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Prediction of the effects of methanol and competing ion concentration on retention in the ion chromatographic separation of anionic and cationic pharmaceutically related compounds

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ABSTRACT

The mixed-mode separation of a selection of anionic and cationic pharmaceutically related compounds is studied using ion-exchange columns and eluents consisting of ionic salts (potassium hydroxide or methanesulfonic acid) and an organic modifier (methanol). All separations were performed using commercially available ion-exchange columns and an ion chromatography instrument modified to allow introduction of methanol into the eluent without introducing compatibility problems with the eluent generation system. Isocratic retention prediction was undertaken over the two-dimensional space defined by the concentration of the competing ion and the percentage of organic modifier in the eluent. Various empirical models describing the observed relationships between analyte retention and both the competing ion concentration and the percentage of methanol were evaluated, with the resultant model being capable of describing the separation, including peak width, over the entire experimental space based on six initial experiments. Average errors in retention time and peak width were less than 6% and 27%, respectively, for runs taken from both inside and outside of the experimental space. Separations performed under methanol gradient conditions (while holding the competing ion concentration constant) were also modelled. The observed effect on retention of varying the methanol composition differed between analytes with several analytes exhibiting increased retention with increased percentage methanol in the eluent. An empirical model was derived based on integration of the observed t_R vs. %methanol plot for each analyte. A combination of the isocratic and gradient models allowed for the prediction of retention time using multi-step methanol gradient profiles with average errors in predicted retention times being less than 4% over 30 different 2- and 3-step gradient profiles for anions and less than 6% over 14 different 2- and 3-step gradient profiles for cations. A modified peak compression model was used to estimate peak widths under these conditions. This provided adequate width prediction with the average error between observed and predicted peak widths being less than 15% for 40 1-, 2- and 3-step gradients for anions and less than 13% over 14 1-, 2- and 3-step gradients for cations.

1. Introduction

Chromatography plays a major role in the pharmaceutical industry and is used for analysis at all stages of drug development, testing and final production. The most common form of chromatography used is reversed-phase high performance liquid chromatography (RPLC) due to its high efficiency and applicability to a wide range of solutes [1]. The retention mechanism relies on solvophobic effects and analyte selectivity therefore derives primarily from these effects. Alternative stationary phases offering different retention mechanisms are desirable as they offer the potential to easily separate compounds that show similar retention characteristics on reversed-phase materials. Ion-exchange chromatography (IC) is a potentially useful separation system in that it can exhibit separation selectivity that is complimentary to RPLC. However, the use of IC based on ion-exchange interactions in the pharmaceutical industry is limited, since it is perceived as exhibiting low separation efficiencies and involves more complex method development procedures than for RPLC.

Application of ion-exchange to the separation of ionogenic organic compounds has been reported by several groups [2–14], although detailed investigations into the effects of eluent composition have generally not been addressed in these studies. The primary means of manipulating selectivity in IC are by varying the nature of the charged functional group on the ion-exchange

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stationary phase or the type and/or concentration of competing ion present in the eluent. When separating organic species, hydrophobic interactions between the analyte and the unfunctionalized parts of the stationary phase are also potentially present offering the possibility for further selectivity control by the addition of an organic modifier. This dual-mode retention was noted by Murawski [2] in the IC separation of nicotinic acid and niacinamide.

Chen et al. [5] demonstrated the separation of theobromine, theophylline and caffeine by anion-exchange IC. It was found that increasing the concentration of hydroxide in the eluent decreased the retention time of theobromine and theophylline, which are both anionic under the experimental conditions used, but had no effect on the retention of caffeine. Further increase in hydroxide concentration resulted in increased retention for caffeine which was attributed to the conversion of caffeine to its anionic form at elevated pH. The effect of organic modifier was also investigated and a decrease in retention was observed for all compounds on increasing the organic modifier concentration. It was suggested that ion-exchange and hydrophobic adsorption interactions were both present for theobromine and theophylline under the conditions investigated although a detailed investigation into the mechanisms was not undertaken. Chen and co-workers also concluded that ion-exchange and adsorption mechanisms were likely to be present in the separation of artificial sweeteners using anion-exchange chromatography [4,9]. In both reports it was found that an increase in the organic modifier concentration, being either methanol or acetonitrile, led to reduced retention for the compounds investigated.

Although it has been noted by several authors that the ion-exchange separation of organic compounds is likely to result from a combination of electrostatic as well as reversed-phase (adsorption) mechanisms, as discussed above, a detailed investigation into the nature of these interactions, and the feasibility to model and exploit these for selectivity improvements, has yet to be reported. Previous work within our laboratory has successfully applied ion-exchange theory, and in particular the Linear Solvent Strength Model (LSSM) and related algorithms [15,16], to the retention modelling and subsequent simulation of separations of a series of anionic [17] and cationic [manuscript in preparation] pharmaceutical compounds in the presence of variable concentrations of the ionic salt and fixed methanol concentrations. In the case of anions, and at the levels of methanol investigated (<25%, v/v) selectivity changes induced by the presence of methanol were minor and the major benefit from the addition of the methanol was improved peak shapes. However, for cations a higher methanol concentration was needed to facilitate reasonable separation times. For both systems it was found that at a fixed concentration of methanol in the eluent, the previously used ion-exchange retention models based solely on electrostatic interactions were still applicable and could be used to accurately model retention over varied hydroxide or hydronium ion concentrations.

