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Two-dimensional ion chromatography using tandem ion-exchange columns with gradient-pulse column switching

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ABSTRACT

A two-dimensional ion chromatography (2D-IC) approach has been developed which provides greater resolution of complex samples than is possible currently using a single column. Two columns containing different stationary phases are connected via a tee-piece, which enables an additional eluent flow and independent control of eluent concentration on each column. The resultant mixed eluent flow at the tee-piece can be varied to produce a different eluent concentration on the second column. This allows analytes strongly retained on the first column to be separated rapidly on the second column, whilst maintaining a highly efficient, well resolved separation of analytes retained weakly on the first column. A group of 18 inorganic anions has been separated to demonstrate the utility of this approach and the proposed 2D-IC method provided separation of this mixture with resolution of all analytes greater than 1.3. Careful optimisation of the eluent profiles on both columns resulted in run times of less than 28 min, including re-equilibration. Separations were performed using isocratic or gradient elution on the first column, with an isocratic separation being used on the second column. Switching of the analytes onto the second column was performed using a gradient pulse of concentrated eluent to quickly elute strongly retained analytes from the first column onto the second column. The separations were highly repeatable (RSD of 0.01–0.12% for retention times and 0.08–2.9% for peak areas) and efficient (typically 8000–260,000 plates). Detection limits were 3–80 ppb.

1. Introduction

Ion chromatography (IC) is a mature technique for the separation of inorganic anions and cations. Separation is dependent on the interaction of ionic analytes with ion-exchange functionalities present on the stationary phase and separation selectivity can be optimised through manipulation of the functional group on the stationary phase and also the type of eluent ion and its concentration. Separation of multi-component samples requires careful optimisation of the eluent operating conditions. Thus isocratic, gradient and multi-step gradients are employed widely in IC. Notwithstanding the undoubted utility and flexibility of these separation modes, IC often suffers from insufficient peak capacity to achieve the desired degree of resolution to fulfil analytical requirements for complex multi-component samples.

Multidimensional separations are ideally suited to the separation of multi-component samples because selectivity can be tuned for individual parts of the sample. Multidimensional chromatography generally refers to the use of two separation dimensions, combined in a way that the individual separations are largely independent of each other. Allowing the eluent to simply flow from the outlet of one separation column into the inlet of another does not constitute a multidimensional separation; this is in effect the same as using a mixed-mode stationary phase. Mixed-mode separations have some use. An example is phase optimised liquid chromatography (POPLC) [1–5] where the desired selectivity can be reached by mixing necessary length sections of different columns. These columns may contain stationary phases of very different selectivity, such as C18, phenyl, or cyanopropyl. This allows different selectivity to be achieved in comparison with only one column. This approach is not as well suited to IC due to the limited orthogonality in IC stationary phases.

The most common multidimensional chromatography modes are heart-cutting and comprehensive two-dimensional chromatography. Both modes employ two separation dimensions and draw on all of the available resolving power of these dimensions to improve separation of sample components. The primary difference between these two multidimensional separation modes is that heart-cutting provides a multidimensional separation of discrete parts of the first dimension chromatogram (targeted analysis),

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while comprehensive multidimensional separation approaches apply the two-dimensional advantage to the entire sample. A literature survey reveals some interesting applications of multi-dimensional ion-exchange/ion-exchange techniques. Trace anion determination in concentrated hydrofluoric acid has been achieved using 2D-IC [6,7], with ion-exclusion performed in the first dimension and the target analytes directed to an ion-exchange second dimension column by heart-cutting. Sensitive analysis of perchlorate has been performed using 2D matrix diversion methods [8]. One method used a Cryptand C1 concentrator column, which preferentially retained perchlorate over matrix anions. Following a rinse to waste to remove the matrix ions, perchlorate was then analysed on a Dionex AS16 or AS20 column. A second method used a first dimension separation to partially resolve perchlorate from matrix ions, with perchlorate then being diverted onto the second dimension for further separation on a Dionex AS16 or AS20 column. This approach was further refined [9] to develop a 2D-IC method for the analysis of perchlorate, published as EPA Method 314.2. We have recently described a comprehensive two-dimensional IC approach [10] in which ion-exchange columns were used in both dimensions of the separation.

In the present investigation, a series-coupled column two-dimensional IC approach was developed that used a gradient pulse for selectivity tuning and for decoupling the two separation dimensions. Coupling of columns in series for optimisation of separations in gas chromatography (GC) and thermally tuned HPLC column arrangements has been described extensively [11–19]. Deans and Scott [11] connected two GC columns containing different stationary phases in series and varied the ratios of the gas velocities in the two columns to adjust retention times of eluted compounds. The pressure at the junction between the two columns could be adjusted independently to optimise previously difficult to separate hydrocarbon mixtures. In a similar approach, Grall and Sacks [16] used an electronic pressure controller between two columns to enable selectivity tuning. A non-polar and a polar column were connected in series, and variation of the junction point pressure allowed the separation of a 19 component mixture to be enhanced. Similar principles have been demonstrated using temperature changes in HPLC by Mao and Carr [20], who connected two columns of very different selectivity in series and used independent temperature control of each column to manipulate the overall selectivity of the system.

