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Demonstration of simultaneous cation-exchange and reversed-phase mechanisms on a strong-acid cation-exchange column

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Abstract

It is demonstrated in this report that a conventional strong-acid cation-exchange column can exhibit reversed-phase chromatographic behavior simultaneously with ion-exchange. Adjusting the pH to control cation retention has no effect on the retention of neutral organic analytes. Likewise, changes in the methanol content of the mobile phase to adjust organic analyte retention causes only a small decrease in retention of metal ions in the 0 to 10% (v/v) methanol range, and no significant effect beyond that. Linear calibration behavior of both metal cations and neutral organic analytes is found on this column over three-order of magnitude. Examples of simultaneous metal cation–neutral organic separations in both the isocratic and gradient modes are shown, with conductivity detection for the metal ions and UV for the organic analytes. An isocratic separation of metal ions and neutrals in a vitamin pill is also demonstrated.

Keywords: Retention mechanisms; Stationary phase, LC; Ion exchangers; Mobile phase composition; Metal ions; Vitamins

1. Introduction

Our laboratory has been interested for several years in the simultaneous liquid chromatographic separation of mixed classes of analytes, especially inorganic ions and neutral organic compounds. Samples that contain both inorganic and organic species of interest might then be analyzed in one run rather than with two or more chromatographic or other methods. Our initial studies utilized high-performance liquid chromatographic (HPLC) phases synthesized in our laboratory [1] or ion interaction (ion-pairing) chromatographic approaches [2]. More recently we have employed commercially available HPLC phases to simultaneously separate ionic and neutral analytes [3]. That report [3] also describes work on both simultaneous and sequential mixed-class separations by other laboratories. Little et al. [4] have given both a theoretical and an applied treatment of sequential multimode elution using silica-based reversed-phase columns.

In order to effect such mixed-class separations three criteria must be met. First, the stationary phase must have interactions suitable for each class of analyte, for example exchange sites for ions, and nonpolar moieties to provide dispersion interactions with neutral compounds. Second, the mobile phase must be compatible with each class of analyte. For

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example, it might be water based so that both ionic analytes as well as eluting ions are soluble, and incorporate a miscible organic cosolvent to regulate retention and provide solubility for neutral organic analytes. Third, detection capability must be available for each analyte class, for example conductance for ionic analytes and UV for organic species.

It turns out that reversed-phase type mobile phases and ion-exchange stationary phases permit the first two criteria to be met. Both ions and neutrals are soluble in these mobile phases, and the stationary phases are of the mixed-mode type, containing both ionic and nonpolar sites. Afrashtehfar and Cantwell state that nonionic interactions are the same on an organic polymer-based ion-exchange phase as on the neutral parent polymer itself [5]. Also, Dumont and Fritz [6] utilized a sulfonated polystyrene–divinylbenzene copolymer to perform solid-phase extraction of polar organic analytes from aqueous samples, the bound ionic group providing hydrophilic character to the phase. We have been interested in demonstrating similar mixed-mode interactions on silica-based phases. The most recent report from this laboratory described the simultaneous separation of mixed anion–organic and cation–organic analytes primarily on silica-based weak ion-exchange phases [3]. Some preliminary results were also included on a typical silica-based strong cation-exchange phase which afforded better efficiency for metal ion separation than the weak-acid cation-exchangers studied. The present report provides follow-up work on the simultaneous separation of mixtures of metal cations and neutral organic compounds on a strong-acid benzene-sulfonate cation-exchange phase. We also report data on the effect that retention adjustment of cations has on the retention of the organic analytes, and vice versa. In addition, application of the method to a multivitamin sample is demonstrated.

2. Experimental

2.1. Apparatus

The chromatographic system consisted of a Shimadzu LC-10AD solvent delivery module; a Shimadzu FCV-10AL low-pressure gradient unit; and a Rheodyne 7010 injection valve with a 10-μl sample loop. An Alltech Model 320 conductivity detector and an ISCO Model V^4 variable-wavelength ultraviolet–visible detector were connected in series to monitor cations and neutral organic compounds, respectively. The UV wavelength was generally set about 250 nm. Detector output was analyzed with EZChrom data acquisition software (version 6.7) from Scientific Software, Inc.