In the present study we have increased the amount of methanol in the eluent so as to introduce selectivity changes through modification of the reversed-phase (adsorption) interactions between the analytes and the stationary phase. Independent control of both the reversed-phase and ion-exchange interactions over a two-dimensional experimental space through concurrent changes in methanol percentage and competing ion concentration have been exploited to allow additional selectivity control of the system. The possibility of predicting analyte retention over this two-dimensional system has been investigated to permit computer simulation of separations and optimisation of eluent composition. Finally, the possibility of performing separations using linear and multi-step methanol gradients at constant competing ion concentration has been assessed.

2. Experimental

2.1. Materials

All the organic compounds used as test analytes were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Chromatographic grade methanol was obtained from Merck (Darmstadt, Germany). Methanol was filtered through 0.2 μm nylon filters (Millipore, Bedford, MA, USA) prior to use. All other chemicals were used as supplied. Bulk standard solutions of the test analytes were prepared directly in methanol and diluted into working standard mixtures with further methanol. Potassium hydroxide eluents were prepared electrolytically using a Dionex (Sunnyvale, CA, USA) Elu-Gen eluent generator. All water was purified using a MilliQ system (Millipore, Bedford, MA, USA). Eluents were filtered through 0.2 μm nylon filters (Millipore, Bedford, MA, USA) prior to use.

2.2. Chromatographic separations

A Dionex ICS-3000 Ion Chromatography system consisting of a dual gradient pump unit (Dionex ICS-3000 DP), dual eluent generator unit (Dionex ICS-3000 EG), dual column and detector compartment (Dionex ICS-3000 DC), variable wavelength UV detector (Dionex ICS Series VWD) and autosampler (Dionex AS) was used for all separations. Methanol gradients were prepared using the Dionex quaternary pump to mix streams of 100% methanol with water in the appropriate amounts to generate the required eluent. Subsequent mixing of this stream with the electrolytically generated hydroxide containing stream was performed using a T-piece followed by a gradient mixer to ensure complete mixing. All final concentrations and flow-rates reported took into account any dilutions that occurred as a result of this mixing. A Dionex isocratic ICS-3000 DP dual pump was used to supply the external water supply to the Dionex Continually Regenerating Trap Column (CR-TC). All separations were performed using a 2 mm × 250 mm Dionex AS11 anion-exchange analytical column or a CS14 cation-exchange analytical column, with associated 2 mm × 50 mm Dionex AG11 or CG14 guard columns. Data were acquired using Chromeleon Version 6.80.

2.3. Calculations

All predictions were performed using Microsoft Office Excel 2007. Experimental data used to derive the models were run in triplicate with average retention times and peak widths used. Correlation data used to assess the performance of the models were run singly with observed retention times and peak widths directly compared to that predicted using the appropriate model.

3. Results and discussion

3.1. Selection of analytes

Previous work within our group has investigated the ion chromatographic separation of a number of UV-absorbing organic anions [17] and cations [manuscript in preparation]. For the current work a subset comprising 12 of the anions and 5 of the cations was chosen, ranging from relatively small compounds (phenol, MW = 94.1) with low retention up to relatively large compounds (dipyridamole, MW = 504.6) exhibiting stronger retention; see Table 1 for a complete listing of analytes. pK_a values for the acidic compounds can be found in [17]. The cations with associated pK_b values (calculated using ACD/Labs 7.00, Advanced Chemistry Development Inc., Toronto, Canada) consisted of sulfamethoxazole (1.39), dipyridamole (1.20, 8.78), propranolol (9.14), diphenhydramine (8.76) and doxepin (9.25). The selected list included

Table 1
Slope and intercept values from log[OH or MSA] vs. log *k* plots at 25, 50 and 75% methanol.

	%MeOH								
	25%			50%			75%		
	Slope	Intercept	Correlation coefficient[a]	Slope	Intercept	Correlation coefficient[a]	Slope	Intercept	Correlation coefficient[a]
Anions									
Tropic acid	−0.79	0.52	0.9805	−0.94	0.68	0.9968	−1.02	0.85	0.9997
Phenol	−0.97	1.34	0.9998	−0.86	1.07	0.9983	−0.89	1.00	0.9995
Captopril	−2.14	2.49	0.9997	−1.96	2.61	0.9979	−1.75	2.95	1.0000
Naphthoic acid	−0.98	1.74	1.0000	−0.89	1.20	0.9988	−0.96	1.07	1.0000
Naphthol	−0.96	2.48	1.0000	−0.85	1.81	0.9987	−0.88	1.45	1.0000
Hydrocortisone	−0.10	−0.35	0.8102	−0.19	−1.00	0.6845	0.37	−2.15	0.6845
Unknown	−2.00	3.72	1.0000	−2.19	4.27	1.0000	−2.03	4.61	0.9981
Diclofenac	−0.97	3.25	0.9921	−0.89	2.12	0.9987	−0.79	1.25	0.9887
Furosemide	−2.00	5.08	0.9963	−2.12	4.84	0.9999	−1.99	4.63	0.9990
Chlorothiazide	−2.01	4.36	0.9986	−2.14	4.45	0.9999	−2.06	4.68	0.9981
Ibuprofen	−0.95	1.85	0.9979	−0.93	1.15	0.9979	−0.83	0.48	0.9970
Tolfenamic acid	N/A	N/A	N/A	−0.91	2.60	0.9992	−0.81	1.58	0.9921
Cations									
Sulfamethoxazole	−0.15	0.63	N/A	−0.09	0.17	N/A	−0.05	−0.26	N/A
Dipyridamole	−0.13	0.59	N/A	−0.09	0.30	N/A	−0.05	0.00	N/A
Propranolol	−0.31	−1.06	N/A	−0.25	−0.27	N/A	−0.17	1.27	N/A
Diphenhydramine	−0.35	0.00	N/A	−0.28	0.10	N/A	−0.19	0.23	N/A
Doxepin	−0.40	0.18	N/A	−0.33	0.20	N/A	−0.21	0.25	N/A

[a] Straight line plots for cationic separations were prepared using the experimental raw data used for modelling and as such only consist of two points.

monovalent and divalent cations, as well as weakly charged compounds.