In this paper we present the coupling of two IC columns via a tee-piece, with employment of a gradient pulse to decouple the two columns, thereby achieving separation under different eluent concentrations on each column. Retention on each column is statistically independent; therefore this is a true two-dimensional separation system. Careful optimisation has resulted in improved resolution of troublesome analyte pairs with highly efficient separations, whilst retaining total run times comparable with traditional one-dimensional separations.

2. Experimental

2.1. Instrumentation

A Dionex ICS-3000 (Sunnyvale, CA, USA) ion chromatograph was used throughout this work. Instrument control and data acquisition were performed using Chromeleon® software. A diagram showing the column arrangement for 2D separations is shown in Fig. 1.

Separations were performed on a Dionex AS19 first dimension column (250 mm, 2 mm ID) with Dionex AG19 guard column (50 mm, 2 mm ID). The second dimension column was a Dionex AS20 column (250 mm, 4 mm ID). The two columns were joined in series using a tee-piece union. Hydroxide eluent for the first dimension separation was generated online using a Dionex EluGen II KOH cartridge and delivered to the first dimension column at a flow-rate of 0.25 mL/min. Hydroxide eluent generated from an additional eluent generator was provided at the confluence of the first and second dimension columns using a second pump to provide 0.75 mL/min make-up flow for the larger internal diameter column and to modify the eluent strength in the second dimension column. Effluent from the second dimension column (1.0 mL/min) was directed to a Dionex ASRS 300 4 mm suppressor operated at 200 mA to allow suppressed conductivity detection. Continuously regenerated anion trap columns were plumbed in-line immediately after each eluent generator cartridge. Dionex AS19 and AS20 columns were also used individually where specified. A Dionex AS autosampler was used for sample injection with a 15 μL sample loop when two-dimensional separations or one-dimensional separations using the 4 mm ID AS20 column were performed. A 5 μL sample loop was used for separations employing only the 2 mm ID AS19 column set.

2.2. Reagents

Standard solutions of anionic analytes were prepared from sodium or potassium salts of analytical reagent grade wherever possible. The following inorganic anion standard solutions were prepared from their sodium salts: fluoride, formate, acetate, chlorite, bromate, cyanate, benzoate, thiosulfate, and phosphate (all from Aldrich, Sydney, Australia); chlorate, nitrite, sulfate, and perchlorate (all from Sigma, Sydney, Australia); and chloride (BDH, Melbourne, Australia). The remaining inorganic anion standard solutions were prepared from potassium salts: nitrate (Sigma), chromate (Aldrich), and thiocyanate (Sigma). Water treated with a Millipore (Bedford, MA, USA) Milli-Q system was used to prepare standard solutions and eluents.

2.3. Procedures

Detection limits were calculated at a signal to noise ratio of three. Repeatability (expressed as percent relative standard

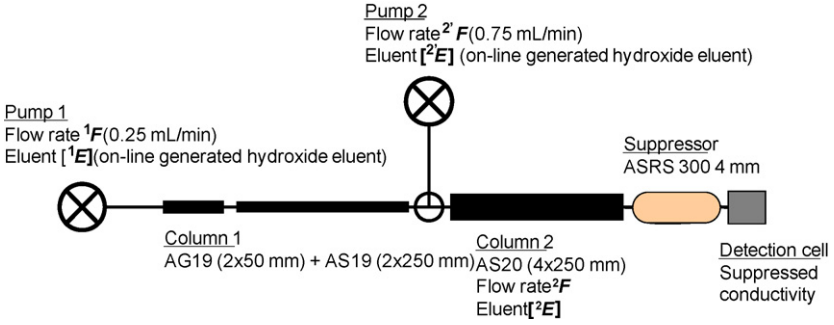

Fig. 1. Diagram of the instrument setup for 2D-IC with gradient-pulse column switching.

deviation, %RSD) was calculated from 10 replicate analyses. Linearity data were calculated over the 0.5–5 mg/L range. Separation efficiencies were calculated based on the peak width at half-height. Peak widths were measured at 5% of peak height. Virtual Column™ software (Dionex) was used to predict possible starting eluent conditions on both columns.

