

Use of temperature programming to improve resolution of inorganic anions, haloacetic acids and oxyhalides in drinking water by suppressed ion chromatography

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Abstract

Temperature programming was used to improve selectivity in the suppressed ion chromatographic (IC) separation of inorganic anions, haloacetic acids and oxyhalides in drinking water samples when using NaOH gradient elution. The programme exploited varying responses of these anions to changes in temperature. Heats of adsorption (ΔH , kJ/mol) for 17 anionic species were calculated from van't Hoff plots. For haloacetic acids, both the degree of substitution and $\log P$ (log of *n*-octanol–water partition coefficient) values correlated well with the magnitude of the temperature effect, with monochloro- and monobromoacetic acids showing the largest effect ($\Delta H = -10.4$ to -10.7 kJ/mol), dichloro- and dibromoacetic acids showing a reduced effect ($\Delta H = -6.8$ to -8.4 kJ/mol) and trichloro-, bromodichloro- and chlorodibromoacetic acids showing the least effect ($\Delta H = -4.7$ to -2.4 kJ/mol). The effect of temperature on oxyhalides ranged from $\Delta H = 8.4$ kJ/mol for perchlorate to $\Delta H = -9.1$ kJ/mol for iodate. The effectiveness of two commercial column ovens was investigated for the application of temperature gradients during chromatographic runs, with the best system applied to improve the resolution of closely retained species at the start, middle and end of the separation obtained using a previously optimised hydroxide gradient, in a real drinking water sample matrix. Retention time reproducibility of the final method ranged from 0.62 to 3.18% RSD ($n = 30$) showing temperature programming is indeed a practically important parameter to manipulate resolution.

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1. Introduction

It can be stated without question, that within both past and present papers detailing the optimisation of high-performance liquid chromatographic (HPLC) separations, the parameter that has, and continues to receive, least attention is temperature. Often temperature is simply fixed at a set point to improve reproducibility of the chromatographic separation, and the effect upon the actual chromatographic selectivity is ignored.

In certain modes of HPLC, such as reversed-phase separations, the general responses to temperature are reasonably

well understood, and the effect of temperature upon the retention and resolution of many organic species can be reliably predicted [1]. Within reversed-phase HPLC, in all but the most unusual cases, increases in temperature results in subsequent decreases in retention. With this in mind a small number of publications detailing the use of temperature programming in reversed-phase separations have appeared, mostly using an increase in column temperature to speed the elution of strongly retained solutes. These studies were the subject of a recently published review [2].

However, in modes of HPLC other than reversed-phase, such as ion chromatography (IC), less predictable behaviour can be observed and contradictory effects and explanations are not uncommon, meaning further investigation is still merited. This is particularly the case with more complex si-

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multaneous separations of inorganic, organic and varyingly charged ions, and even more so when mixed mode retention mechanisms may exist. There has been only a limited number of publications detailing temperature effects in ion exchange chromatography, either focusing on cation exchange [3–8] or anion exchange chromatography [9–13]. In case of cation exchange, the most significant temperature effects upon both retention and selectivity have been seen with those ion exchangers which incorporate some degree of cation complexation [14], for example cation-exchangers bearing carboxylic [15], phosphonic [16,17], and more complex functionalities like iminodiacetic acid [18,19] or aminocarboxylic acids [3,20]. For the separation of anions on anion exchangers, temperature effects could be described as less significant, and are mostly associated with changes in hydration radii of the anions and ion-exchange resins. For example, the enthalpy changes for halide ion-exchange reactions on strong PS-DVB based anion-exchangers are usually not higher than 13.81 kJ/eq. [21].

In most cases the above studies have investigated temperature effects using isothermal separations, with very little attention paid to potential application of temperature programming. However, a limited number of studies have included temperature gradients in ion exchange separations. For example, Smith et al. [22] applied temperature gradients to decrease the capacity of 2,2,1-cryptand based anion exchange columns (the commercial product that has evolved from these early studies is the Dionex IonPac Cryptand A1 column). In this work the column capacity was based upon the nature of the complexed cation within the macrocycle and it was the binding of this cation that was affected most by the temperature gradient, with the effect of temperature upon interaction of the anion with the complex being secondary.

In suppressed IC of anions the use of hydroxide eluents is now common practice and the use of hydroxide eluent generators has also gained in popularity. When using suppression, particularly with hydroxide eluents, the ability to selectively manipulate retention becomes somewhat of a problem, as control of eluent chemistry is obviously limited. Thus, alternative approaches need to be assessed. This is where temperature can prove useful. Temperature programming potentially can be used to adjust selectivity whilst simultaneously running isocratic or gradient hydroxide separations. In addition, this secondary temperature gradient causes little or no disturbance to the background signal because of heat dissipation in the suppressor module and temperature control in the conductivity cell.