3.2. Anion-exchange prediction

Previous work [17] has demonstrated that the retention of small to medium-sized organic anions on an anion-exchange column using eluents containing up to 25% methanol can be described by the equation:

$$\log\ k_i = a_i - b_i \log[E_m^{y-}] \quad (1)$$

where a_i and b_i are constants (with b_i being equal to the ratio of the charges on the analyte and competing ions) and $[E_m^{y-}]$ is the concentration of the competing anion E^{y-} in the eluent. The performance of this retention model at higher methanol concentrations was investigated to determine whether it would be possible to concurrently control the ion-exchange interactions using the competing ion concentration, as well as the hydrophobic (adsorption) interactions by altering the percentage of methanol in the eluent. Table 1 lists the slopes, intercepts and correlation coefficients (for anionic analytes only) of the log[OH] vs. log *k* plots for the test analytes obtained using 25%, 50% and 75% of methanol in the eluent. Separations at 0% methanol resulted in excessive retention time and/or broad peak shapes for many of the described compounds. Furthermore, this data was not required for the successful application of the prediction method and, as such, separations under these conditions were not pursued. It should be noted that the unknown compound appearing in Table 1 is a degradation product from chlorothiazide and has been included to demonstrate the applicability of the described work to compounds that cannot be identified but can be separated.

Table 1 shows that high correlation coefficients were observed for all anionic analytes at the three methanol percentages investigated. This implies that even at elevated percentages of methanol the electrostatic analyte–stationary phase interactions conform to the standard retention relationship existing in aqueous solution. The poor correlation for hydrocortisone results from the very weak electrostatic interactions occurring with this analyte. It can also be seen that the slopes from Eq. (1) are generally close to the expected values of −1 and −2 for mono- and divalent anions, respectively. From this it can be concluded that tropic acid, phenol, naphthoic acid, naphthol, diclofenac, ibuprofen and tolfenamic acid are monovalent at the studied conditions while captopril, furosemide, chlorothiazide and the unknown are all divalent. The deviations from the −1 and −2 values expected from theory likely arise from increased hydrophobic character of some of the compounds [17] due to partial shielding of the ion-exchange interaction. This shielding will decrease the effect from increasing counter-ion concentration in the eluent and lead to a reduction in the observed slopes of the log *k* vs. log[competing ion] plots for the more hydrophobic compounds as was observed previously [17]. This trend is less apparent in the case of cations where observed slopes differ dramatically from those predicted by theory. However, this could be due to far greater hydrophobic interactions present in the cation-exchange system as evidenced by increased retention times and broader peaks when compared to the equivalent anion-exchange system.

3.3. Reversed-phase prediction

The effect of methanol on the separation of the pharmaceutically relevant ions was taken to be hydrophobic in nature rather than a secondary effect, such as a change in the dielectric constant of the eluent since no direct correlation between the change in retention of a compound on varying methanol concentration and its pK_a/pK_b was found. For example, the retentions of chlorothiazide and furosemide were affected differently on increasing the methanol concentration: chlorothiazide showed increased retention while furosemide showed decreased retention, despite both having similar pK_a values of 9.78 and 9.79, respectively [17]. Furthermore the two compounds that exhibited the biggest decrease in retention as methanol concentrations increased, diclofenac and tolfenamic acid, also have the largest log *P* values while chlorothiazide, which exhibits a reversed dependence on methanol, has the lowest log *P* value. These points suggest that hydrophobic interactions were predominantly responsible for the observed effects on retention resulting from changes in methanol concentration.

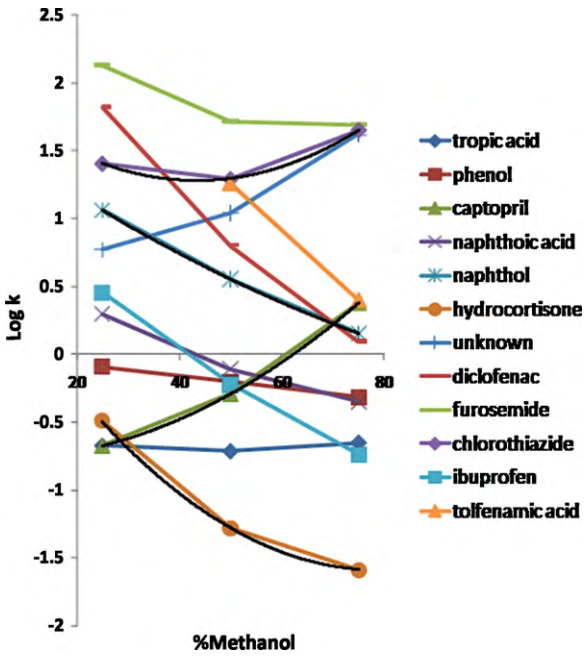

Fig. 1. Log k vs. %MeOH for the 12 anionic test compounds. Conditions: column: 2 mm × 250 mm AS11 with AG11 guard column; eluent: 30 mM KOH; flow rate: 0.28 ml/min. Quadratic trend lines are shown for hydrocortisone, naphthol, chlorothiazide and captopril.

