Robert A. Shellie[1]
Éadaoin Tyrrell[1]
Christopher A. Pohl[2]
Paul R. Haddad[1]

[1]Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Hobart, Australia
[2]Dionex Corporation, Sunnyvale, CA, USA

Original Paper

Column selection for comprehensive multidimensional ion chromatography

This paper discusses the selection of ion chromatography (IC) columns for use in comprehensive multidimensional ion chromatography (IC × IC). First, a single number was determined for a wide range of anions (one number for each anion) using the linear solvent strength model. These numbers were then used to compare the column selectivity characteristics for five different columns. Principal component analysis was used to illustrate selectivity differences between columns. Dionex AS16 and AS20 columns were selected for use in the development of an IC × IC method for the separation of ten anions. To achieve the required speed of analysis in both the first and second separation dimensions, custom column lengths were packed in-house. The use of an eluent suppressor between the first and second columns permits a relatively low flow ratio regime of only <1:20 in the first and second dimensions, respectively, which reduces dilution effects common in comprehensive multidimensional LC. Selection of the second dimension eluent conditions was aided by the development of a spreadsheet based on the linear solvent strength model.

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1 Introduction

There are several fundamental factors which need to be considered in the development of comprehensive 2-D separations. First, the separation selectivity of the separation columns used in each separation dimension should be different, in fact it is often stated that the first and second dimensions in comprehensive multidimensional chromatography should exhibit *orthogonal selectivity*, but a better definition is *that the elution times in the two dimensions can be treated as statistically independent* [1]. In the present study, we wish to focus on the development of a comprehensive 2-D ion chromatography (IC × IC) system. It is not immediately obvious that a 2-D separation in which both separation dimensions involve ion-exchange could satisfy the above selectivity criterion, but even subtle differences in selectivity can be used to advantage, and an automated 2-D heart-cutting column concentration and matrix elimination IC technique has been very successfully applied for perchlorate analysis [2, 3]. The key to developing an IC × IC approach is to identify maximal differences in selectivity among the available stationary phase materials. In this study, we base our column selection approach on the work of van Gyseghem *et al*. [4] who measured retention factors for 68 pharmaceutical compounds using 11 different chromatographic systems and used chemometrics to determine orthogonal chromatographic systems for pharmaceutical analysis [4].

The second important consideration is the ability to perform high-speed separations. This is a critical requirement for any comprehensive multidimensional separation because the second dimension column needs to provide rapid elution to permit contiguous injections into this column. If the separation window of each second dimension analysis exceeds the frequency of injections then the second dimension chromatograms will be intermingled and this will lead to nonsensical results, so each second dimension separation needs to be completed before injection of the next fraction of the first dimension effluent. The maximum acceptable second dimension analysis time is related to the width of peaks in the first dimension column. For some years it was considered that there should ideally be three to four second dimension separations for each peak in the first dimension to ensure that the separated peaks remained resolved throughout the entire process [5]. Recently Marriott and coworkers [6] introduced the term modulation ratio (M_R), which describes the sampling rate of the first dimension

Correspondence: Professor Paul Haddad, Australian Centre for Research on Separation Science (ACROSS), School of Chemistry, University of Tasmania, Private Bag 75, Hobart 7001, Australia
E-mail: paul.haddad@utas.edu.au
Fax: +61-3-6226-2858

separation. The modulation ratio is defined as the ratio of four times the first column peak SD divided by the modulation period. It has been shown that for the analysis of trace components where precise quantitative measurements are being made the comprehensive multidimensional separation should be conducted with $M_R \geq 3$ [6]. For the analysis of abundant solutes, or for semiquantitative analysis, an $M_R \sim 1.5$ is sufficient [6]. Thus, even for semiquantitative analysis, the time of the second dimension separation must be less than about 2/3 of the base width of each first dimension peak. This speed requirement is a major obstacle in comprehensive multidimensional LC. A few specialised studies have employed stopped-flow operation of the first dimension separation to circumvent the need for rapid elution from the second column, but this leads to a major increase in total analysis time. The use of high-speed elution columns for the second dimension is the most usual approach.

Recently, we developed an approach to produce short IC columns specifically for high-speed separations [14]. These short separation columns offered approximately a threefold higher sample throughput for a set of seven analytes, including bromate, chloride, sulphate, chlorate, nitrate, chromate and perchlorate compared to optimised separations performed using commercial columns. Under isocratic conditions, efficiency values of *ca*. 45 000 N/m for chloride were recorded using home-packed 30 mm × 4 mm id IC columns, which compared very favourably with a commercial 250 mm × 4 mm id AS20 column (55 000 N/m). Since these short, home-packed columns can be produced reproducibly and give consistent performance over extended periods of usage they are ideally suited for use as second dimension columns in an IC × IC system.

2 Experimental

2.1 Instrumentation

A Dionex ICS-3000 ion chromatographic instrument with conductivity detection controlled using Chromeleon® software (version 6.80) was used for all analyses and all the instrumental components were obtained from Dionex (Sunnyvale, CA, USA). Separations were carried out using polymeric Dionex AS20 (30 mm × 4 mm id) and AS16 (250 mm × 2 mm id and 62.5 mm × 2 mm id) analytical columns and suppressed conductivity detection was used to monitor the eluted analytes. Custom columns were prepared according to an approach which is described elsewhere [14]. The IC system used two reagent-free eluent generators each with an EGC II KOH cartridge to generate potassium hydroxide eluent of the required composition for isocratic or gradient separations. A continuously regenerated anion trap column was employed to remove trace contaminants from the eluent. Postcolumn eluent suppression was carried out using an anion self-regenerating suppressor. The IC system was fitted with a 25 μL sample loop that was used to introduce the sample *via* an AS autosampler. Chromatographic data were collected at 20 Hz and chromatograms were processed using the Chromeleon software. The modulation interface was constructed using one of the two ten-port switching valves located in the Dionex automation manager unit fitted to the ICS-3000 instrument. The valve was configured in the symmetrical mode described by van der Horst and Schoenmakers [7]. A more detailed description of the modulator is given in Section 3.