In this work, we assess the potential of temperature to improve resolution of anionic species in chlorinated drinking water samples, namely common inorganic anions, haloacetic acids (HAAs) and selected oxyhalides. A number of methods on the application of IC to HAA analysis within drinking water have been published [23]. However, within these publications, those methods using hydroxide eluents with suppressed conductivity detection have all shown resolution of HAAs from excess common anions to be problematical, particularly

when applied to the analysis of real samples. Therefore, here we assess the response of HAAs and other anionic species to temperature both under isocratic and gradient hydroxide conditions and from these responses devise a suitable temperature programme to selectively manipulate retention. The practical ability to do this was investigated with a comparison of two commercial column ovens and the final method applied to real sample matrices.

2. Experimental

2.1. Apparatus

For chromatographic separations, a Dionex model DX500 ion chromatograph (Dionex Corporation, Sunnyvale, CA, USA) was employed comprising of a GP50 gradient pump at a constant flow rate of 0.30 mL/min, LC25 chromatographic oven and a CD20 conductivity detector. The Rheodyne injection valve (Rheodyne, Cotati, CA USA) was fitted with a 100 μ L loop for all work. Separations were carried out using a Dionex AG16 (2.0 mm \times 50 mm) guard and an AS16 (2.0 mm \times 250 mm) analytical column, with a capacity of approximately 42.5 μ eq./column.

All experiments were carried out using suppressed conductivity detection. The suppressor employed for this work was a Dionex Atlas suppressor. Suppression was carried out in the auto-regeneration mode and the applied current for all experiments was 19 mA, which was supplied by a Dionex SC20 suppressor controller. For removal of anionic impurities from eluents, a Dionex continuously regenerating anion trap column (CR-ATC) was used.

The column temperature was regulated using an Dionex LC25 oven, which contained the injection valve, the suppressor, the guard and analytical columns and the conductivity cell. Temperature could be selected in 1 $^{\circ}$ C increments from 30 to 45 $^{\circ}$ C. For temperature gradient studies, actual column and oven temperatures were independently measured using an iButton sensor (Dallas Semiconductor Group, Dallas, TX, USA) placed within the oven to record temperature data at 1 min intervals and was sensitive to 0.5 $^{\circ}$ C changes in temperature. A Spark-Holland Mistral column oven (Spark-Holland, Emmen, The Netherlands) fitted with a monitored temperature readout (sensitive to 0.1 $^{\circ}$ C changes in temperature) was also used, for comparative temperature gradient work. This oven housed the guard and analytical columns, whilst the conductivity cell and injector remained in the Dionex LC25 (programmed with the same temperature profile) to ensure an equal amount of eluent pre-heating was seen with both column ovens and the conductivity cell temperature remained the same during comparative studies.

2.2. Reagents

Analytical grade chloroacetic acid (MCA), bromoacetic acid (MBA), dichloroacetic acid (DCA), dibromoacetic acid

(DBA), trichloroacetic acid (TCA), trifluoroacetic acid (TFA), chlorodifluoroacetic acid (CDFA), bromodichloroacetic acid (BDCA) and chlorodibromoacetic acid (CDBA), as well as the potassium salts of iodate, bromate, chlorate, perchlorate, fluoride, chloride, and sulphate and the sodium salt of nitrate were obtained from Sigma-Aldrich (Gillingham, UK). All oxyhalides and haloacetates were prepared to a stock concentration of 10 mM and stored in a refrigerator at 4 °C until required for use for working standards. All inorganic anion stocks were prepared to a concentration of 1000 µg/L. All eluents were prepared from a 50% solution of sodium hydroxide in water purchased from Sigma-Aldrich. All eluents and standards were prepared using diluent water from a Millipore water purification system (Millipore, Bedford, MA, USA) with a specific resistance of 18.3 MΩ cm. Eluents were passed through a 0.25 µm filter, followed by 15 min sonication prior to use.

2.3. Procedure

Temperature studies on isocratic separations were carried out at ambient, 30, 35, 40 and 45 °C. In all cases, the temperature within the oven was allowed to equilibrate for a 35 min period between temperature changes. When studying the effect of temperature on gradient separations, 37 °C was also included. Temperatures of above 45 °C were not studied to ensure the anion exchange columns were not damaged [13]. The use of microbore guard and analytical columns in this work minimised temperature equilibration times.

The optimum gradient separation method employed both a temperature gradient and a hydroxide concentration gradient. The column was initially allowed to equilibrate for 16 min between runs. Eluent concentration was kept at 1 mM for the first 20 min then ramped to 4 mM hydroxide over a 20 min period, then to 20 mM hydroxide over 5 min. This hydroxide concentration was then maintained for a further 26 min. The initial temperature for the separation of early eluting anions was 30 °C. At 20 min the temperature was set at 45 °C and then returned to 30 °C at either 30 or 40 min, depending upon the sample.