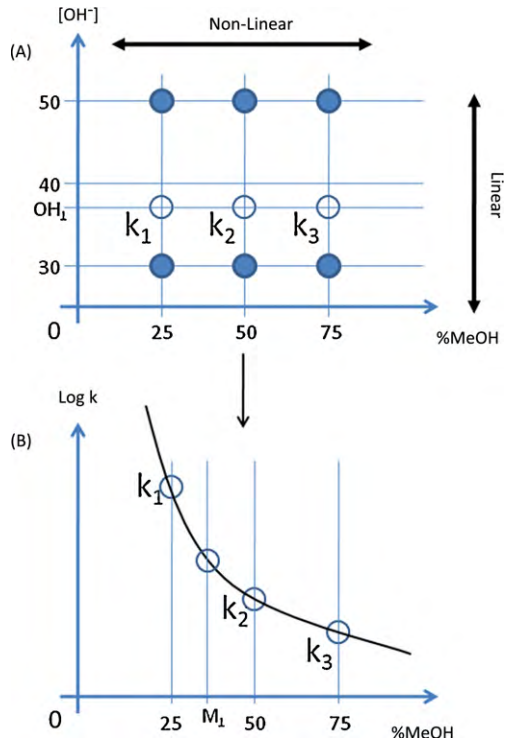

Fig. 2. Two-dimensional experimental space used to concurrently model ion-exchange and reversed-phase interactions. (A) Experimental space and ion-exchange theory to determine points k_1, k_2 and k_3 at the desired hydroxide concentration OH_1. (B) Quadratic curve fit through k_1, k_2 and k_3 to determine log k at the desired methanol percentage M_1.

Reversed-phase theory predicts a linear relationship between the percentage of organic modifier in the eluent and log k, usually written as:

$$\log k = \log k_w - S\varphi \quad (2)$$

where k is the retention factor of the given compound, φ the volume fraction of the solvent and k_w and S are constants [18]. Eq. (2) suggests that an increase in the percentage of methanol will lead to a corresponding linear decrease in log k. However, this behaviour is not observed in the mixed-mode system under study for some of the test analytes. Fig. 1 shows a plot of log k vs. %MeOH for the 12 test analytes and indicates that non-linear plots were observed and in some cases (namely chlorothiazide, the unknown degradation product of chlorothiazide, and captopril) there is actually an increase in retention as the methanol content of the eluent is increased. The origin of this increased retention is unclear, but may be due to a hydrophilic interaction mechanism occurring at high levels of organic modifier. This is supported by the fact that the log P values for chlorothiazide and captopril are amongst the lowest of the investigated compounds at −0.22 and 0.34, respectively [17]. Only tropic acid has a comparatively low log P (0.28) and it also shows a slight increase in retention at higher methanol concentrations, though less significant than the effect observed for chlorothiazide and captopril. The retention behaviour cannot be modelled using a linear relationship, but it was found that a quadratic relationship gave a good fit to the data. Furthermore it was found that the concentration of hydroxide present in the eluent had no effect on the observed log k vs. %MeOH curves, with the same retention relationships being observed for all compounds over the full range of hydroxide concentrations employed.

3.4. Combined anion-exchange and reversed-phase prediction

The independent natures of the ion-exchange and reversed-phase interactions permits concurrent prediction of both interactions over the two-dimensional experimental space defined by the hydroxide concentration and the percentage of methanol as variables. Retention data are measured for each analyte under the conditions defined by the solid circles in Fig. 2A and a linear relationship between log k and log[OH^-] and a quadratic relationship between log k and %MeOH are assumed. The retention behaviour of any of the test analytes over the full experimental space can then be modelled. This involves initially determining k_1, k_2 and k_3 at a specified hydroxide concentration (e.g. at OH_1 in Fig. 2A) by linear interpolation on the [OH^-] axis between the measured data points. A quadratic fit is then applied to these data and used to derive the retention at a specified methanol percentage (e.g. M_1 in Fig. 2B). In this way the retention for each of the compounds can be derived over the entire experimental space. Fig. 3A shows the observed and predicted retention times for the 12 anionic test compounds investigated at 9 eluent compositions, 5 from within the experimental space defined by the solid circles in Fig. 2A and 4 from outside (with respect to hydroxide concentrations). Fig. 3A shows that good agreement was obtained between observed retention times and those predicted using the empirical model, with average errors at each eluent composition being less than 5%.

In a previous report we have noted that the efficiencies observed when separating these relatively large organic anions on IC columns traditionally used for inorganic ions is relatively poor and requires the addition of methanol to the eluent to improve peak shape and resolution [17]. However, even with the addition of methanol however, efficiencies are still lower than for the inorganic anions (~1000–5000 plates over the full range of methanol concentrations compared to ~6000–10,000 plates as reported on the Dionex Quality Assurance Report shipped with the particular column used in this work). This reduced efficiency supports the proposed mechanism involving more than one analyte–stationary phase interaction. Despite the broader peaks, the empirical model can be used to estimate peak widths over the experimental space using the same initial experiments used for retention prediction. In this case a power approximation was used to estimate the effect of hydroxide