The analytical column was 150×4.6 mm, packed with spherical 5-μm, 100-Å pore Nucleosil SA strong-acid cation-exchange material (Machery-Nagel, from Alltech Associates). The stationary phase is trimethylsilyl end-capped. The system was fitted with a 5-cm mobile-phase saturator column packed with plain silica gel, and placed before the injection valve. A guard column packed with RSil (Alltech) strong-acid cation-exchanger material was placed between the injector and analytical column.

2.2. Reagents and solutions

All mobile phases and analyte solutions were made up in water purified with the Nanopure (II) System (Barnstead). Reagents were either HPLC or ACS grade. Analyte salt stock solutions were made up in methanol–water solvent (30:70, v/v). Polar organic analytes and PAHs up to four rings were made up in 100% methanol. Stock solutions of larger PAHs were prepared in methanol–acetone (50:50, v/v). All analyte solutions were diluted in mobile phase before injection. Mobile phases and analyte solutions were filtered through 0.45-μm membrane filters. The pH values of aqueous–organic mobile phases are apparent values as the pH meter was calibrated with aqueous buffers.

The ''One-a-Day'' multivitamin was ground up with a mortar and pestle and sonicated in mobile phase for a few minutes. The mixture was further diluted with mobile phase and filtered through a 0.45-μm membrane filter to remove insoluble filler material.

2.3. Chromatographic procedures

All chromatography was performed at ambient temperature. Flow rate was 1.0 ml/min. The column

dead time (t_o) was determined from the injection of methanol. Although the methanol injection peak retention time decreased by 10–15% on going from 0 to 60% (v/v) methanol in the mobile phase it provided a convenient "t_o" marker, and allowed self-consistent retention factor data to be obtained. The column was generally equilibrated for 1 h with mobile phase before injections were made. Each week the column was flushed with a methanol–water mix (50:50, v/v) and then by 100% methanol, for about 30 min each.

3. Results and discussion

The key to any chromatographic experiment, of course, is the differential interactions of analytes with the stationary phase. As mentioned above, to separate mixed classes of analytes from each other and, perhaps, from other classes, stationary-phase interactions must be present for each of the classes. In an earlier study we achieved this condition using a C_{18} column in the ion-interaction (ion-pair) mode where mobile-phase surfactants adsorbed on the stationary phase provided a temporary ion-exchange site and the C_{18} groups allowed dispersion interactions [2].

The strong-acid cation-exchange column employed in this study is a conventional silica-gel-supported benzene-sulfonate phase. The benzene moiety is linked to the silica surface through a propyl chain, and residual silanols are trimethylsilyl end capped. The $-SO_3^-$ group on the benzene ring is the cation-exchange site, of course, but also makes the propyl benzene surface linker more compatible with aqueous mobile phases, as discussed further below. The organic appendage, ostensibly present only to connect the ion-exchange site to the silica surface, in fact provides dispersion interactions needed to allow differential phase transfer of neutral species. The trimethylsilyl end-capping groups are, in effect, a C_1 phase, providing additional dispersion interactions. And so, this strong-acid cation-exchange material is a mixed-mode, mixed-ligand HPLC phase. In this report we show that it and, by implication, other strong-acid cation-exchangers, can be used to separate cations and a wide range of neutral organic analytes in one run, or can simply be used in the conventional ion-exchange or reversed-phase modes.

In order to separate metal cations from each other by k regulation the pH and/or ionic strength of the mobile phase is adjusted, and metal complexors are often added. The present work employed ethylenediammonium ion as the competing cation and tartrate as a differentiating metal complexor [3,7]. We discuss below the effect of mobile phase pH changes, not only on metal ion retention, but also on that of neutral organic analytes. Methyl alcohol was used as the reversed-phase cosolvent and we likewise describe its effect on retention of both the organic and metal cation analytes.