Drinking water samples, where necessary, had sulphate and chloride removed by passing through a series of Alltech Maxi Clean cartridges (Alltech Associates, Deerfield, IL, USA) in the barium, silver and acid forms. These cartridges were preconditioned with 10 mL Milli-Q water, passed through at a flow rate of 1 mL/min, followed by the samples at the same flow rate. Samples of approximately 25 mL were taken by leaving the laboratory tap running for a period of 3 min, then rinsing the container three times with drinking water prior to collection, followed by immediate chloride and sulphate removal and injection. A preconcentration procedure based on hypercrosslinked polystyrene LiChrolut EN (Merck, Germany) cartridges detailed previously [24] was used for selected water samples.

3. Results and discussion

3.1. Effect of temperature on ion chromatography—theoretical considerations

There are kinetic and thermodynamic effects in IC, which can be attributed to changes of temperature. The kinetic effects are mainly associated with changes in the diffusion coefficients of solutes in mobile and stationary phases, and with changes in the rate of solute–sorbent reactions. These can be slower in ion exchange than in other modes of liquid chromatography, particularly if complexation is involved in the ion exchange process. Such kinetic effects are responsible for variations in column efficiency and peak symmetry.

The thermodynamics of the IC process is potentially more important to the chromatographer because of different solute responses due to temperature changes, which means temperature can be utilised as a real tool for the variation of separation selectivity.

The effect of temperature, T , on retention factor, k , can be described by the van't Hoff equation:

$$\ln k = \frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \varphi \quad (1)$$

where ΔH is enthalpy change for the ion-exchange reaction; ΔS , the entropy change; R , the molar gas constant and φ is the phase volume ratio, a characteristic constant for a given column. If ΔS is a constant under the studied range of temperature, a plot of $\ln k$ versus the reciprocal temperature $1/T$ should have a slope equal to $-\Delta H/R$. However, it should be noted that values of enthalpy calculated from the van't Hoff equation should not be considered as standard enthalpy values ΔH° , as all data used for corresponding calculations cannot be obtained under conditions close to standard. For example, elevated pressure (50–70 bar) is always experienced within the chromatographic system.

The suitability of using the van't Hoff equation for the evaluation of enthalpy of sorbate–sorbent interactions is also limited by a number of other factors. Ideally the effects of temperature should be associated with solute–sorbate interactions of only a single definite type. In practice such single type interactions are rarely seen during chromatographic separations, so in the observed data it is necessary to take into account all possibilities for multi-mode interactions. To understand other limitations of the van't Hoff equation, possible changes in both the mobile and stationary phases should be considered as well as the possible changes in solute environment.

3.2. Ion-exchanger

Depending on rigidity and chemical nature of stationary phase, increases in temperature can change the porous structure of matrix, resulting in changes in basic parameters, such as dead volume and retention factors. The most dramatic effect of temperature upon the conformational mobility of poly-

mer chains should take place in those ion-exchangers having low cross-linkage or in so-called gel-type ion-exchangers. The increased conformational flexibility of polymer chains combined with subsequent repulsion effects of charged functional groups attached to the polymer, may cause a partial redistribution of ion-exchange functional groups at the surface (or within the ion-exchanger) and hence induce changes in ion-exchange selectivity. If any such changes in the structure of the ion-exchanger take place it represents a change of ΔS , which causes difficulties in the evaluation of ΔH from the van't Hoff equation. To simplify this, the ΔS is usually accepted to be constant under experimental conditions.

In this particular investigation a Dionex IonPac AG16 (2 mm \times 50 mm) guard and an IonPac AS16 (2 mm \times 250 mm) analytical column were used for the separation of a mixture inorganic anions and HAAs. The IonPac AS16 column contains a 9 μ m agglomerated anion-exchanger, consisting of a moderately sulfonated macroporous (200 nm) poly(ethylvinylbenzene-divinylbenzene) (EVB/DVB) polymer substrate bead with 55% cross-linking, coated with a layer of alkanol quaternary ammonium functionalised 80 nm latex particles, with only 1% cross-linking. According to the producer's data, the IonPac AS16 has an ion-exchange capacity of approximately 42.5 μ eq./column and an ultra low hydrophobicity (related to the latex layer). The IonPac AG16 guard column is filled with 13 μ m resin based of similar ion-exchange structure but based on micro-porous substrate and having a much lower capacity of 0.875 μ eq./column (despite slight differences between the analytical and guard column resins, the two columns were used in conjunction in this study

to reflect current practice in IC. However, due to the relatively low capacity of the guard column in relation to the analytical column, removal of the guard column had no effect upon the selectivity effects discussed later in this work).

The low degree of cross-linking of the latex particles at the outer layer of the IonPac AS16 means the flexibility of the polymer chains bearing charged groups is increased at higher temperatures, so it is possible to expect redistribution of functional groups relative to each other or relative to the oppositely charged, partly sulfonated surface of the more rigid central substrate with temperature changes.