3. Results and discussion

3.1. Optimisation of eluent profiles in a single dimension

A group of 18 anions was selected as the test mixture of analytes. This particular group of ions is relevant to the post-blast analysis of inorganic explosive devices [21]. In order to provide a benchmark to evaluate the multidimensional separations, one-dimensional separations of the 18 anion mix were investigated. Simulations using Virtual Column™ software were performed to estimate the optimal isocratic and ramp gradient conditions for separation of the analyte mix on a range of Dionex anion-exchange columns, namely AS11-HC, AS16, AS19, and AS20. Optimisation criteria were employed to find the column which offered best resolution of the mixture and to identify which column provided the shortest possible run time with a minimum resolution of 1.0. This simulation indicated that an AS19 stationary phase would give the best separation for the 18 anion mixture using isocratic conditions (15 mM KOH). The predicted optimal conditions were then tested experimentally and where necessary slight modifications to the eluent composition were performed in order to improve the separation. Separation of the 18 anions (by employing 15 mM KOH mobile phase) was completed in 90 min, giving a minimum resolution of 0.87 between perchlorate and thiosulfate (see Fig. 2).

In previous work [21] on an AS20 column a complex gradient profile was found to be necessary to separate the 18 anion mix in 21 min, including re-equilibration, although marginal resolution resulted for some peak pairs (R_S values of 0.74 for carbonate/sulfate, 0.70 for phosphate/thiosulfate and 0.65 for thiosulfate/chromate). It is noteworthy that this separation was performed at a higher than usual flow-rate of 0.375 mL/min on a 2 mm ID column. A similar separation performed at a more typical flow-rate of 0.25 mL/min took 25 min. The use of multi-step gradients in IC is now commonplace and although the new ability to simulate IC separations involving multiple isocratic and gradient steps [22] facilitates method development, in the present study we propose that the improved selectivity offered by 2D-IC can exceed the resolution achieved using one-dimensional separations without recourse to multi-step eluent profiles, leading to more straightforward method optimisation. The use of isocratic separations in both dimensions should also lead to more robust methods which do not require re-optimisation to accommodate slight changes in column performance. Ramp gradients could also be used if improved selectivity and performance were provided.

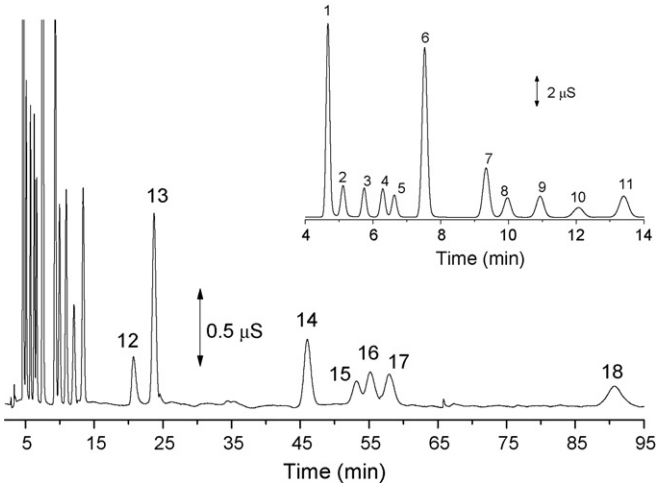

Fig. 2. Isocratic separation (15 mM KOH) of 18 analyte mix on AS19 column only. *Conditions*: Column - 2 mm × 50 mm AG19 and 2 mm × 250 mm AS19, sample injection loop - 5 μL, temperature - 35 °C, detection-suppressed conductivity (ASRS 300 2 mm, current 100 mA), flow-rate - 0.25 mL/min, eluent - on-line generated 15 mM hydroxide. 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, 7 = nitrite, 8 = cyanate, 9 = chlorate, 10 = benzoate, 11 = nitrate, 12 = carbonate, 13 = sulfate, 14 = thiocyanate, 15 = perchlorate, 16 = thiosulfate, 17 = chromate, 18 = phosphate.

A series of experiments was performed to determine suitable conditions for the separation of the first group of 13 analytes (fluoride, acetate, formate, chlorite, bromate, chloride, nitrite, cyanate, chlorate, benzoate, nitrate, carbonate, sulfate) on a 2 mm ID Dionex AG19/AS19 column set. Isocratic conditions were optimised and it was found that an eluent of 20 mM KOH provided a minimum resolution of 1.0 (with chlorite/bromate as the critical peak pair), with a run time of 13 min. Gradient separations were also optimised and a starting concentration of 17 mM KOH with a linear gradient ramp of 2.0 mM/min was found to provide a minimum resolution of 1.15 (between chlorite and bromate) and an 11.5 min run time. Although gradient elution provided marginally better performance than isocratic separation for these 13 anions, an isocratic eluent regime was considered favourable because it did not require a post-run re-equilibration period.