2.2 Methods

Characterisation of the columns was performed using isocratic elution conditions using monodimensional chromatography at 30°C. The 2 mm id AS16 and 4 mm id AS20 columns were operated using a range of eluent flow-rates and separations of nitrate were performed using a 10 mM KOH eluent so that van Deemter plots for the columns could be constructed. The IC × IC experiment was performed using a gradient in each separation dimension. The flow-rate in the first dimension column was 80 μL/min and the KOH eluent was programmed from 1.0 to 31 mM in 38.4 min, starting 2.6 min after injection and was then held constant at 31 mM for 1.6 min. The first dimension column was housed in the lower chromatography compartment in the ISC-3000 instrument and was thermostatically controlled at 23°C. The flow-rate in the second dimension column was 1400 μL/min and the KOH eluent was programmed from 2.0 to 7.0 mM in 5 min starting 7.6 min after injection; 7.0 to 9.2 mM in 1.4 min; 9.2 to 50 mM in 27 min and was then held constant at 50 mM for 1.6 min. The second dimension column was housed in the upper chromatography compartment in the ISC-3000 instrument and was thermostatically controlled at 30°C. Modulation was controlled by the Chromeleon software which caused valve actuation every 40 s starting at 3.0 min.

2.3 Reagents

All chemicals used were of analytical reagent grade and were used as supplied by Sigma – Aldrich (Sydney, Australia) unless stated otherwise. The eluent and standard solutions were prepared using deionised 18.2 MΩ water from a Millipore Milli-Q water purification system (Bedford, MA, USA). Working standards used for IC × IC evaluation were prepared from 1000 mg/L stock standard solutions. The chloride, chlorate, fluoride, nitrate, perchlorate, phosphate and thiocyanate standard solutions were all prepared from their respective sodium salts, while the standard solutions of bromate, sulphate and chromate were prepared from their potassium salts.

2.4 Chemometrics

Isocratic retention data acquired using three different eluent concentrations and three different temperatures (23, 30, 35°C) using five different stationary phases had been collected previously (Private communication, Dionex Corporation, Sunnyvale, CA, 2005) for acetate, acrylate, arsenate, azide, benzenesulphonate, benzoate, bromate, bromide, bromoacetate, butanesulphonate, butyrate, carbonate, chlorate, chloride, chlorite, chloroacetate, chromate, *cis*-aconitate, citrate, dibromoacetate, dichloroacetate, difluoroacetate, ethanesulphonate, fluoride, fluoroacetate, formate, fumarate, glutarate, glycolate, heptanesulphonate, hexafluorophosphate, hexanesulphonate, iodate, iodide, iso-citrate, lactate, malate, maleate, malonate, methacrylate, methanesulphonate, molybdate, monofluorophosphate, *n*-butyrate, nitrate, nitrite, *n*-valerate, octanesulphonate, oxalate, *p*-chlorobenzenesulphonate, pentanesulphonate, perchlorate, perrhenate, phosphate, phthalate, propanesulphonate, propionate, pyrophosphate, pyruvate, quinate, selenate, selenite, selenocyanate, sorbate, succinate, sulphate, sulphite, tartrate, tetrafluoroborate, thiocyanate, thiosulphate, trans-aconitate, tribromoacetate, trichloroacetate, trifluoroacetate, trimetaphosphate, tripolyphosphate and tungstate. A table of retention factors for these anions was constructed for PCA using XLSTAT 2006 Version 2006.3 (Addinsoft, New York, NY) to investigate column selectivity differences.

2.5 Column packing procedures

The column packing procedure has been described elsewhere [14] in short, a slurry mixture was prepared using 15% w/v of packing material in a slurrying solvent consisting of appropriate volumes of acetic acid, ethylenediamine and PEG mono(nonylphenyl) ether in deionised water. The slurry was stirred for 10 min, and then placed in an ultrasonic bath for 10 min, followed by a further 10 min of stirring, after which it was poured into the reservoir of the packing assembly that was connected to the column bodies. Four empty PEEK column bodies were joined end to end using stainless steel unions to form a longer column so that the packed bed could be divided into shorter analytical columns after the packing process was complete. A Haskel 40102 air driven amplification pump (Haskel, Brisbane, Australia) used in conjunction with a standard cylinder of air was employed to pack the columns at 4000 psi with Milli-Q water used as the driving solvent. A retaining frit was placed at the outlet of the final column body to hold the stationary phase packing material in place and allow the driving liquid to pass through. After the packing process was completed, the pressure within the columns was allowed to dissipate (~1 h) and then the column bodies were separated and a porous retaining frit was placed at both ends of each prior to capping with suitable end-fittings.

