved from both carbonate and chloride.

3.4. Optimisation of column temperature

With a given mobile phase (in this case 3 mM phthalate, pH 6.0) the column temperature was increased from ambient (22 °C) to 45 °C. Retention of each anion was plotted versus temperature, resulting in little or no effect on the retention of most of the test mixture of anions except a reduction in retention for bromide and nitrate. This selective effect was most advantageous as it allowed the excessive retention of bromide and nitrate to be reduced without affecting the resolution of the earlier eluting anions. In addition, it was interesting to note that as the retention of these other anions was not affected by an increase in temperature, the stability of the column coating, even at elevated temperatures was likely being maintained.

3.5. Optimised eluent for fast anion separations

From the above studies, it was clear a phthalate eluent of pH 7.5 and column temperature of 45 °C resulted in the complete resolution of the test anion mixture. It was found that retention times could be further reduced by increasing the eluent concentration to 5 mM without significantly compromising resolution or increasing background noise. Under

these conditions at room temperature, and a flow-rate of 1 ml/min retention times were fluoride (0.69 min), carbonate (0.74 min), phosphate (1.0 min), chloride (1.4 min), bromate (1.7 min), nitrite (2.1 min), sulphate (2.3 min), bromide (4.9 min), nitrate (7.0 min). The next step was to increase the flow-rate to 2 ml/min and column temperature to 45°C, to obtain the final conditions. As the flow-rate was increased the effect upon overall peak resolution was monitored. The limiting factor was the resolution of the peaks for nitrite and sulphate, although at a flow-rate of 2 ml/min both were still completely baseline resolved. For the optimised separation of the mixed anion standard run under the conditions described above, see Fig. 1.

3.6. Rapid analysis of water samples

During optimisation of chromatographic conditions, a loop size of 25 μ l was used. However, an injection volume of 50 μ l was chosen for use with real samples, to improve detection limits. No significant reduction in peak efficiencies was observed with the larger size loop.

To illustrate an application of the developed method, seawater samples were collected for the determination of bromide. These were diluted with Milli-Q water by a factor of 10, and syringe-filtered before injection. Depending on location and sam-

pling depth (i.e. surface or sea-bed), chloride and sulphate levels can be 240 and 14 times higher than bromide ion, respectively [7]. Therefore, with conventional anion-exchange chromatographic techniques, some sample preparation may be necessary to avoid interference with bromide ion determinations. In addition, runtimes can be relatively long without the use of gradient systems to shorten the retention of the late-eluting sulphate peak. However, with the chromatography developed in this work, bromide ion can be rapidly determined (150 s) and quantified in the presence of high levels of chloride and sulphate, using an isocratic system, with minimal sample preparation. Fig. 2 is a seawater sample, overlaid with a standard. Despite the high ionic strength of the sample, bromide retention matches that in a standard very well, illustrating the unusually high exchange capacity of the column. Bromide was crudely quantified at a level of \sim 48 mg/l which correlates quite well with literature values for bromide in seawater, of 39–56 mg/l [7].

In addition, the rapid chromatography developed in this work was used to analyse river water from a sampling point known to contain significant levels of phosphate. With a phosphate retention time of only 30 s, linearity and system precision were determined in minutes rather than hours, permitting the rapid construction of standard curves for system calibration. The method quantification limit for phosphate was calculated to be of the order 0.5 mg/l, which was again suitable for the rapid screening of river water samples. The method was linear from 0.5 mg/l

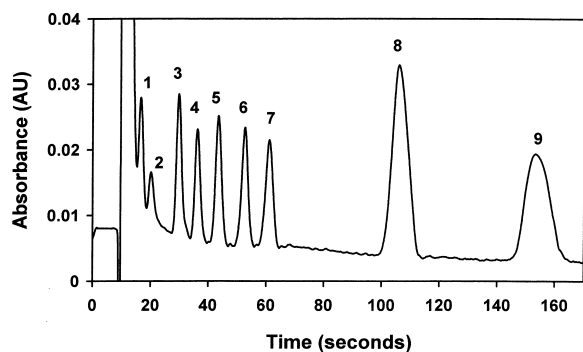


Fig. 1. Optimised separation of nine anions. Peaks: 1: fluoride (5 mg/l), 2: carbonate (12 mg/l), 3: phosphate (12 mg/l), 4: chloride (9 mg/l), 5: bromate (85 mg/l), 6: nitrite (15 mg/l), 7: sulphate (14 mg/l), 8: bromide (44 mg/l), 9: nitrate (56 mg/l). Chromatographic conditions: mobile phase: 5 mM phthalate, pH 7.5. Flow-rate: 2 ml/min. Column temperature: 45°C. Loop volume: 50 μ l.

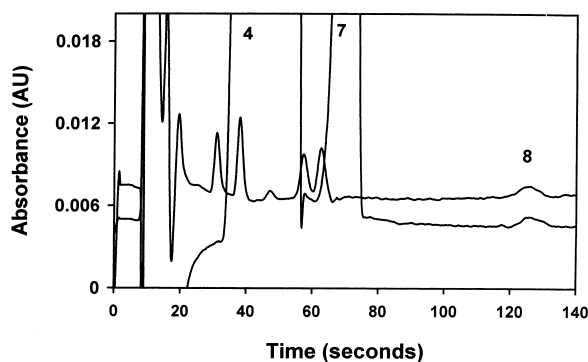


Fig. 2. Analysis of seawater (diluted 1:10) for bromide ion. The upper trace is an overlay of a standard mix solution. For chromatographic conditions, see Fig. 1.

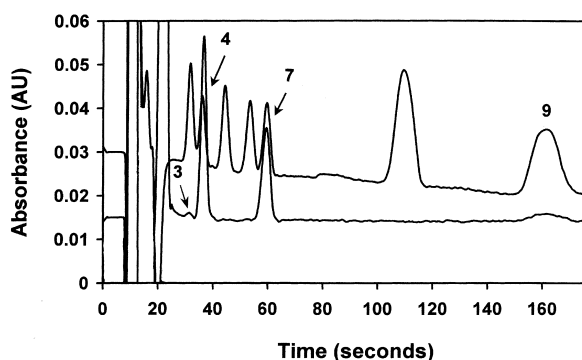


Fig. 3. Analysis of river water for phosphate. The upper overlay is a standard mix solution. For chromatographic conditions, see Fig. 1.

up to at least 100 mg/l ($n=6$, $R^2=0.9997$), with a system precision determined at %RSD=0.98 for $n=8$ consecutive injections of a 5 mg/l phosphate standard. For the analysis of the river water sample, nine repeat injections of the sample were carried out and phosphate levels determined to be 0.64 mg/l. Fig. 3 shows an overlay of the river water sample with a standard.

4. Conclusion

A simple rapid chromatographic method has been described for analysis of nine common anions in <180 s, using a short 3-cm analytical column

permanently coated with didodecyldimethylammonium bromide, and a phthalate eluent for indirect UV detection. The potential application of the developed method has been illustrated with analysis of natural water samples, with the column performing particularly well with samples of considerable ionic strength, due to its high exchange capacity.

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