3.3. Mobile phase

It is well established [25] that water based mobile phases exhibit a higher elution ability for hydrophobic solutes in reversed-phase separations under increased temperatures. On this basis, the use of overheated pure water as the mobile phase (up to 150 °C) has become popular in RP HPLC as a potential environmentally friendly replacement for water-organic solvent mixtures. In this current study there exists a definite probability of some degree of secondary hydrophobic interactions between particular HAAs and the surface of EVB-DVB substrate of the IonPac AS16 resin. Therefore one can expect that the impact of such hydrophobic interactions will be less expressed at high temperatures. Mobile phase temperature effects on non-hydrophobic interactions, namely pure ion-exchange, are in the most part only seen with weak acid/basic eluents, where for example the dissociation constant is dependent upon

Table 1
Slope data from van't Hoff plots, correlation coefficients, and ΔH values for oxyhalides, haloacetates and inorganic anions

Anions	$\log P$	van't Hoff slope ^b	Correlation coefficient R^2 ($n = 5$) ^c	ΔH (kJ/mol) ^d
Inorganic anions				
Fluoride	–	–744	0.993	–6.18
Chloride	–	–636	0.998	–5.29
Nitrate	–	–263	0.998	–2.18
Sulphate	–	–2169	0.999	–18.03
Haloacetates				
MCA	0.22	–1282	0.989	–10.7
MBA	0.41	–1247	0.988	–10.4
TFA	0.50 ^a	–1119	0.989	–9.3
CDFa	0.82 ^a	–1037	0.991	–8.6
DCA	0.92	–1010	0.991	–8.4
DBA	1.69	–818	0.990	–6.8
TCA	1.33	–566	0.989	–4.7
BDCA	2.31	–428	0.984	–3.6
CDBA	2.91	–284	0.975	–2.4
Oxyhalides				
Iodate	–	–1090	0.983	–9.1
Bromate	–	–763	0.993	–6.4
Chlorate	–	–332	0.998	–2.8
Perchlorate	–	1008	0.999	8.4

^a Calculated in accordance with [27] all other taken from [28].

^b Data over 30–45 °C ($n = 5$), each data point collected in triplicate.

^c Calculated from linear regression of averaged retention data from triplicate injections.

^d Calculated using van't Hoff slope = $\Delta H/R$ and assuming ΔS to be constant.

temperature, which will subsequently affect eluent elution strength.

3.4. Solutes

There is a general conclusion that charged ions should have a decreased hydration radii at elevated solution temperatures. With all other conditions constant, this then would be the main reason for solute heat effects observed during ion-exchange interactions.

3.5. van't Hoff plots

Initial temperature experiments were carried out on the IonPac AS16 column using isocratic conditions with a 20 mM hydroxide eluent. A relatively small temperature range from 30 to 45 °C was investigated ($n=5$) to avoid any possible column damage at higher temperatures [13], and to keep well within most operating parameters of most common column ovens. At each of the five temperatures investigated injections were carried out in triplicate to insure column equilibration had been achieved (see Table 1).

Hatsis and Lucy [12] have shown previously that the temperature effect upon anion exchange is dependent upon both eluent type (sodium carbonate or sodium hydroxide) and concentration, with the former having the larger effect. In the same study two anion exchange columns (IonPac AS11 and IonPac AS14) were compared using the same eluent and again considerable variation was seen.

Here the effect of temperature upon a number of common inorganic anions, HAAs and oxyhalides was investigated to provide information on the potential use of temperature and temperature gradients to improve resolution of anions from within each of these classes during ion chromatographic analysis of drinking waters. The test mixture included fluoride, chloride, nitrate, sulphate, MCA, MBA, TFA, DCA, CDFA, DBA, TCA, BDCA, CDBA, iodate, bromate, chlorate and perchlorate. The results of this study are shown in Fig. 1 with corresponding solute data, correlation coefficients, slopes and ΔH values shown in Table 1.

Hatsis and Lucy in their study with IonPac AS11 made some conclusions about general temperature effects upon anions separated using the above anion exchangers [12]. These

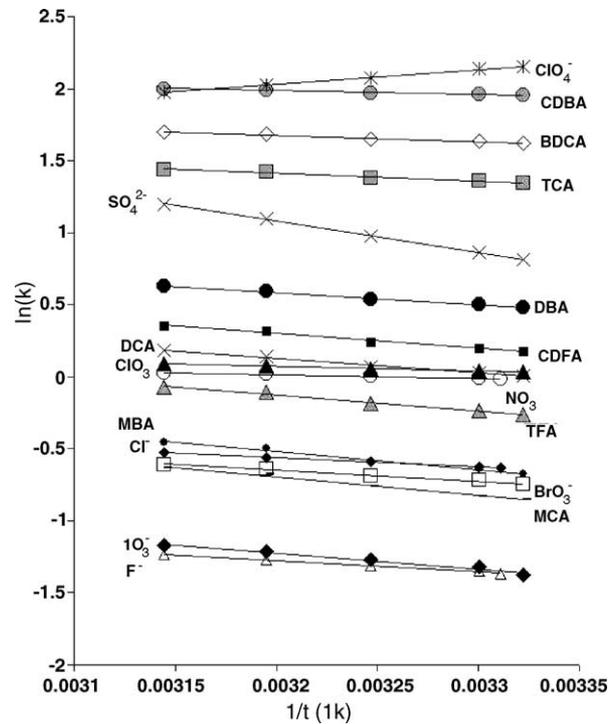


Fig. 1. van't Hoff plots for four oxyhalides, nine HAAs and four inorganic anions using AS16 column with isocratic elution. Eluent = 20 mM NaOH.