3 Results and discussion

3.1 Column selection for IC × IC

In our first investigation of IC × IC, we have restricted column selection to hydroxide-selective anion-exchange columns. The primary reasons for this relate to the routine use of electrolytic eluent generators in which water used as eluent is converted *via* an electrolysis step into the desired potassium hydroxide eluent and the use of a suppressor in which the eluent is converted back to water prior to the conductivity detection step. The columns investigated in this study included Dionex AS11-HC, AS16, AS18, AS19 and AS20 columns. A summary of each column's characteristics is given in Table 1 (Hydroxide-selective anion columns, Dionex Corporation, Sunnyvale, CA, 2008, http://www1.dionex.com/en-us/columns_accessories/ICcols/hydrox/lp60634.html). In order to classify these columns by using a PCA approach, it was first necessary to establish a reliable method to quantitatively compare the selectivity of a range of IC columns. Fortunately, retention behaviour in IC is very well understood and the retention factor (k) of an analyte anion in IC under isocratic conditions is described by the linear solvent strength model [8]. We have recently shown that the linear solvent strength model can be used to predict analyte retention in ion chromatography separations performed using elution profiles comprising multiple isocratic and gradient steps [9].

In anion exchange IC a plot of log k *versus* log $[E^{y-}]$ is linear for isocratic separations, with the intercept, a, and the slope, b, being determined by only a few chromatographic parameters.

Figure 1 shows the log k *versus* log $[OH^-]$ plot for iodide for separations performed using three eluent concentrations with Dionex AS11-HC, AS16, AS18, AS19 and AS20 IC columns. Estimates of the a and b values for iodide were made using these data. The mean and RSD of the a value was 229 (69%), and the mean and RSD of the b value was 1.0 (1.6%). As theory predicts, the b values are very similar across all columns. The effective charge on both the analyte and the eluent are not affected by changing the column, and the b value has no value in this study as a quantitative classifier of column selectivity. However, the a values for each column differ markedly and this value is potentially ideally suited as a quantitative classifier of column selectivity. Thus, the a values were calculated for a large set of anions using each of the columns available for the study. Data were available at three different column compartment temperatures (23, 30 and 35°C). Principal component analysis was performed on these data and the PC scores plot for the first and second

Table 1. Stationary phase characteristics of the columns investigated for selectivity differences in this study

Column	Ion exchange group	Functional group characteristics	Column capacity (μequiv./col)
AS11-HC	Alkanol quaternary ammonium ion Latex crosslinking: 6% Latex diameter: 70 nm	Medium low hydrophobicity	290
AS16	Alkanol quaternary ammonium ion Latex crosslinking: 1% Latex diameter: 80 nm	Ultralow hydrophobicity	170
AS18	Alkanol quaternary ammonium ion Latex crosslinking: 8% Latex diameter: 65 nm	Low hydrophobicity	285
AS19	Alkanol quaternary ammonium ion	Low hydrophobicity	240
AS20	Alkanol quaternary ammonium ion	Ultralow hydrophobicity	310

All columns were of 250 mm × 4 mm id format.

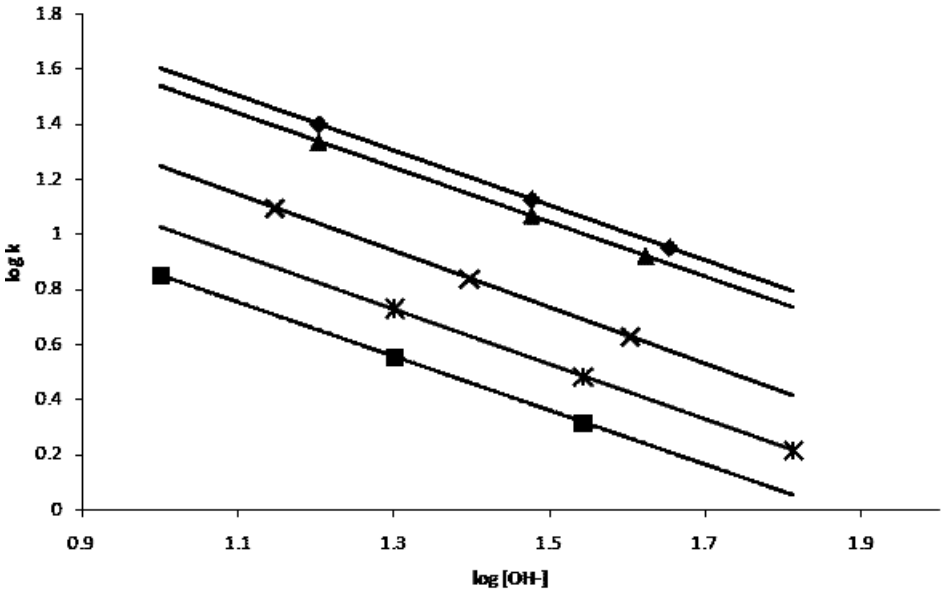

Figure 1. Log *k versus* log [OH^-] plot for iodide using Dionex AS11-HC ♦, AS18 ▲, AS19 ×, AS20 * and AS16 ■ columns.