3.2. Two-dimensional separations using isocratic eluents

The eluent concentration in the second dimension column $[^2E]$ is determined by the concentrations and flow-rates of the two eluent streams shown in Fig. 1, according to

$$[^2E] = \frac{[^1E] \times {}^1F}{{}^2F} + \frac{[^{2'}E] \times {}^{2'}F}{{}^2F} \quad (1)$$

where ${}^1F$ and ${}^2F$ are the eluent flow-rates in the first and second dimension columns, respectively, $[^1E]$ is the eluent concentration in the first dimension column, and $[^{2'}E]$ and ${}^{2'}F$ are the make-up eluent concentration and flow-rate applied to the tee-piece union between the first and second dimension columns. Careful manipu-

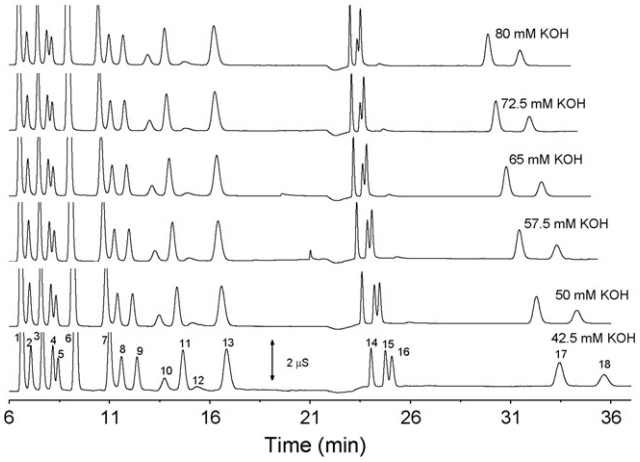

Fig. 3. Variation of second dimension concentration. Separation of 5 ppm anion standards on a Dionex AG19 and AS19 2 mm ID column connected in series via a tee-piece to a Dionex AS20 4 mm column. Separations are performed isocratically at 20 mM on the AG19/AS19 column set, and at concentrations as shown on the AS20 column. A step gradient pulse from 20 to 100 mM is introduced on the AG19/AS19 column set at 15 min. *Conditions*: Columns - 2 mm × 50 mm AG19 and 2 mm × 250 mm AS19 in series with a 4 mm × 250 mm AS20, sample injection loop - 15 μL, temperature - 35 °C, detection-suppressed conductivity (ASRS 300 4 mm, current 200 mA), flow-rate-*V*(1) 0.25 mL/min, *V*(2) 0.75 mL/min, eluent - on-line generated hydroxide gradient: *EGC*(1) 0–15 min: 20 mM, 15 min: 20–100 mM, 15–23 min: 100 mM, 23 min: 100–20 mM, 23–29 min: 20 mM, *EGC*(2) concentrations are varied to produce the second dimension concentration shown in the figure. Key: 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, 7 = nitrite, 8 = cyanate, 9 = chlorate, 10 = benzoate, 11 = nitrate, 12 = carbonate, 13 = sulfate, 14 = phosphate, 15 = thiosulfate, 16 = chromate, 17 = thiocyanate, 18 = perchlorate.

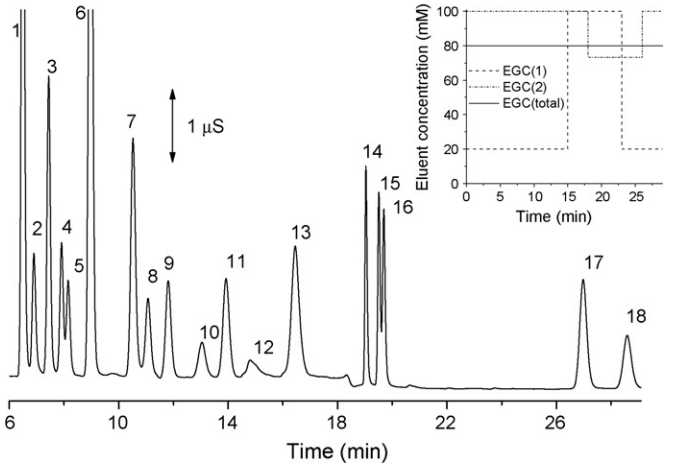

Fig. 4. Separation of 5 ppm anion standards on a Dionex AG19 and AS19 2 mm ID column connected in series via a tee-piece to a Dionex AS20 4 mm column. Separations are performed isocratically at 20 mM on the AG19/AS19 column set, and at 80 mM on the AS20 column. A step gradient pulse from 20 to 100 mM is introduced on the AG19/AS19 column set at 15 min. *Conditions*: Columns - 2 mm × 50 mm AG19 and 2 mm × 250 mm AS19 in series with a 4 mm × 250 mm AS20, sample injection loop - 15 μL, temperature - 35 °C, detection-suppressed conductivity (ASRS 300 4 mm, current 200 mA), flow-rate - *V*(1) 0.25 mL/min, *V*(2) 0.75 mL/min, eluent - on-line generated hydroxide gradient: *EGC*(1) 0–15 min: 20 mM, 15 min: 20–100 mM, 15–23 min: 100 mM, 23 min: 100–20 mM, 23–29 min: 20 mM, *EGC*(2) 0–18 min: 100 mM, 18 min: 100–73.33 mM, 18–26 min: 73.33 mM, 26 min: 73.33–100 mM, 26–29 min: 100 mM. Key: 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, 7 = nitrite, 8 = cyanate, 9 = chlorate, 10 = benzoate, 11 = nitrate, 12 = carbonate, 13 = sulfate, 14 = phosphate, 15 = thiosulfate, 16 = chromate, 17 = thiocyanate, 18 = perchlorate.

lation of $[^1E]$ and $[^{2'}E]$ permits selectivity tuning between the two dimensions.