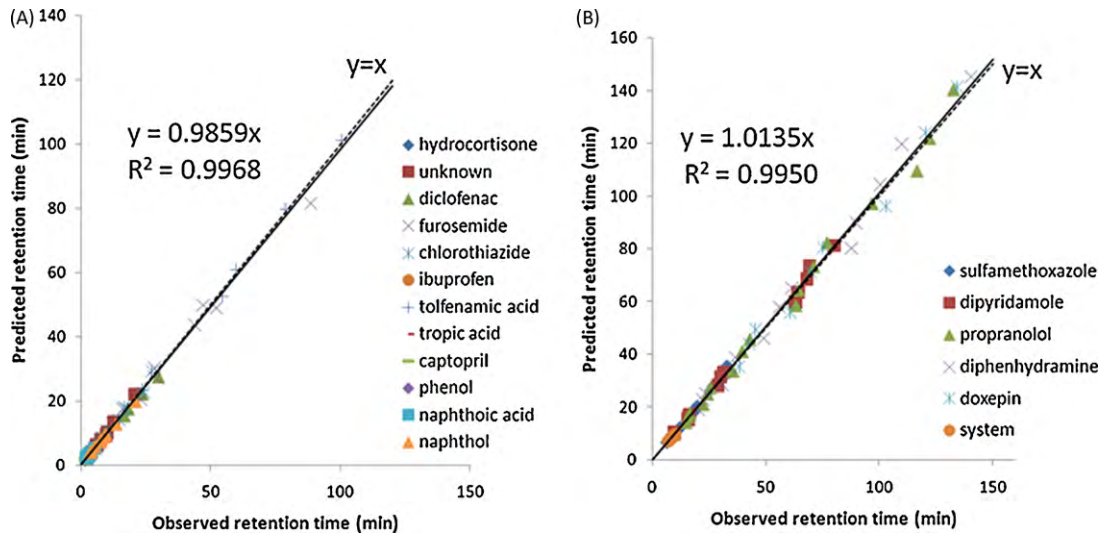

Fig. 3. Correlation between observed and predicted retention times. (A) 11 anionic and one neutral organic compound separated using ion chromatography over the two-dimensional experimental space with %MeOH and [hydroxide] as variables. Conditions: column: 2 mm × 250 mm AS11 with AG11 guard column; eluent: 25–75% methanol, 10–50 mM hydroxide for tropic acid, captopril, phenol, naphthoic acid, naphthol and 30–70 mM hydroxide for remaining compounds. Correlation was performed for 9 eluent compositions. (B) 5 pharmaceutical cations and a system peak, over the two-dimensional experimental space with %MeOH and [MSA] as variables. Correlation was performed for 19 eluent compositions (13 of which fall outside the experimental space used to obtain retention data). Conditions: column: 2 mm × 250 mm CS14 with CG14 guard column; eluent: 40–75% methanol, 20–75 mM MSA.

concentration on peak width, and a quadratic approximation was used to estimate peak width as a function of methanol percentage. Agreement between predicted and observed peak widths was generally acceptable, although for more highly retained compounds, such as tolfenamic acid, predicted peak widths were broader than the observed values. Average error over all the eluent compositions tested was less than 20%.

The methodology used above was also applied to the separation of pharmaceutically relevant cations. The separation of such compounds by ion chromatography has been performed previously within our group (manuscript in preparation) and a subset of 5 of these compounds (sulfamethoxazole, dipyridamole, propranolol, diphenhydramine and doxepin) consisting of both weakly and strongly retained compounds was investigated. Fig. 3B shows the correlation between observed and predicted retention time for the 5 test compounds as well as a system peak that was observable for all samples injected. It can be seen that good correlation was obtained, especially considering that 13 of the 19 eluent compositions used were taken from outside the experimental space of the model, highlighting its robustness for retention time prediction. Predicted peak widths showed an average error of 27%.

3.5. Methanol gradient prediction

In previous studies we have successfully modelled the ion-exchange separation of organic anions using a competing ion gradient in the presence of a constant methanol concentration [17]. A system using a methanol gradient at constant hydroxide concentration has yet to be modelled, but has the potential to take advantage of the differing selectivities offered by varying the amount of organic modifier in the eluent, as shown by the isocratic studies presented above.

A set of test analytes comprising hydrocortisone, diclofenac, furosemide, ibuprofen, tolfenamic acid, chlorothiazide and the chlorothiazide degradation compound, was selected to investigate the methanol gradients. This set comprised strongly retained compounds which are likely to respond to the use of methanol gradients. From Fig. 1 it is evident that the retention behaviour of the test analytes on the anion-exchange columns was both complex and unpredictable. Modelling of such behaviour from a theoretical standpoint would be unlikely to succeed because of the requirement to elucidate the precise combination of mechanisms contributing to the retention of each analyte. In view of this, an empirical approach was used to model retention, based on the isocratic retention data used for the two-dimensional studies presented in Section 3.4. This approach involved fitting the retention data for the full range of methanol concentrations to a quadratic relationship ($ax^2 + bx + c$), power relationship (ax^b), or to a series of linear steps ($ax + b$) consisting of the straight line segments between each measured data point. An expression for retention time in terms of the percentage methanol in the eluent, $t_R(M)$, can then be obtained. If linear methanol gradients are used, Eq. (3) shows the percentage of methanol in the eluent with respect to time:

$$M(t) = M_i + rt \quad (3)$$

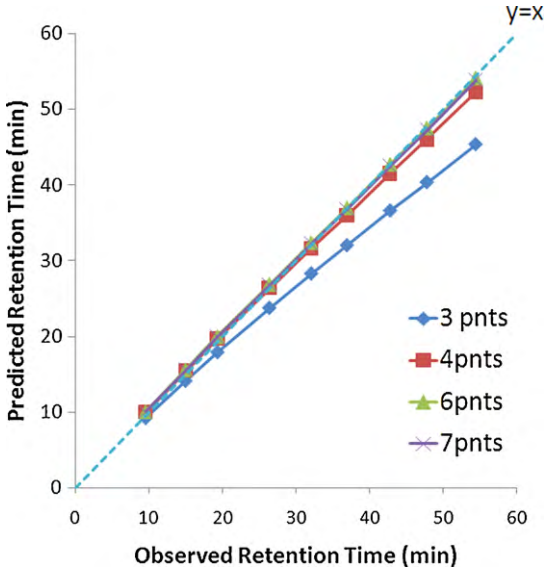

Fig. 4. Effect of the number of points used to derive the power approximation $t_R(M)$ on the ability of the model to predict the gradient retention times for diclofenac over a series of linear gradients. Gradients run from 25 to 75% methanol over ramp times of 10–200 min.

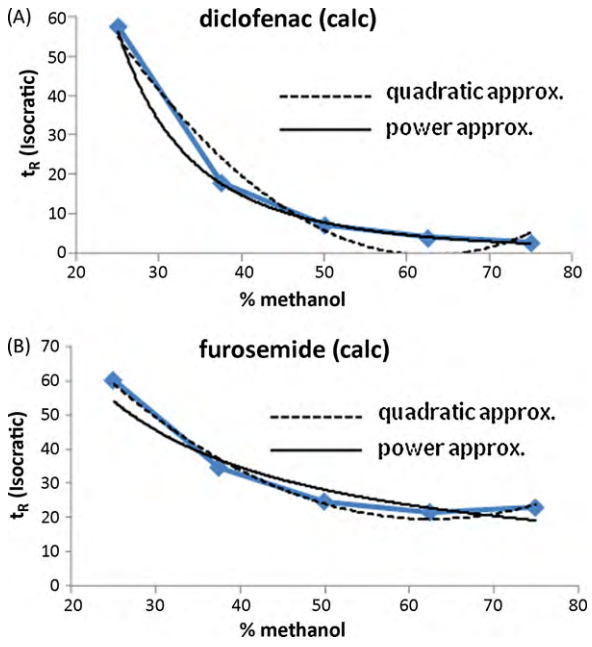

Fig. 5. Comparison of approximation curves to describe t_R vs. %MeOH plots for (A) diclofenac and (B) furosemide.

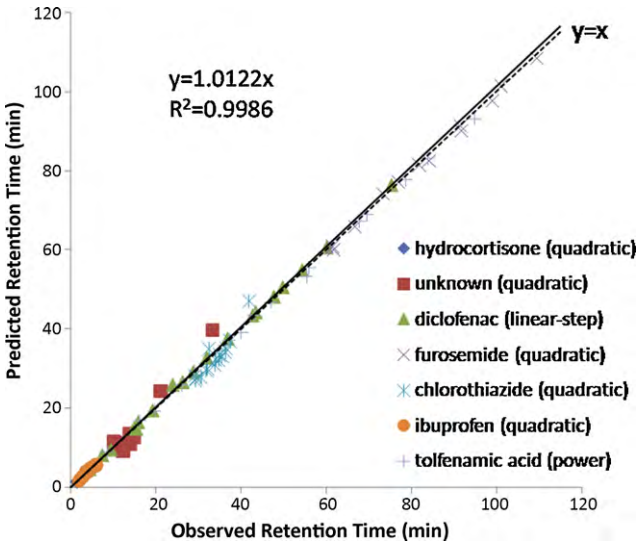

Fig. 6. Correlation between observed and predicted retention times for 19 linear methanol gradient conditions comprising differing starting and ending conditions and ramp rates. The best approximation curve for each compound was chosen by visual inspection of the curve fit and is shown on the figure. Conditions: column: 2 mm × 250 mm AS11 with AG11 guard column; eluent: 30 mM KOH; flow rate: 0.28 ml/min.

where M_i is the initial methanol percentage and r is the gradient ramp in %/min. Substituting Eq. (3) into $t_R(M)$ gives an expression for retention time in terms of t, $t_R(t)$. This expression can be used to derive the velocity of the analyte at any time along the separation, as shown by Eq. (4):

$$V(t) = \frac{L}{t_R(t)} \tag{4}$$

where L is the length of the column. The displacement, S, of the analyte in terms of t is simply the integral of $V(t)$ with respect to time, as given by Eq. (5):

$$S(t) = \int V(t)\,dt = \int \frac{L}{t_R(t)}\,dt \tag{5}$$

Setting $S(t) = L$ and solving Eq. (5) for t yields the retention time for the analyte under the gradient defined by Eq. (3). The general solutions for the 3 approximations for $t_R(M)$ employed in this work are shown in Eqs. (6)–(8) for the quadratic, power and linear-step approximations respectively.

$$t_1 = \frac{\sqrt{4ac - b^2}\,\tan[((l\sqrt{4ac - b^2})/2L) + \tan^{-1}((2at_0 - b)/\sqrt{4ac - b})] - b}{2a} \tag{6}$$

$$t_1 = \frac{[((lar(1-b))/L) + (M_i + rt_0)^{1-b}]^{1/1-b} - M_i}{r} \tag{7}$$

$$t_1 = \frac{e^{(lar/L)+\ln|art_0+[aM_i+b]|} - [aM_i + b]}{ar} \tag{8}$$

where a, b and c refer to the relevant constants for the curve approximation used to obtain $t_R(M)$ while l is the distance to be travelled