principal components is shown in Fig. 2. We have recently used short AS20 columns for fast IC [14], so these were a logical choice for investigation of suitability as second dimension columns in the IC × IC experiment. From the PCA scores plot, it is apparent that AS11-HC or AS18, or AS16 are potential candidates for use as the first dimension column. The *a* values were compared column by column and correlation coefficients of 0.81, 0.91 and 0.83, respectively (at 30°C). From the PCA scores plot, AS19 is apparently most similar to the AS20 column, and the correlation coefficient for *a* values was calculated to be 0.98 (at 30°C) for this column pair. While van Gyseghem *et al.* [4] concluded that "the interpretation of PCA plots to determine the relationships between the systems, was not always found to be straightforward" using retention factors to characterise the columns, but the inference of column selectivity differences from the PCA scores plot is quite clear in IC when the *a* values are used to quantify selectivity. The *a* value incorporates the ion-exchange selectivity coefficient (as well as ion exchange capacity, and phase ratio) and therefore, reflects selectivity on that particular stationary phase. The ion exchange selectivity coefficient could be deconvolved from this *a* value by careful experimentation, but the results show that this was unnecessary in the present investigation. In the present investigation, we specifically wanted to include the polarisable anion perchlorate, and AS16 was selected as the most suitable stationary phase out of the three candidates for this reason.

The selection of columns with appropriate selectivity difference is only the first part of the column selection process. Next one needs to determine the order that

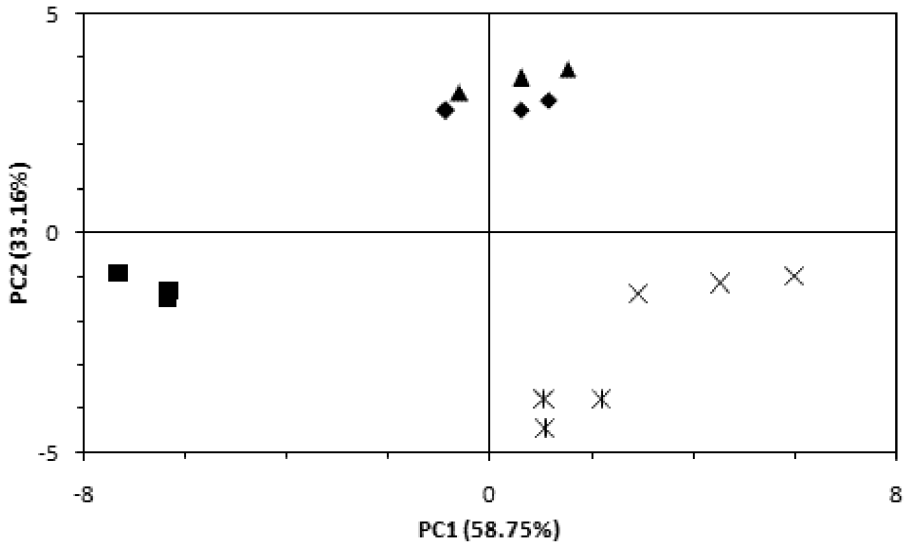

Figure 2. PC scores plot of *a* values. Dionex AS11-HC ♦, AS18 ▲, AS19 ×, AS20 * and AS16 ■ columns.

these two stationary phases should be used in the multidimensional system and the eluent conditions also need to be optimised. There are several distinct possibilities for performing the first and second dimension separations in this IC system. The potassium hydroxide eluent in each separation dimension is electrolytically generated and it is possible to run each dimension in isocratic elution mode, in ramped eluent concentration mode, or it is possible to program a multistep eluent profile in each separation dimension. Ikegami *et al.* [10] showed that a gradual gradient in the second dimension column has real benefits. Rather than program an individual gradient for each second dimension separation, they increased the second column eluent strength using a linear gradient over the duration of the first column separation time. Since the *a* and *b* values can be used to predict analyte retention in IC using isocratic or programmed gradient elution [9], a spreadsheet was developed in Microsoft Excel using the theory described in ref. [9] to give an estimate of the utilisation of the 2-D separation space when these two columns are employed. In this instance the first dimension retention times were manually entered into the spreadsheet to expedite method development of only the second dimension eluent profile. However, it is not necessary to know the retention times of the first dimension separation in advance because these retention data can be predicted easily [9]. Figure 3 illustrates a typical output from the spreadsheet showing the predicted utilisation of the separation space from the coupling of an AS16 first dimension column to an AS20 second dimension column. The alternate arrangement (*i.e.* with the columns reversed) is not shown here, but utilisation of the separation space was far less optimal than this arrangement. This spreadsheet also provides insight into suitable eluent profiles for the IC × IC experiment. The second dimension eluent profile can be changed in the spreadsheet to predict the effect on second dimension retention. Only suitable 'hits' are then tried in the IC × IC instrument. These conditions can be used as a starting point for the IC × IC method and fine-tuning performed manually to maximise resolution of critical pairs. In the present investigation, the final eluent profile was selected using iterative optimisation.

3.2 Preliminary separations

Eluent flow-rates in both the first and second dimensions are very important considerations for proper operation of a comprehensive multidimensional separation. These are intimately linked to the size of the sample loops used in the modulator and are therefore also strongly correlated with the modulation frequency. Several recent studies have utilised a narrow-bore first dimension column comprehensively coupled with a fast elution (high flow-rate) second dimension column [11]. This arrangement is favourable for multidimensional LC systems, where the two dimensions are operated in normal phase and RP mode, because the introduction of large volumes of an incompatible solvent yields broadened and distorted peaks [12]. The use of a microbore column in the first dimension permits the injection of a small volume onto the secondary column, making the transfer of incompatible solvents from the first to the second dimension possible without significant peak shape deterioration or resolution losses [13]. A high-flow second dimension column operated in gradient elution mode leads to effective peak focusing in the second dimension column. Dugo *et al.* [13] used a flow ratio of 1:200 in the first and second dimensions, respectively. Unfortunately, this large flow difference resulted in dilution and therefore increased LODs. In IC × IC with KOH eluents, a suppressor is placed between the first dimension column