There are two features of the proposed 2D system that were essential to its successful operation. First, it was necessary to maximise the extent to which the separations obtained in the two dimensions were decoupled. Second, it was necessary to ensure that the longer retained analytes to be separated on the second column were transferred to this column as compact bands in order to attain acceptable separation efficiency. A fully decoupled system permitted the separations performed in each dimension to be optimised independently. The simplest way to accomplish decoupling in the proposed system was for the analytes separated on the first column to pass unretained through the second column, thereby preserving any separation already achieved on the first column. Some band-broadening on the second column could be expected but this is minimised when retention on the second column is low. Transfer of the more strongly retained analytes (in this case, analytes 14–18) to the second column can be achieved using a step pulse of concentrated eluent (e.g. increasing $[^1E]$ to 100 mM KOH for a suitable period) to rapidly sweep analytes 14–18 off the first dimension column. However, upon application of this gradient pulse, $[^{2'}E]$ must be reduced commensurately according to Eq.

Table 1
Figures of merit for the isocratic-step gradient pulse-isocratic two-dimensional separation (Fig. 4) and an optimised isocratic dimensional separation for peaks 1–13 (shaded area).

Analyte	2D separation						1D separation		
	Retention time		Peak area %RSD	Peak width	R_s	LOD (ng/L)	Retention time	Peak width	R_s
	Mean	%RSD							
Fluoride	6.45	0.09	0.08	0.27	1.81	3	4.22	0.24	1.68
Acetate	6.86	0.10	1.05	0.29	2.27	18	4.56	0.26	2.20
Formate	7.40	0.07	0.65	0.29	2.04	36	5.03	0.26	1.90
Chlorite	7.87	0.09	1.73	0.30	0.98	19	5.45	0.28	1.10
Bromate	8.12	0.09	1.74	0.31	3.05	27	5.70	0.28	2.84
Chloride	8.94	0.07	0.42	0.33	5.32	3	6.39	0.31	4.97
Nitrite	10.50	0.06	0.49	0.45	1.65	11	7.76	0.38	1.53
Cyanate	11.05	0.06	1.04	0.47	2.02	27	8.25	0.42	2.12
Chlorate	11.78	0.05	0.60	0.61	2.84	27	8.97	0.44	2.19
Benzoate	13.00	0.05	1.93	0.54	1.87	79	9.86	0.54	2.23
Nitrate	13.91	0.05	0.61	0.88	1.56	25	10.84	0.51	3.65
Carbonate	14.94	0.10	2.63	0.70	2.44	70	12.92	0.85	2.27
Sulfate	16.46	0.12	2.05	0.20	7.36	19	14.42	0.74	
Phosphate	19.03	0.06	1.39	0.24	2.94	12			
Thiosulfate	19.50	0.05	2.49	0.25	1.00	17			
Chromate	19.68	0.04	1.51	0.27	21.26	14			
Thiocyanate	27.05	0.03	0.79	0.60	3.03	31			
Perchlorate	28.68	0.03	1.50	0.65		45			

(1) to maintain a constant eluent strength in the second dimension column. Changes in $[^{2'}E]$ were delayed by 3 min to account for the additional time for changes in $[^{1}E]$ to reach the central tee-piece. Installation of a gradient mixer column immediately after the tee-piece and prior to the AS20 column was evaluated but this resulted in substantial peak broadening and all further work was carried out without any mixing column.

To investigate both the decoupling of the two dimensions and the effectiveness of the transfer of analytes 14–18, $[^{1}E]$ was set to 20 mM KOH and the make-up eluent concentration ($[^{2'}E]$) passed into the tee-piece by pump 2 was varied such that the final eluent concentration in the second column ($[^{2}E]$) varied over the range 47.5–80 mM KOH. A 8.0 min pulse of 100 mM KOH was used to transfer analytes 14–18 to column 2. The chromatograms obtained are shown in Fig. 3, from which it can be seen that the separation of analytes 1–13 was relatively unaffected over the range studied, although there was evidence of slightly increased retention for peaks 7–13 at the lowest value of $[^{2}E]$. This confirmed that their separation occurred predominantly on the first column and at the higher concentrations present on the second dimension column these analytes passed rapidly though column 2. Separation of analytes 14–18 on the second dimension column (AS20) showed only minor changes over the $[^{2}E]$ range tested, although run-time was shorter at the higher values of $[^{2}E]$.