3.1. Sample chromatograms

The earlier report from this laboratory on mixed ion–neutral separations using ion-exchange phases showed the separation of four metal cations and six neutral organic compounds in one run on a strong-acid cation-exchanger [3]. The cations were detected conductimetrically and the organic analytes spectroscopically at 254 nm. It was noted that the separation was effected at a relatively low concentration of methanol (15%, v/v) for the polar organic analytes tested. In Fig. 1 here we show further exploitation of the ability of this strong-acid cation-exchanger to also operate in the reversed-phase mode with relatively low amounts of organic cosolvent. In this ethylenediammonium–tartrate mobile phase anthracene is eluted at about 4 min with only 60% methanol. It should be noted that the metal–ion complexes are less conducting than that of the background mobile phase. This results in negative peaks when a metal ion–tartrate complex is exchanged for an ethylenediammonium ion in the analyte zone [7].

Fig. 2 further illustrates this low cosolvent advantage of the ion-exchange phase. Here three metals and two organic analytes are simultaneously separated by the ethylenediammonium–tartrate mobile phase with no added methyl alcohol cosolvent. The presence of the $-SO_3^-$ exchanger group on the organic appendage makes it compatible with the aqueous phase and minimizes self-assembly or collapse of the bonded groups such as would occur with C_{18} or C_8 groups in an all-aqueous mobile phase [8].

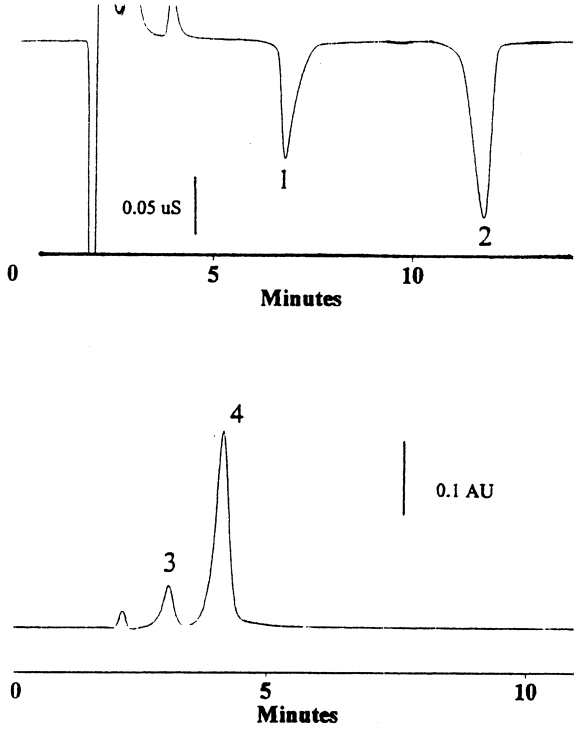

Fig. 1. Simultaneous determination of metal cations and PAHs on a strong-acid cation-exchange column. Mobile phase: methanol–water (60:40, v/v), 5 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4. Detection: top, conductivity; bottom, UV at 254 nm. Analytes: (1) Zn(II), (2) Mg(II), (3) naphthalene, (4) anthracene.