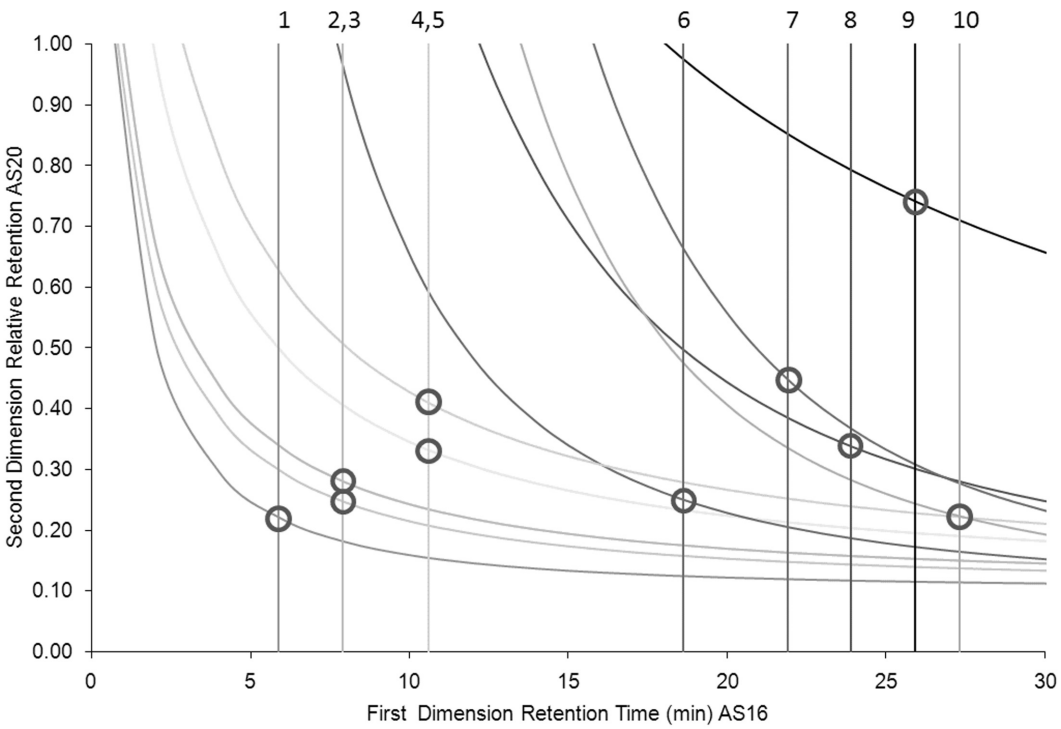

Figure 3. Peak trajectory plot for the IC × IC separation of nine anions. The vertical lines represent the positions of peak maxima in the first dimension separation. A slow gradient is applied in the second dimension column, so each second dimension separation is essentially performed under pseudo isocratic conditions. The curved lines represent analyte retention in the second dimension column at the analysis time dependent pseudo isocratic concentration. The point where these lines intersect (marked with a circle) represents the position in the 2-D separation space where these analytes are predicted to be eluted. Peak identities are (1) fluoride, (2) bromate, (3) chloride, (4) chlorate, (5) nitrate, (6) sulphate, (7) thiocyanate, (8) chromate, (9) perchlorate, (10) phosphate.

and the modulator, so the extremely different flow-rates are not necessary. The flow ratio in the present investigation was <1:20 in the first and second dimensions, respectively. Peak focusing is achieved because the analytes are rapidly injected as a narrow sample plug into the second analytical dimension dissolved in water, which is a very weak ion-exchange eluent so the analyte ions accumulate onto the head of the second dimension column before being eluted with the KOH eluent. We initially attempted a series of separations without the intermediate suppressor, but it was impossible to obtain the suitable efficiency on the second dimension column.

A 2 mm id column was chosen for the first dimension separation in the present investigation and an initial characterisation of the column was performed by constructing a van Deemter curve (Fig. 4). 2 mm id columns packed with the same stationary phase material as 4 mm id columns have different capacity and phase ratio and analyte retention is not exactly the same, but retention factors are strongly correlated between these columns (Fig. 5) so the generalised selectivity differences described above are maintained. The recommended flow-rate for a 2 mm id AS16 column is 250 μL/min, but we found that the optimum flow-rate is significantly lower (80 μL/min; u = 0.43 mm/s). This optimum flow-rate determines that the modulation loop volumes should be 80 μL for 60 s modulation period or 40 μL/min for a 30 s modulation period. However, operating the commercial 250 mm × 2 mm id column at optimum flow-rate led to excessively long analysis time. The void time of the 250 mm × 2 mm id column operated at 80 μL/min was 10 min. To bring the analysis time in line with monodimensional IC separations, the first dimension column was shortened by 75%. Thus, a 62.5 mm × 2 mm id column was packed according to the procedure described in ref. [14]. The column was coated with AS16 functionalised latex nanoparticles immediately after the packing procedure. Equivalent plate heights were achieved using the long commercial columns and the short custom columns.