were (i) weakly retained anions, such as iodate, bromate, nitrite, nitrate and bromide, were unpredictable and either showed increased or decreased retention with increasing temperature; (ii) multiply charged anions such as sulphate and phosphate showed significant increases with increasing temperature; and (iii) strongly retained singly charged anions such as iodide and perchlorate showed significant decreases in retention with increasing temperature. From Table 1 it is clear that in the work carried out here with the IonPac AS16 column, some similarities to these trends can be seen, particularly for the inorganic anions, where the more strongly retained nitrate is least affected by temperature and the doubly charged sulphate shows the largest increase in retention.

Upon close consideration of the oxyhalide and HAAs data some interesting trends in temperature effects can also be observed. With the HAAs a strong correlation between retention and temperature effects can be clearly seen, with negative

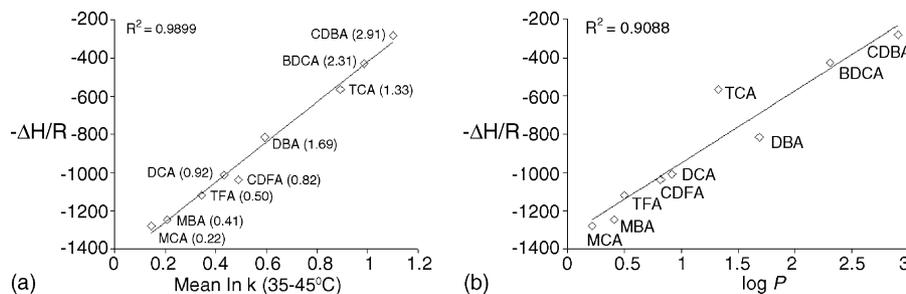


Fig. 2. (a) Plot showing the slope from van't Hoff plot against mean $\ln k$ for each haloacetate over the temperature range 35–45 °C. Data shown in brackets = $\log P$ value for each anion. (b) Plot showing the slope from van't Hoff plot against $\log P$ for each haloacetate.

van't Hoff slope decreasing almost linearly with increased retention. To fully illustrate this, Fig. 2(a) shows a plot of slope (obtained from van't Hoff plots shown in Fig. 1, with retention data for each haloacetate taken at five different column temperatures in triplicate) against mean retention factor over the temperature range studied (average $\ln k$ for each haloacetate over five column temperatures). As can be seen from the data shown the correlation is remarkable high, with a coefficient of $r^2 = 0.9899$.

Interestingly the retention order of the HAAs and hence the magnitude of the temperature effect correlates very closely ($r^2 = 0.9088$) with the lipophilicity of the haloacetates ($\log P$, \log of *n*-octanol–water partition coefficient), with the least lipophilic species, namely MCA, showing the largest temperature effect, and the most lipophilic species, namely CDBA, showing the least effect. This is shown graphically as Fig. 2(b). It is worthy of note that the lipophilicity of some of the haloacetates is in excess of some common organic acids typically considered to exhibit considerable lipophilicity, e.g. propionic acid ($\log P = 0.33$), butyric acid ($\log P = 0.79$) and heptanoic acid ($\log P = 2.42$). With this in mind it is reasonable to assume that even on what is described as an 'ultra-low hydrophobicity column' such as the IonPac AS16, the lipophilic nature of the haloacetates still contributes somewhat to retention and selectivity and this is reflected in the relative responses to temperature. Unlike retention due to pure ion exchange, which for the most part increases with increasing column temperature (as for the common inorganic anions), retention due to such lipophilic interactions would be reduced at higher column temperatures, hence the relatively smaller increases in retention seen for the more lipophilic haloacetates.

3.6. Effect of elevated temperature upon gradient IC separation

A previously optimised (room temperature) hydroxide gradient separation of common inorganic anions, HAAs and oxyhalides was run under increasing column temperatures between 30 and 45 °C ($n = 5$) to evaluate effects upon selectivity, resolution and efficiency. As eluent strength has been shown previously to effect van't Hoff slopes [12] the data collected in Section 3.2 could not be used directly to predict changes in selectivity seen under eluent gradient conditions. The effect upon the optimised separation of the applied increase in column temperature can be seen in Fig. 3.