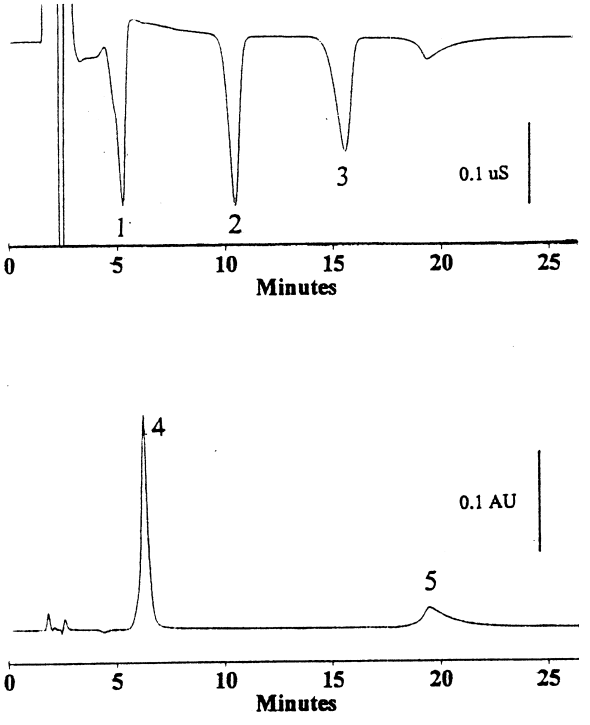

Fig. 2. Simultaneous determination of metal cations and neutral organics on a strong-acid cation-exchange column in an all-aqueous mobile phase. Mobile phase: 100% water, 5 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4. Detection: top, conductivity; bottom UV at 254 nm. Analytes: (1) K(I), (2) Zn(II), (3) Mg(II), (4) *p*-nitrophenol, (5) *p*-chloroaniline.

3.2. Mobile-phase effects

In order to be able to exploit ion-exchange phases for reversed-phase or mixed exchange–reversed-phase separations a reasonably good understanding of mobile-phase effects is needed for these systems. That is, in adjusting *k* values of ions by pH and/or ionic strength changes, what is the effect on the *k* of neutral organics? Likewise, how do changes in the organic cosolvent concentration affect retention of charged species?

It is to be expected that increases in either the ethylenediammonium eluting ion concentration or that of the tartrate metal–ion complexor would decrease retention of metal cations. This has been demonstrated in our earlier report [3] and in the work of others [7,9,10]

Further, an increase in pH above the pH 3–4 range used in this work would have the same result as increasing the tartrate concentration, as the metal–tartrate effective formation constant would increase, and retention would decrease [11]. These effects are well established and were not repeated in this study.

3.2.1. pH effects on organic retention

While it is also well known that the retention of neutral organic compounds in reversed-phase HPLC on nonpolar stationary phases is unaffected by mobile phase pH changes [12,13], whether this would remain so on the strong-acid cation-exchange phase had to be demonstrated. Fig. 3 shows that retention is essentially unaffected by pH changes in the range of 2.4–6.4. Note that the points at pH 5.1 are an average of three sets of runs and those at pH 2.4 were run 1 month later than the rest. Neither the

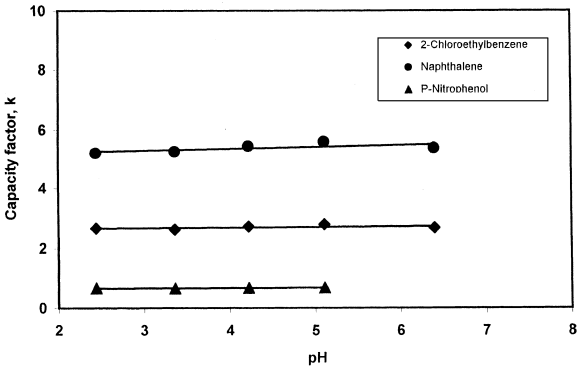

Fig. 3. Effect of pH on retention factor of neutral organic analytes on a strong-acid cation-exchange column. Mobile phase: methanol–water (20:80, v/v), 5 m*M* tartrate, 2.5 m*M* ethylenediammonium. Data at pH 5.1 are average of three sets of three runs each. Data at pH 2.4 were run 1 month later than the rest.

polar nor the nonpolar organic analytes showed any pH effect.