Initially a short 2 mm id second dimension column was used, but the sample volume (40 μL) was apparently too large for the column and results were unsatisfactory, but this problem was alleviated by using a short 4 mm id column in the second dimension, which permitted a greater injection volume. The column dimensions were

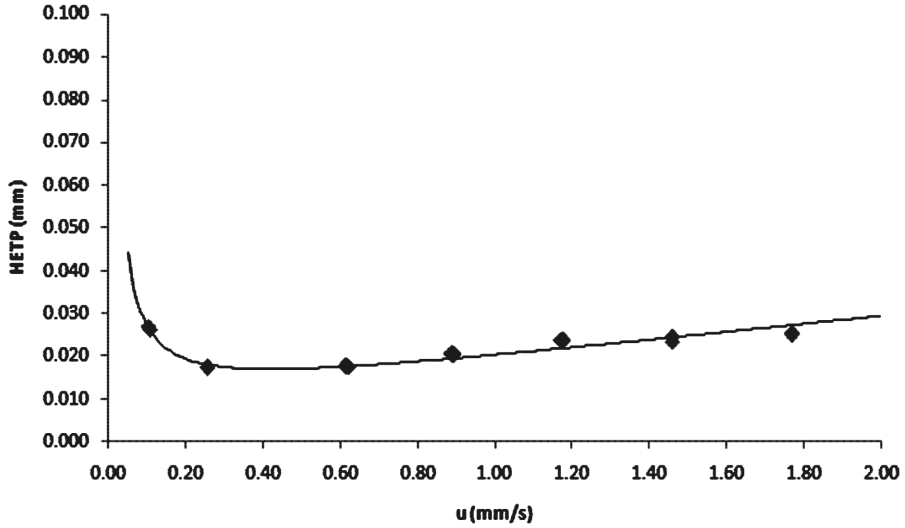

Figure 4. Van Deemter plot for the 250 mm × 2 mm id AS16 column.

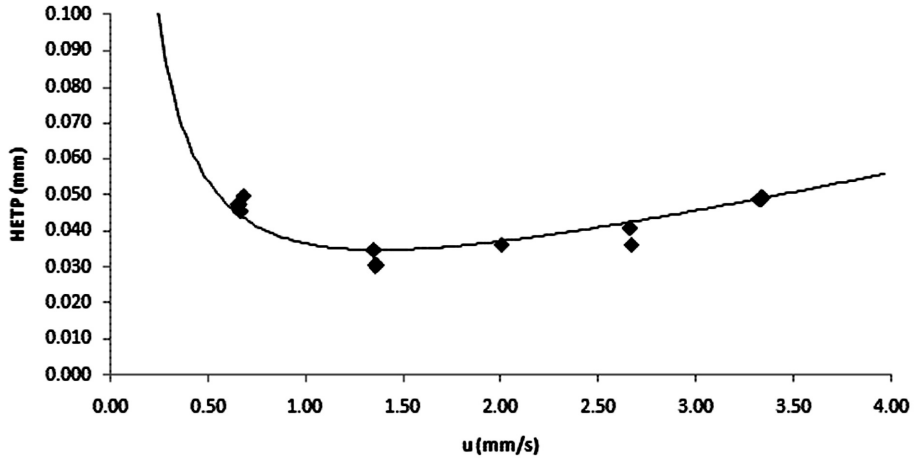

Figure 5. van Deemter plot for the 30 mm × 4 mm id AS20 column.

30 mm × 4 mm id and it was initially operated at its optimum flow-rate of 1000 μL/min (u = 0.99 mm/s), but as the van Deemter curve for this column shows (Fig. 5), it is possible to increase the column flow-rate to 1400 μL/min (u = 1.39 mm/s) to speed up the separation without significantly reducing column efficiency. This higher flow-rate was therefore used for all IC × IC analyses. The performance of short custom AS20 columns like those described here, compared with commercial columns is discussed in more detail in ref. [14].

3.3 Instrument setup

Having determined ideal column dimensions and eluent flow-rates, the volume of the modulator sample loops could be set. An optimal modulation period of 40 s was reached by iteration, with modulator sample loops of 50 μL. The IC × IC setup is illustrated in Fig. 6, showing the positions of the columns and the two electrolytic suppressors as well as the configuration of the ten-port valve.

3.4 Application

The IC × IC separation of a mixture of ten anions is shown in Fig. 7. The first thing to notice is the remarkable similarity in peak elution order in the second dimension column compared to the predicted result in Fig. 3. The 2-D peak shapes for most analytes are highly satisfactory, although some of the analytes (peaks 6, 8 and 10) appear as downward sloping spots in the 2-D separation space. This odd peak shape is caused by the increasing eluent strength produced by operating the second dimension column with a gradual gradient. This is most obvious for the sulphate anion (peak 6). The retention of the divalent sulphate anion is affected strongly by changes in eluent strength, and this is reflected by a steep slope in the log k *versus* log [OH^-] plots. Figure 8 shows an expanded region of 2-D separation showing