The chromatograms obtained show that complete resolution of all anions was not possible under any of the conditions investigated. It was also noticed that increasing temperature led to a decrease in resolution of peaks 1 to 6 (with the subsequent co-elution of MCA and bromate), an increase in the resolution of peaks 7–13 (allowing the resolution of chlorate and DCA), and a decrease in resolution of peaks 14–19 (causing coelution of carbonate and TCA, and CDBA and perchlorate). In summary, elevated column temperature had a detrimental affect upon resolution and the start and end

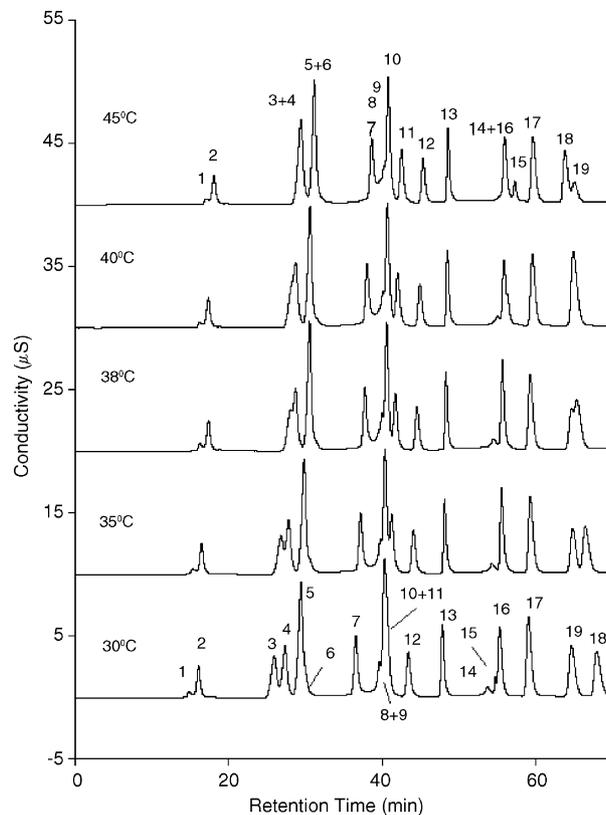


Fig. 3. Effect of increased column temperature upon the isothermal gradient separation of inorganic anions, oxyhalides and HAAs. Eluent conditions = 1 mM NaOH 0–20 min, increased to 4 mM hydroxide over next 20 min, then increased to 20 mM hydroxide over next 5 min. Peaks 1 = acetate 2 = fluoride, 3 = MCA, 4 = bromate, 5 = chloride, 6 = MBA, 7 = TFA, 8 = nitrate, 9 = bromide, 10 = chlorate 11 = DCA, 12 = CDFA, 13 = DBA, 14 = carbonate, 15 = sulphate, 16 = TCA, 17 = BDCA, 18 = CDBA, 19 = perchlorate.

of the chromatogram, whilst improving resolution of those peaks eluting at the centre. Clearly the observed trends indicated that an application of a temperature programme could be the solution to obtaining complete resolution.

3.7. Column oven performance

In order to apply a temperature gradient programme during a chromatographic run it is of great importance to be able to accurately and rapidly control column temperature [26]. Practically it is not trivial to accurately assess temperatures within analytical columns, as air temperatures within column ovens may not accurately reflect column temperatures. Therefore, to apply temperature programmes correctly the performance of the column oven in controlling column temperature needs to be assessed. In an attempt to achieve a more accurate assessment of column temperature over time, a temperature sensor (iButton) was used which allowed surface temperatures to be monitored. This sensor was adhered to the analytical column itself, which was housed within a column oven whilst temperature programmes were applied. The sensor continuously measured the temperature of the ac-

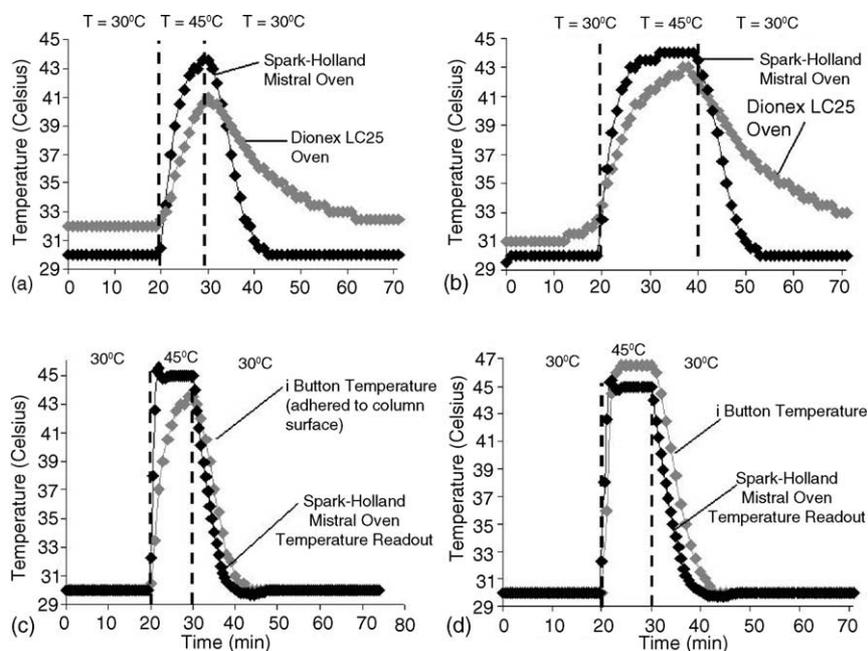


Fig. 4. (a and b) Column temperatures for columns held within column ovens during temperature programming cycles, recorded using iButton temperature sensor adhered to column surface. (c) Difference between the column oven readout and the column surface temperature. (d) Difference between the column oven readout and the column oven wall temperature.