Both the timing and magnitude of the gradient pulse were investigated and it was found that a step gradient in the first dimension separation from 20 to 100 mM at 15 min in conjunction with adjustment of $[^{2}E]$ from 100 to 73.33 mM (also step gradient) at 18 min provided optimal performance, with a run-time of 28 min and minimum resolution between thiosulfate and chromate of 0.98 (Fig. 4, with figures of merit listed in Table 1). For comparison, performance data for an isocratic one-dimensional separation of the first 13 analytes at 20 mM performed on the AS19 column is also included in Table 1. The performance data for the isocratic and 2D-IC separations are very similar, with peak widths being increased only marginally and resolution decreased slightly by the addition of the second column. Calibration linearity for all analytes was in the range $R^2 = 0.992–1.000$ for concentrations of 0.5–5 mg/L. The first dimension eluent concentration was stepped back down to 20 mM at 23 min and the AS19 column was therefore re-equilibrated and ready for the next injection as soon as the second dimension separation was complete. Total effective eluent concentration on the AS20 column was maintained at 80 mM by varying $[^{2'}E]$ to take into account the gradient pulse delivered to the first dimension AS19 column, as shown in the inset to Fig. 4. Finally, variation of the shape of the gradient pulse was investigated to determine if this could be used to help improve the separation of analytes 14–16. The intention was to obtain a partial separation of these analytes on column 1, prior to their transfer to column 2. Addition of a ramp gradient pulse (20–60 mM KOH step gradient at 9 min, a ramp gradient from 60 to 100 mM KOH over the next 4 min, followed by 100 mM maintained for a further 10 min, as shown in the inset to Fig. 5, with commensurate modification of the $[^{2'}E]$ profile) gave significantly improved separation of analytes 14–16 and reduced overall run-time. Resolution between thiosulfate/chromate was improved to 1.36 with a run time of 27 min. The separation of the first group of 13 analytes was unchanged. Linearity over a 0.5–5 mg/L range varied from 0.984 to 1.000. Figures of merit for this separation are shown in Table 2.

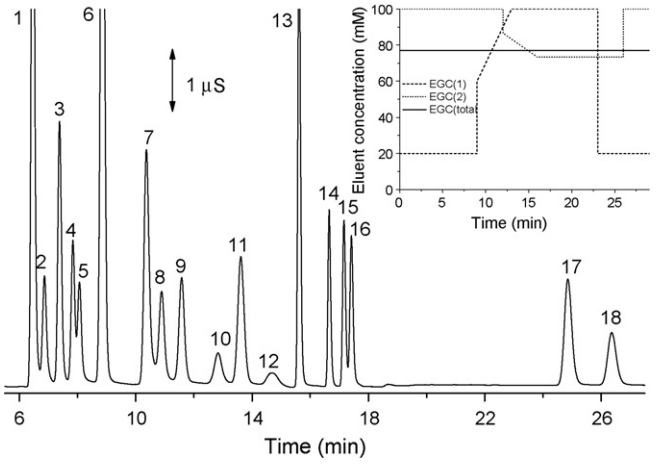

Fig. 5. Separation of 5 ppm anion standards on a Dionex AG19 and AS19 2 mm ID column connected in series via a tee-piece to a Dionex AS20 4 mm column. Separations are performed isocratically at 20 mM on the AG19/AS19 column set, and at 80 mM on the AS20 column. A ramped gradient pulse from 20 to 100 mM is introduced on the AG19/AS19 column set at 9 min. *Conditions*: Eluent - on-line generated hydroxide gradient: *EGC*(1) 0–9 min: 20 mM, 9 min: 20–60 mM, 9–13 min: 60–100 mM, 13–23 min: 100 mM, 23 min: 100–20 mM, 23–29 min: 20 mM, *EGC*(2) 0–12 min: 100 mM, 12 min: 100–86.66 mM, 12–16 min: 86.66–73.33 mM, 16–26 min: 73.33 mM, 26 min: 73.33–100 mM, 26–29 min: 100 mM. All other conditions as in Fig. 3. Key: 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, 7 = nitrite, 8 = cyanate, 9 = chlorate, 10 = benzoate, 11 = nitrate, 12 = carbonate, 13 = sulfate, 14 = phosphate, 15 = thiosulfate, 16 = chromate, 17 = thiocyanate, 18 = perchlorate.

Table 2
Figures of merit for the isocratic-ramped gradient pulse-isocratic two-dimensional separation (Fig. 5).