3.2.2. Methanol effects on cation retention

The effect of changes in methanol cosolvent concentration on both the cations and organic analytes also had to be examined. Fig. 4 shows the effect on the *k* of Zn(II) and Mg(II) from 0 to 60% (v/v) methanol. To be sure that the slight decrease in *k* between 0 and 10% methanol was real the 0% methanol experiment was run three times on different days with different batches of mobile phase. The

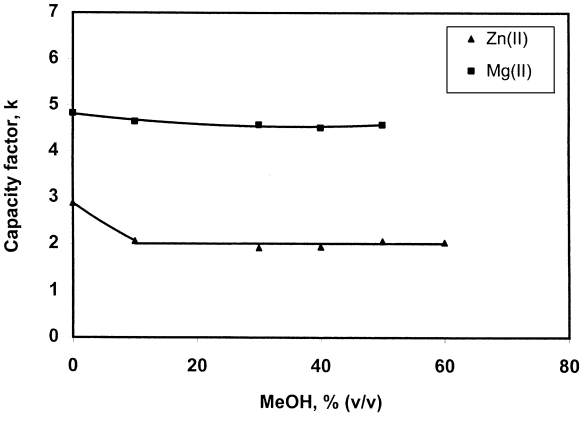

Fig. 4. Effect of methanol concentration on retention factor of metals on a strong-acid cation-exchange column. Mobile phase: 5 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4. Points at 0% methanol are the average of three sets of data.

relative standard deviation of this data was 0.3% for Zn(II) and 0.1% for Mg(II). The deviations of the other points were similar. The data in Fig. 4 indicate that *k* decreases slightly in the 0–10% (v/v) methanol range, but is essentially constant after that. A similar decrease in *k* for alkaline earth cations in the 0–20% methanol range has been observed on a silica gel column [13, p. 447; 9]. Two competing effects apparently operate here. First, the added methanol decreases the mobile-phase dielectric constant, resulting in increased interaction of the cations with the exchange sites. Secondly, the methanol causes an increase in the metal ion–tartrate formation constant leading to decreased retention [9]. This second effect seems to slightly predominate at low methanol concentrations but the two effects essentially balance out as more methanol is added. Earlier work shows only an increase in retention for alkali metal ions which do not form tartrate complexes [14].

3.2.3. Methanol effects on organic retention

It was of interest to determine if reversed-phase behavior of neutral organic analytes is followed on the cation-exchange stationary phase in the presence of the ionic eluting agents in the mobile phase. Fig. 5 shows the results for both polar and nonpolar organic analytes with added methanol cosolvent. Note again that the more polar analytes, nitrophenol and chloroaniline, were run at 0% methanol. The data in Fig. 5 show a 2–3-fold decrease in *k* with a 10% increase in methanol concentration, typical for reversed-phase interactions [13, p. 260]. It appears, then, that reversed-phase behavior is exhibited by organic analytes on this cation-exchange column even in the presence of mobile-phase conditions tailored to cation-exchange. Manipulating the mobile phase to adjust *k* for one class of analyte has little or no effect on *k* of the other class. The ion-exchange and reversed-phase mechanisms operate essentially independently of each other.

3.3. Gradient separation

Having established that reversed-phase behavior is exhibited by both polar and nonpolar organic species it was of interest to attempt a mixed-class separation with neutral analytes that exhibit a wide range of polarities. As in conventional reversed-phase LC this