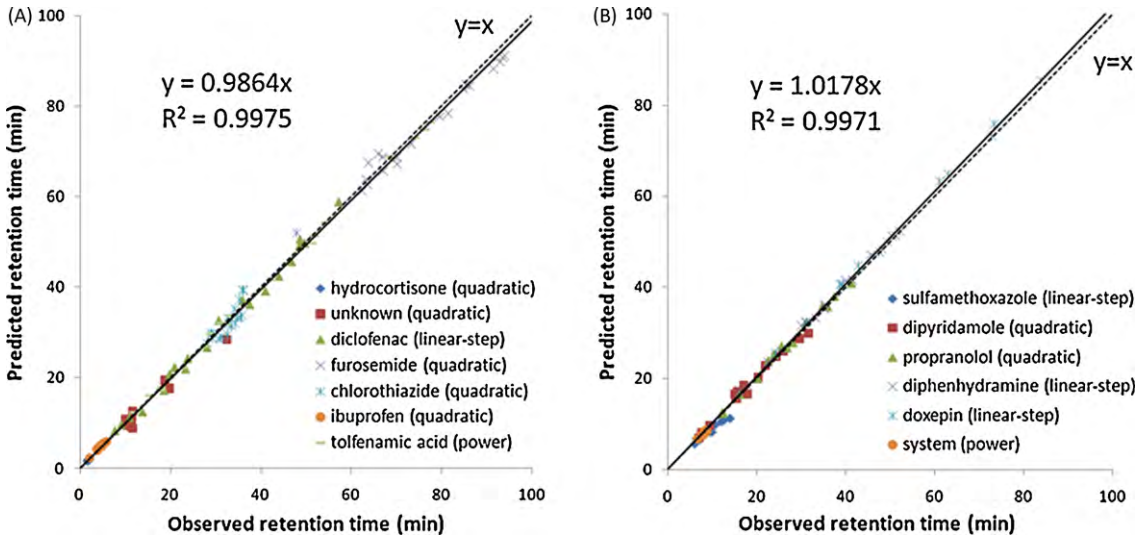

Fig. 7. Correlation between observed and predicted retention times using the multi-step isocratic and gradient models. (A) 20 multi-step conditions comprising 2- and 3-step methanol gradient profiles at 30 mM hydroxide. The best approximation curve for each compound was chosen by visual inspection of the curve fit. Conditions: column: 2 mm × 250 mm AS11 with AG11 guard column; flow rate: 0.28 ml/min. (B) 14 multi-step conditions comprising 2- and 3-step methanol gradient profiles at 20 mM MSA. The best approximation curve for each compound was chosen by visual inspection of the curve fit. Conditions: column: 2 mm × 250 mm CS14 with CG14 guard column; flow rate: 0.20 ml/min.

between t_0 and t_1. In solving for retention time over the whole column $t_0 = 0$, $t_1 = t_R$ and $l = L$.

Eqs. (6) and (7) can be used directly to determine the distance travelled by an analyte between t_0 and t_1, while Eq. (8) needs to be applied to each straight line segment in the linear-step approximation of $t_R(M)$. For example, if 5 points were used to derive $t_R(M)$, then Eq. (8) would need to be applied individually to the 4 linear segments between these points, followed by a summation to yield the total distance moved by the analyte.

Fig. 4 illustrates the effect on the predictive ability of the power approximation approach for $t_R(M)$ using diclofenac as a test compound with various numbers of data points to derive the power approximation. It can be seen that accuracy of the prediction increases with the number of points used to derive the curve approximation. The use of only 3 points leads to substantial errors, especially at longer elution time, while the use of 4 or more points provides reliable prediction of retention behaviour as the percentage of methanol in the eluent is varied. For the remainder of this study, 5 data points were employed for all gradient predictions.

The choice of the most appropriate approximation method (quadratic, power, or linear step) is vital for the accurate performance of the model. Fig. 5 shows quadratic and power approximations for the retention behaviour of both diclofenac and furosemide. The power approximation gives a better fit than the quadratic approximation for diclofenac, but the reverse is true for furosemide. In the situation where neither the power nor the quadratic approaches yield a sufficient fit, the linear-step approximation method is then used. In cases where the quadratic or power functions give a negative value for retention (such as the quadratic curve for diclofenac in Fig. 5A), an alternative approximation method, in this case the linear-step approximation, is used. Fig. 6 shows the correlation observed for 19 linear methanol gradient separations in which the best approximation method was used for each analyte, as indicated on the figure. The choice of the appropriate approximation curve was made by a visual inspection of the fit of each curve with the t_R vs. %MeOH. However, this choice could potentially be automated using appropriate criteria and software.

Peak width was modelled using a peak compression approach:

$$w = \left(\frac{4t_R}{\sqrt{N}}\right)\left(\frac{t_R}{t_{R_i}}\right)^2 \quad (9)$$

where N is the efficiency of the column measured in theoretical plates and under isocratic conditions, t_R is the observed gradient retention time and t_{R_i} is the equivalent isocratic retention time measured under the gradient starting conditions. This equation was derived experimentally and is based on a similar equation reported by Shellie et al. for inorganic ions [19] with the square root dependence replaced by a squared dependence to better reflect observed peak widths. Errors in predicted peak widths were < 17%, except for tolfenamic acid for which isocratic data at 25% MeOH could not be obtained due to excessively long retention times, requiring Eq. (9) to be extrapolated outside measured boundary points. As a general comment, peak width prediction is particularly problematic in cases where multiple analyte–stationary phase interactions are present and where the relative magnitudes of these interactions are likely to differ between analytes. In practice Eq. (9) yielded predicted peak widths which were generally slightly larger than the experimentally observed values. The exact reason for this is unknown, however, given the empirical nature of the model it does not affect its applicability to the described systems.