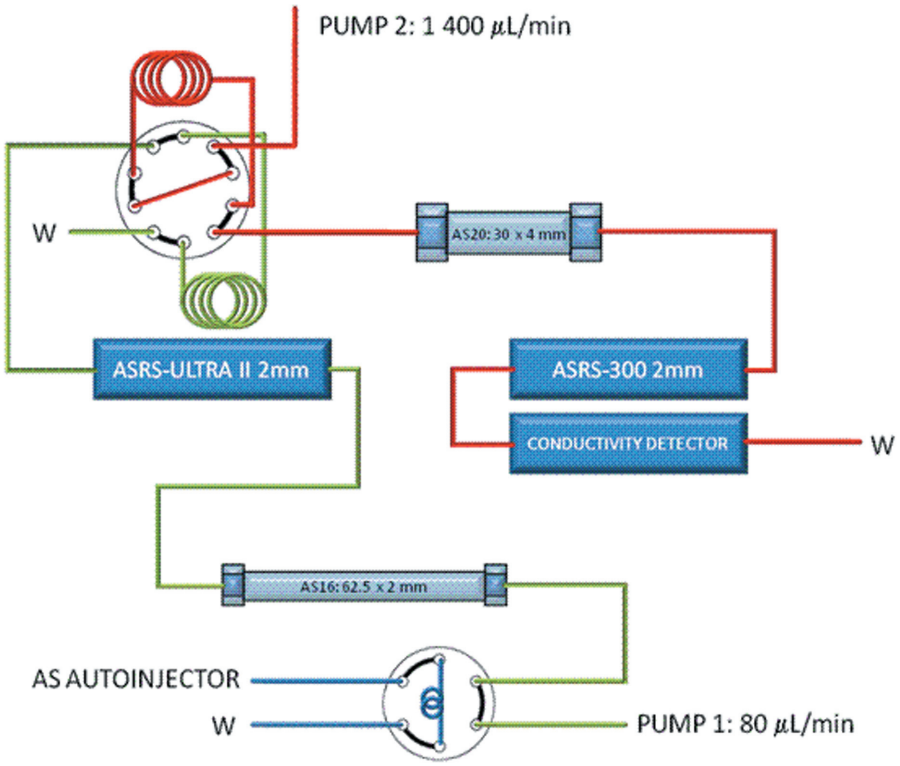

Figure 6. Diagram of the IC × IC system, including the column arrangement, two electrolytic suppressors and the ten-port switching valve fitted with dual 50 µL sample loops.

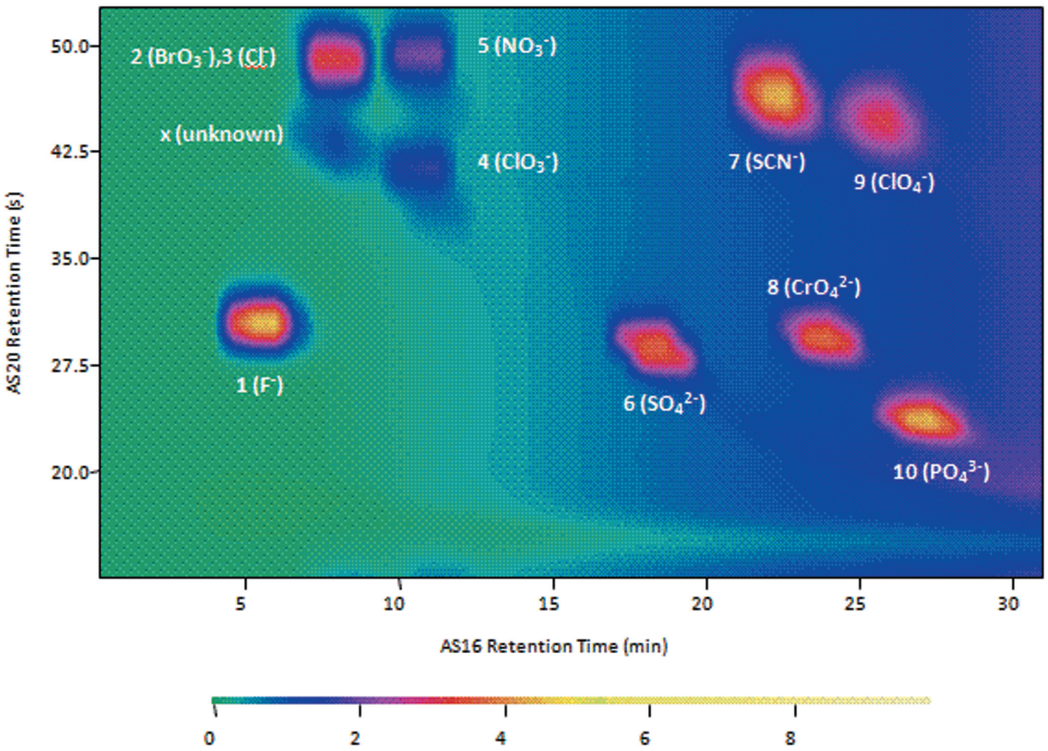

Figure 7. First demonstration of a comprehensive 2-D ion chromatography separation. Peak identities are (1) fluoride, (2) bromate, (3) chloride, (4) chlorate, (5) nitrate, (6) sulphate, (7) thiocyanate, (8) chromate, (9) perchlorate, (10) phosphate and (x) unknown.

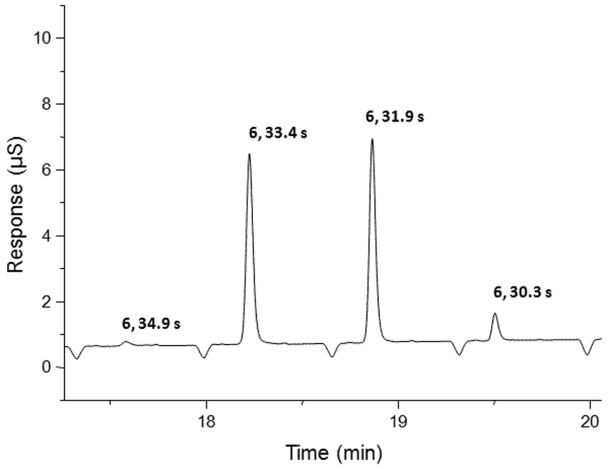

Figure 8. Expanded region of the comprehensive 2-D ion chromatogram shown in Fig. 7 showing the second dimension chromatograms for the sulphate anion. Note that the peak is eluted closer to the void in successive second dimension separations because the eluent strength gradually increases during the analysis. Second dimension retention times of the individual sulphate peak slices are given in the figure.