tual column housing, whilst the column oven air temperature was simultaneously monitored by the column oven itself. Two commercial column ovens were available within this study and so their relative performance was assessed, these being the Dionex LC25 oven and a Spark-Holland Mistral oven (the Mistral oven contains peltier elements for more efficient heating and cooling, whereas the Dionex LC25 uses a simpler air conditioning scheme). To obtain an accurate comparison, lengths of pre-column tubing housed within each oven were kept equal and all samples were injected through a temperature controlled injector. With the responses to increased temperature shown in Fig. 3 in mind, temperature programmes starting at 30 °C at $t=0$ and stepping to 45 °C at $t=20$, and decreasing back to 30 °C at either $t=30$ (10 min programme) or $t=40$ min (20 min programme), were investigated. Fig. 4(a) and (b) show the column temperatures as measured by the iButton sensor over the two programmes for the two column ovens. As is clear from the figures shown, the performance of the two column ovens differed markedly, particularly upon cooling. In addition, it can be seen (Fig. 4(a)) that the actual column never reached the desired 45 °C temperature in either oven within the 10 min programmed time, although the column within the Spark-Holland Mistral oven did reach a steady temperature of close to 45 °C after 10 min during the 20 min programme. Fig. 4(c) shows the comparison of the column temperature within the Spark-Holland Mistral oven, with the actual oven temperature readout over the 10 min programme. From the comparison shown, it is clear that the column oven temperature readout cannot be relied upon for accurate temperature programming, and that the actual column temperature should be known if the depend-

able use of temperature programming is required. Fig. 4(d) shows the comparison of the iButton data when attached to the Spark-Holland Mistral oven wall and the Spark-Holland Mistral oven readout, showing good correlation.

3.8. Application of temperature program

With the use of the above performance data it was possible to apply temperature programmes to separations of standard solutions and spiked drinking water samples. Three programmes were applied, namely 30 °C at $t=0$ and stepping to 45 °C at $t=20$, and decreasing back to 30 °C at either $t=30$ (10 min programme) or $t=35$ min (15 min programme), or 40 min (20 min programme). Fig. 5 shows a chromatogram of a drinking water sample spiked with 20 μM of each of the HAAs and selected oxyhalides resulting from the latter 20 min programme, obtained using the Mistral column oven. As can be seen from Fig. 5, improved resolution of peaks 3–6 was obtained through delaying the temperature increase for the first 20 min. After which the resolution of peaks 7–11 was gradually improved through increasing the time set at 45 °C, with the 20 min programme (shown here) proving optimum. Maintaining the higher temperature for the full 20 min also results in the improved resolution of TCA and sulphate (as under these conditions TCA elutes after the large sulphate matrix peak), without negatively affecting the resolution of the CDBA and perchlorate.

An unspiked chlorinated drinking water sample was then analysed using the combined hydroxide and temperature programme. The freshly acquired sample was analysed directly following sulphate and chloride removal, and also

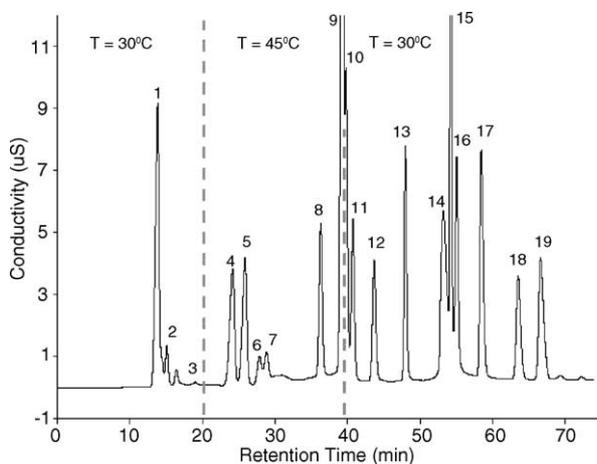


Fig. 5. Separation of HAAs and oxyhalides in a drinking water sample (20 μM spike of each HAAs and oxyhalide, ex. CDBA = 32 μM , BDCA = 40 μM) using a hydroxide gradient (as Fig. 3 with applied column temperature programming). Peaks; 1 = fluoride, 2 = iodate, 3 = chlorite, 4 = MCA, 5 = bromate, 6 = chloride, 7 = MBA, 8 = TFA, 9 = nitrate, 10 = chlorate, 11 = DCA, 12 = CDFA, 13 = DBA, 14 = carbonate, 15 = sulphate, 16 = TCA, 17 = BDCA, 18 = CDBA, 19 = perchlorate.