Analyte	Retention time		Peak area	Peak width	Resolution	Efficiency	LOD (ppb)
	Mean	%RSD	%RSD				
Fluoride	6.47	0.04	0.21	0.28	1.71	15,000	3
Acetate	6.86	0.02	1.16	0.32	2.12	11,000	17
Formate	7.39	0.03	0.37	0.30	1.96	16,000	9
Chlorite	7.85	0.03	2.90	0.31	0.94	18,000	19
Bromate	8.08	0.02	2.68	0.31	3.12	17,000	25
Chloride	8.87	0.03	0.33	0.32	5.18	19,000	3
Nitrite	10.38	0.03	0.79	0.42	1.54	16,000	10
Cyanate	10.92	0.03	1.09	0.43	1.97	16,000	24
Chlorate	11.61	0.03	0.61	0.43	3.12	18,000	23
Benzoate	12.85	0.03	1.71	0.58	1.79	12,000	73
Nitrate	13.65	0.03	0.63	0.50	1.99	18,000	19
Carbonate	14.64	0.12	2.39	0.69	2.28	8,000	62
Sulfate	15.63	0.02	0.49	0.23	5.90	124,000	5
Phosphate	16.67	0.02	0.70	0.23	2.78	141,000	14
Thiosulfate	17.20	0.03	1.78	0.26	1.31	130,000	13
Chromate	17.44	0.03	1.44	0.25	21.91	117,000	15
Thiocyanate	24.94	0.02	0.89	0.59	2.95	43,000	25
Perchlorate	26.46	0.02	1.64	0.66		37,000	45

Table 3
Figures of merit for the ramp gradient-ramped gradient pulse-isocratic two-dimensional separation (Fig. 6) and for a one-dimensional AS19 gradient separation of analytes 1–13 (shaded area).

Analyte	2D separation							1D separation			
	Retention time		Peak area	Peak width	Resolution	Efficiency	LOD (ppb)	Retention time	Peak width	Efficiency	Resolution
	Mean	%RSD	%RSD								
Fluoride	7.92	0.10	0.24	0.29	2.38	19,000	3	5.66	0.25	12,000	2.39
Acetate	8.49	0.08	0.99	0.32	3.05	18,000	21	6.19	0.28	11,000	2.98
Formate	9.24	0.08	0.39	0.31	2.49	24,000	9	6.87	0.27	15,000	2.46
Chlorite	9.84	0.07	1.66	0.29	1.29	27,000	22	7.42	0.28	17,000	1.46
Bromate	10.15	0.07	0.87	0.30	3.71	28,000	26	7.75	0.30	18,000	3.42
Chloride	11.07	0.07	0.46	0.32	5.91	30,000	3	8.54	0.29	21,000	5.70
Nitrite	12.67	0.06	0.34	0.39	1.77	31,000	10	9.97	0.33	22,000	1.65
Cyanate	13.19	0.05	0.79	0.38	2.19	31,000	23	10.42	0.35	22,000	2.30
Chlorate	13.84	0.05	0.58	0.38	3.14	33,000	20	11.08	0.36	23,000	2.24
Benzoate	14.93	0.05	1.70	0.49	1.91	23,000	52	11.83	0.46	15,000	2.25
Nitrate	15.62	0.04	0.42	0.41	1.75	35,000	15	12.61	0.39	26,000	4.01
Carbonate	16.26	0.03	2.80	0.46	1.85	26,000	34	14.14	0.44	15,000	1.70
Sulfate	16.85	0.02	1.24	0.30	7.80	83,000	6	14.76	0.36	44,000	
Phosphate	18.42	0.01	0.74	0.22	2.68	184,000	13				
Thiosulfate	18.90	0.01	1.24	0.23	1.29	162,000	40				
Chromate	19.15	0.01	1.61	0.25	21.57	164,000	16				
Thiocyanate	26.41	0.01	0.96	0.59	3.01	47,000	23				
Perchlorate	27.96	0.01	1.53	0.66		41,000	43				

3.3. Two-dimensional separations using gradient elution

Potentially, gradient elution can be used in either or both of the first and second dimension separations. In the case of a gradient being applied to the first dimension, the major objective would be to improve the separation of the weakly retained analytes (1–13), whereas a gradient in the second dimension would focus on the separation of the strongly retained analytes (14–18). Both avenues were explored.

Some potential gradient profiles for the first dimension separation, based on starting concentrations of 1, 9 and 17 mM KOH, were available from the initial screening experiments described in Section 3.1 and these were tested in the two-dimensional system. The ramp slopes were varied to provide optimised separations of the first 13 analytes for all three starting concentrations and an isocratic separation was maintained on the second dimension column by adjusting the make-up eluent concentration accordingly. After the gradient separation of the first group of 13 analytes was completed, a step change in the eluent composition to 100 mM KOH was applied to sweep the second group of five strongly retained analytes onto the AS20 column. The best separation achieved using this approach is shown in Fig. 6, with figures of merit reported in Table 3. A ramp gradient starting at 9 mM KOH, slope 2 mM/min was applied on the AS19 column up to 10 min, followed by a ramp from 29-100 mM over 3 min (slope 23.67 mM/min). The make-up eluent concentration was decreased accordingly to maintain an effective total concentration of 77 mM KOH on the second dimension column. The same gradient was applied using only the AS19 column set with peak data listed in the furthest 4 right-hand columns of Table 3 for further comparisons.