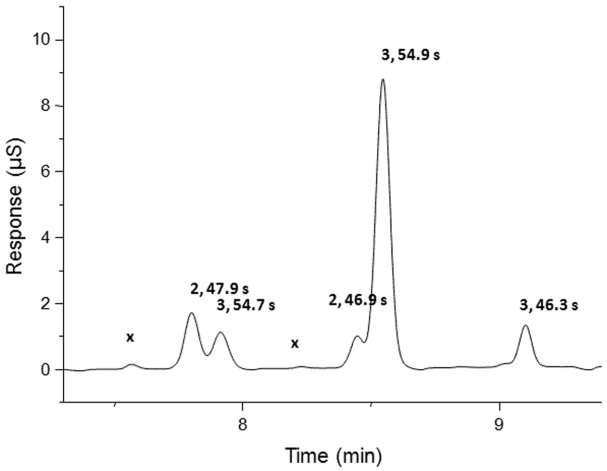

Figure 9. Expanded region of the comprehensive 2-D ion chromatogram shown in Fig. 7. Peak identities are (2) bromate, (3) chloride and (x) unknown; second dimension retention times of the individual bromate and chloride peak slices are given in the figure.

four modulations spanning the sulphate peak. The retention factor of the sulphate peak visibly decreases in the later second dimension separations because the eluent strength in the second dimension column gradually increases during the duration of the analysis. Similarly, the peak shapes for chromate (8) and phosphate (10) are sloping downwards in the 2-D separation space.

Bromate (peak 2) and chloride (peak 3) do not appear to be separated in the 2-D separation space and although they are almost completely overlapped in the first dimension separation, close inspection of a an expanded region (Fig. 9) shows that the short second dimension column provides sufficient selectivity difference to the first dimension column to partially resolve these anions. The first modulation slice has a resolution of 0.96, but the resolution is poorer in the next slice. The separation of equal amounts of bromate and chloride is problematic using the conditions employed here, but further method optimisation might improve the 2-D separation. Another expanded section of the 2-D chromatograms is presented in Fig. 10 to illustrate the excellent peak shape and performance characteristics of the fast second dimension column as well as to show the separation of the chlorate (peak 4) and nitrate (peak 5) peak pair. These analytes are completely co-eluted in the first dimension AS16 column, but have $R_s > 1$ in all second dimension separations. Three or four modulation slices are achieved for all anions in this mixture so the choice of 40 s modulation period is appropriate. The modulation ratio could be increased by making more frequent injections into the second dimension column, and a 30 s modulation period

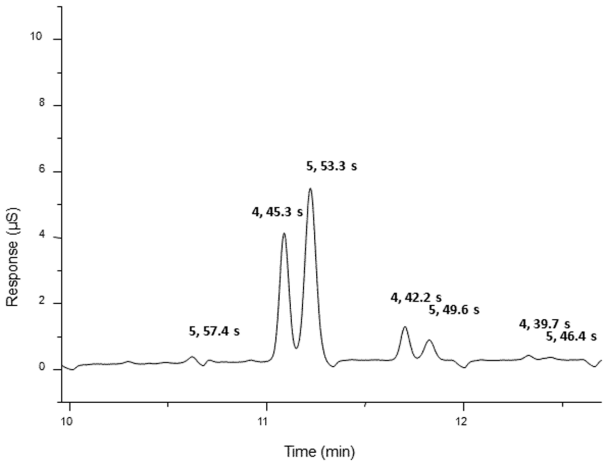

Figure 10. Expanded region of the comprehensive 2-D ion chromatogram shown in Fig. 7 showing the second dimension chromatograms for the chlorate (4)/nitrate (5) peak pair. Second dimension retention times of the individual chlorate and nitrate peak slices are given in the figure.

was also trialed, but several of the second dimension peaks were eluted at the same time as the void from the subsequent separation leading to poor peak shapes.

4 Conclusions

This investigation has shown for the first time that it is possible to perform comprehensive multidimensional ion chromatography separations and has demonstrated that PCA is a useful way to select columns for IC × IC separations. Preceding column selectivity studies have used

retention factors to classify column selectivity, but k is dependent on eluent strength and as a consequence classification using retention factors are highly dependent on the separation system used, which includes the specific analysis conditions. In the present investigation, we have used a single parameter from the simplified linear solvent strength equation that describes column selectivity very well. The data used in the PCA experiment were derived from three different eluent concentrations and three different column temperatures for each of the columns investigated. The column selectivity difference between the chosen AS16 and AS20 should therefore be very robust and permits the use of different conditions to enable optimisation of challenging anion separations. Selected applications of IC × IC will be reported elsewhere. A great deal of method development for IC × IC can be performed *in silico* which is highly advantageous, because comprehensive multidimensional separations can be very difficult to optimise otherwise. This study has also shown a potentially very useful application of short IC columns.

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