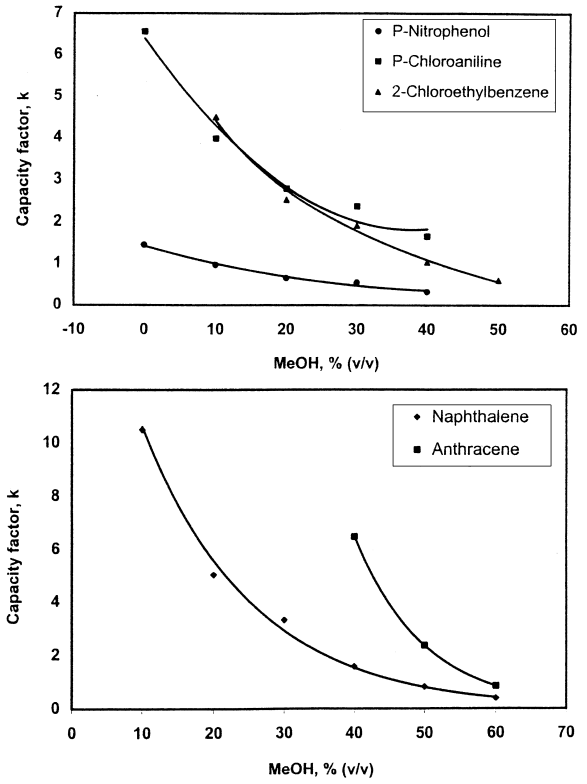

Fig. 5. Effect of methanol concentration on retention factor of organic analytes on a strong-acid cation-exchange column. Mobile phase: 5 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4.

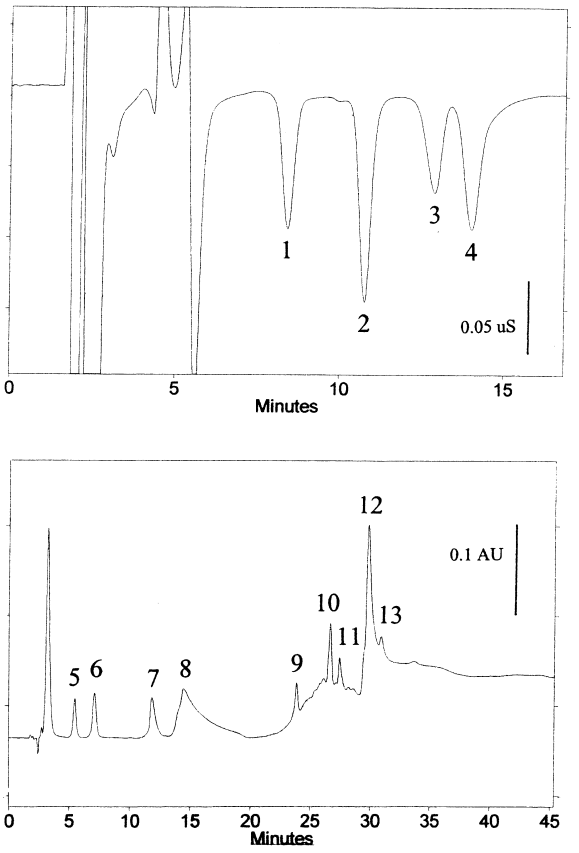

Fig. 6. Gradient chromatogram of a mixture of metal cations and polar and nonpolar organics on a strong-acid cation-exchange column. Mobile phase: A, 100% methanol; B, methanol–water (5:95, v/v), 8 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4. Detection: top, conductivity; bottom, UV at 260 nm. Gradient program: 0 min, 0% A; 12 min, 0% A; 20 min, 65% A (linear change); 30 min, 75% A (linear change); 50 min, 75% A. Analytes: (1) Pb(II), (2) Zn(II), (3) Co(II), (4) Ca(II)/Mg(II) coeluted, (5) *p*-nitrophenol, (6) *p*-nitroaniline, (7) acetophenone, (8) *p*-chloroaniline, (9) naphthalene, (10) anthracene, (11) pyrene, (12) benzo[*a*]pyrene, (13) benzo[*ghi*]perylene.

requires a gradient approach. Fig. 6 demonstrates this experiment. The results here again demonstrate the reversed-phase behavior of neutral organic compounds on the strong-acid exchanger in a cation-eluting mobile phase.

Note that the gradient was started after 12 min to allow the cations to elute isocratically so as to minimize base-line disturbance in the conductivity mode. Gradient elution commonly causes base-line changes for bulk-property detectors, as we observed with the conductance detector. As the cations in this sample eluted in the same time range (5–15 min) as the early eluting organic analytes, selective (conductance) detection was necessary. It turned out that a gradient was not needed for these early-eluting analytes and so base-line disturbance was avoided. If a mobile phase can be chosen such that cations and organics elute as groups in different time windows (see Ref. [3], anion/organic mixtures) then an alternate detection mode for cations, e.g. indirect UV, can be chosen that would be less susceptible to gradient base-line effects. However, more studies will be needed to fully test this concept.