Prediction of retention for multi-step elution profiles containing sequential isocratic and gradient steps was achieved using the same approach as that reported in previous papers [17,19]. This involved mapping the position of the analyte on the column at the end of each individual step in the elution profile, until elution of the analyte from the column occurred. Fig. 7 shows the correlation between observed and predicted retention times using 20 multi-step eluent profiles for anions (Fig. 7A) and 14 multi-step eluent profiles for cations (Fig. 7B). The average error in retention times was 3.8% and 4.5% for anions and cations respectively and for peak width was 14.3% and 12.7% for anions and cations respectively.

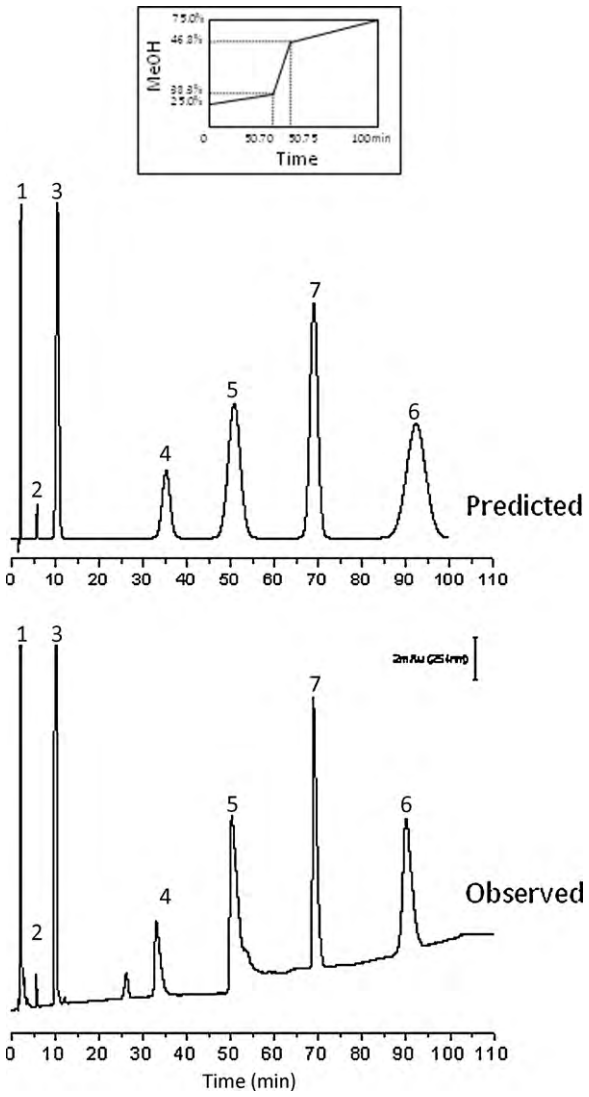

Fig. 8. Optimised (A) predicted and (B) observed separation of a 7-component mixture using the normalised resolution product criterion. Conditions: column: 2 mm × 250 mm AS11 with AG11 guard column; eluent: 30 mM hydroxide, methanol gradient as shown; flow rate: 0.28 ml/min; detection: UV at 254 nm. Peaks are: 1, hydrocortisone; 2, ibuprofen; 3, unknown associated with chlorothiazide; 4, chlorothiazide; 5, diclofenac; 6, furosemide; 7, tolfenamic acid.

3.6. Optimisation of separation using multi-step elution profiles

Optimisation of the separation of a mixture of a 7-component mixture of anions was performed using an iterative approach and the Solver function within Microsoft Excel. The search area was constrained to 3-step eluent profiles within the range of 25–75% methanol and a total separation time of 100 min was used. A normalised resolution product criterion was applied and this criterion is maximised when peaks are spread evenly over the chosen time window. Several starting points for the iterative optimisation process were used to avoid finding local maxima on the response surface.

Fig. 8 shows the optimised separation conditions, the predicted chromatogram, and the chromatogram obtained experimentally. Good agreement between the predicted and observed chromatograms is evident and there was an average error of 2.4% in the predicted retention times. Furthermore, it can be seen that the peaks are distributed evenly over the separation window, as would be expected from use of the normalised resolution product criterion. Peak widths for the experimental chromatogram were less than those predicted using Eq. (9).

4. Conclusions

Mixed-mode ion chromatographic separation of small- to medium-sized organic anions and cations of pharmaceutical interest has been demonstrated. Separation selectivity can be modified by either varying the concentration of competing ion (hydroxide or methanesulfonic acid) in the eluent, or changing the percentage of organic modifier (methanol) in the eluent. The effects on selectivity of each of these variables can be controlled and modelled independently, with the result that retention can be predicted accurately over the two-dimensional experimental space comprising [hydroxide] or [MSA] and %MeOH as variables. Extension of the model to incorporate linear methanol gradients has been demonstrated using an approach based on isocratic t_R vs. %MeOH data fitted to an appropriate curve approximation equation. Multi-step elution profiles consisting of isocratic and gradient steps were modelled successfully by mapping the analyte position on the column at the end of each step of the elution profile. Optimisation of the multi-step elution profile was achieved using an iterative approach and the normalised resolution product criterion. Further studies are continuing with the intention of improving the prediction by using more accurate curve approximations as well as an algorithm to select the best fitting curve automatically.

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