The outcomes arising from the use of a gradient in the first dimension separation can be evaluated by comparison of Figs. 4–6 and Tables 1–3. All three chromatograms are of similar length, but the use of a ramp gradient to separate analytes 1–13 (Fig. 6) results in a significantly improved separation of chlorite from bromate (R_s = 1.29), as well as some improvement in the separation of thiosulfate from chromate in the group of strongly retained analytes. Table 3 shows that separation efficiency was improved substantially for all peaks when a ramp gradient was employed in the first dimension. It is noteworthy that re-equilibration of the first dimension column could be completed before the overall run time was reached, meaning that a new sample could be injected immediately after the end of the run.

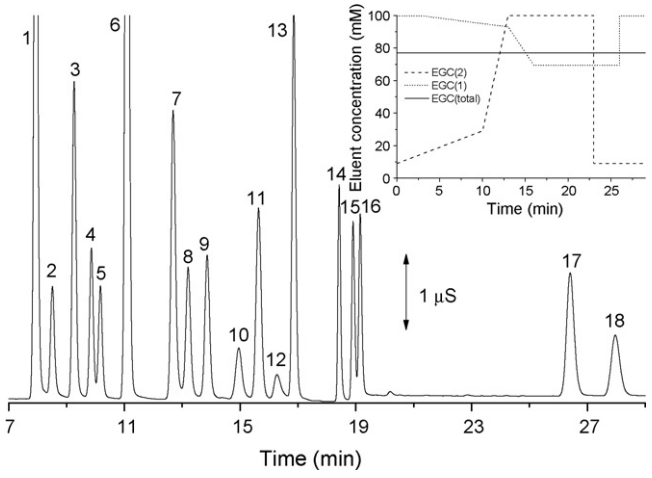

Fig. 6. Separation of 5 ppm anion standards on a Dionex AG19 and AS19 2 mm ID column connected in series via a tee-piece to a Dionex AS20 4 mm column. Gradient separation is performed on the AG19/AS19 column set, and an isocratic separation at 77 mM is performed on the AS20 column. A ramped gradient pulse from 20 to 100 mM is introduced on the AG19/AS19 column set at 10 min. *Conditions*: Eluent - on-line generated hydroxide gradient: *EGC*(1) 0–10 min: 9–29 mM, 10–13 min: 29–100 mM, 13–23 min: 100 mM, 23 min: 100–9 mM, 23–29 min: 9 mM, *EGC*(2) 0–3 min: 99.67 mM, 3–13 min: 99.67–93.00 mM, 13–16 min: 93.00–69.33 mM, 16–26 min: 69.33 mM, 26 min: 69.33–99.67 mM, 26–29 min: 99.67 mM. All other conditions as in Fig. 3. Key: 1 = fluoride, 2 = acetate, 3 = formate, 4 = chlorite, 5 = bromate, 6 = chloride, 7 = nitrite, 8 = cyanate, 9 = chlorate, 10 = benzoate, 11 = nitrate, 12 = carbonate, 13 = sulfate, 14 = phosphate, 15 = thiosulfate, 16 = chromate, 17 = thiocyanate, 18 = perchlorate.

The approximately 7 min elution window between chromate and thiocyanate suggested that there was an opportunity to reduce the retention times of thiocyanate and perchlorate by employing a gradient to 100 mM KOH in the second dimension column. However, this step cannot be introduced earlier than 16 min without changing the earlier optimised ramp gradient. As a result, there was only a modest reduction in run time of around 1 min when a second dimension gradient was applied. The use of gradients in the second dimension starting at $[^2E] < 40$ mM KOH resulted in peak

de-focusing at the confluence of the two columns. For this reason, and also the fact that significant re-equilibration times would be required, the use of gradient elution in the second dimension separation was not pursued further.

4. Conclusions

The use of a tee-piece to join two ion-exchange columns of differing selectivity and to then introduce an additional eluent flow stream was found to be suitable to independently control eluent concentration on both columns. Resolution between difficult to separate analyte pairs in a complex mixture has been improved significantly using this approach in comparison to a traditional single column arrangement. Isocratic eluent profiles can be applied in both dimensions to achieve overall resolution which would normally require multi-step gradients on a single column. Highly efficient separations have been achieved without lengthening total run times substantially. This approach requires no specialised equipment (apart from an additional pump, eluent generator, and tee-piece) to provide a multidimensional ion chromatographic separation.

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