analysed following a preconcentration method previously described by Barron and Paull [24]. The resultant chromatograms are shown overlaid as Fig. 6(a) and (b), respectively. From the sample chromatograms it is clear that the combined using of the hydroxide gradient and the temperature programming results in excellent resolution of almost all peaks of interest. In the chromatograms shown the 10 min temperature programme was used to improve the resolution of the 8 peaks eluting after sulphate. As discussed previously by Barron and Paull [24] the preconcentration on method used led to a reduction in sample fluoride, chloride, nitrate, bromide and carbonate, whilst recoveries for the HAAs ranged from 16% for TFA to 88% for CDFA. As the procedure uses sample acidification with sulphuric acid, there is also an increase in weak acid inorganic anions such as nitrite and phosphate, together with the appearance of several unknown peaks, probably due to weak organic acids. Quantification of the haloacetates was achieved using

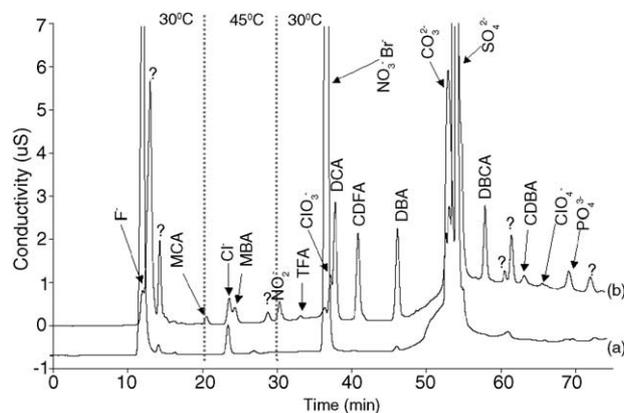


Fig. 6. Chromatograms showing the hydroxide gradient separation of inorganic anions, organic acids, oxyhalide and HAAs in a chlorinated drinking water (a), and the same sample following preconcentration (b). Hydroxide gradient, as Fig. 3.

a three point standard addition. The results of which were MCA = 0.09 μM ($R^2 > 0.999$), DCA = 0.61 μM ($R^2 = 0.980$), MBA = 0.36 μM ($R^2 = 0.987$), DBA = 0.56 μM ($R^2 = 0.987$), TFA = 0.05 μM ($R^2 > 0.999$), CDFA = 0.50 μM ($R^2 = 0.985$), BDCA = 0.40 μM ($R^2 = 0.980$), CDBA = 0.15 μM ($R^2 = 0.959$).

3.9. Reproducibility of dual gradient method

Clearly evaluation of precision is important if the application of temperature programmes (in combination with eluent gradients) is to be used routinely. Within the system used here this was assessed through 30 repeat gradient runs, each carried out with the temperature programme applied. Fig. 7 shows the resulting retention data graphically with %RSD values for all retention times given. Given the long run times of the developed method, the data shown represents retention time reproducibility over an approximate 30 h period and is clearly most satisfactory. It is also worthy of mention that through limiting the column temperatures to below 50 $^{\circ}\text{C}$, no reduction in column capacity or performance was noticed for the length of the study.

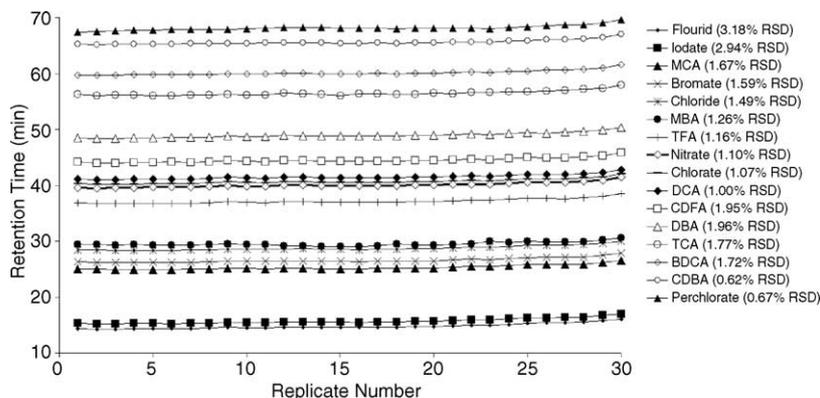


Fig. 7. Retention time repeatability for 30 repeat gradient runs with applied temperature programme.

4. Conclusions

Better understanding of the temperature effects upon the ion exchange separation of oxyhalide and haloacetates has led to a practical and useful application of temperature programming to significantly improve the resolution of analyte peaks within a particularly complex sample. Some evidence for hydrophobic interactions between haloacetates and anion-exchanger IonPac AS16 were found. The final method, although longer than ideal, exhibits the resolution, efficiency and sensitivity required to determine the above species in actual drinking water samples (as shown), and as such is a real practical alternative to gas chromatographic methods for monitoring such disinfection by-product species.

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