3.4. Efficiency considerations

The efficiencies of the ion-exchange and reversed-phase modes obtained in this study are modest. Plate

counts were about 15–20 000 plates per meter for organic analytes and about 20 000 plates per meter for ionic species. These values, especially for the reversed-phase mode, would be cause for concern for complex mixtures containing many neutral compounds. It should be mentioned, however, that we did not attempt to optimize efficiency in this study, and it is possible that improvement can be obtained with suitable choice of column packing and mobile phase.

3.5. Quantitation studies

In order to establish efficacy for this mixed-class approach routine quantitation studies were necessary. Log–log plots were obtained for Pb(II), Zn(II) and Mg(II), as well as for *p*-nitrophenol and *p*-nitroaniline. Plots were obtained using both peak height and peak area from near the detection limit to the concentration at which column overload began to appear (as evidenced by retention time changes).

Table 1 gives the regression information. Standard deviations of slope and intercept are mostly in the 1–3% relative range. Correlation coefficients are at least two ''9s'', and slopes are close to the value of unity expected for log–log plots. Metal ions showed linear behavior from about 10^{-2} to 10^{-5} *M*, while the organics were linear in the 10^{-2}–10^{-6} *M* range. Table 2 gives limits of detection based on a signal-to-noise ratio of 3, where the noise was taken as the peak-to-peak base-line fluctuations.

3.6. An example application

The simultaneous chromatographic determination of both ionic and neutral analytes has potential applications in environmental, medical and food analysis, among others. In order to demonstrate the utility of the approach we ran a number of samples, including a red wine in which at least four ionic and about 10 neutral species were seen at relatively high levels (not shown). Fig. 7 shows another example, that of a ''One-A-Day'' vitamin pill. The peaks seen are those which resulted from a mobile-phase extract of a crushed pill. Zn(II), Ca(II) and Mg(II) were positively identified, as were vitamin C and niacin (nicotinamide). Peak 3 has the same retention time as Mn(II), which is present according to the label, but this assignment was not confirmed. Like Mn(II), other mineral ions and vitamins were present as minor constituents. As the sensitivity of the detectors was set for the major components mentioned above, minor-component peaks are not seen.

Quantitation of several of these major components was performed by standard addition. Good recoveries (compared to label values) were obtained for Zn(II) and niacin, but low results for Ca(II) and Mg(II). As the point was to demonstrate the potential of the simultaneous ion–neutral separation approach

Table 2
Limit of detection of analytes on a strong-acid cation-exchange column in mobile phase in Table 1

Analyte	limit of detection (ng)[a]
Pb(II)	104
Zn(II)	3.3
Mg(II)	12
p-Nitrophenol	7
p-Nitroaniline	7

[a] Based on $S/N=3$; N=peak-to-peak base-line fluctuations; 10 μl injection volume.

Table 1
Calibration curve regression information for a three metal–two organic mixture on a strong-acid cation-exchange column[a]

Analyte	Slope±SD	Intercept±SD	Correlation coefficient	Concentration range (mol/l)
Pb(II)	0.86±0.04	8.6±0.1	0.996	5×10^{-5}–1×10^{-2}
Zn(II)	0.91±0.03	9.3±0.1	0.997	1×10^{-5}–1×10^{-2}
Mg(II)	1.02±0.001	9.4±0.04	0.999	1×10^{-5}–1×10^{-2}
p-Nitrophenol	0.96±0.02	8.8±0.1	0.999	1×10^{-6}–1×10^{-3}
p-Nitroaniline	0.90±0.03	8.8±0.2	0.996	1×10^{-6}–1×10^{-3}

[a] Log peak area vs. log concentration. Mobile phase: methanol–water (5:95, v/v), 5 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4.

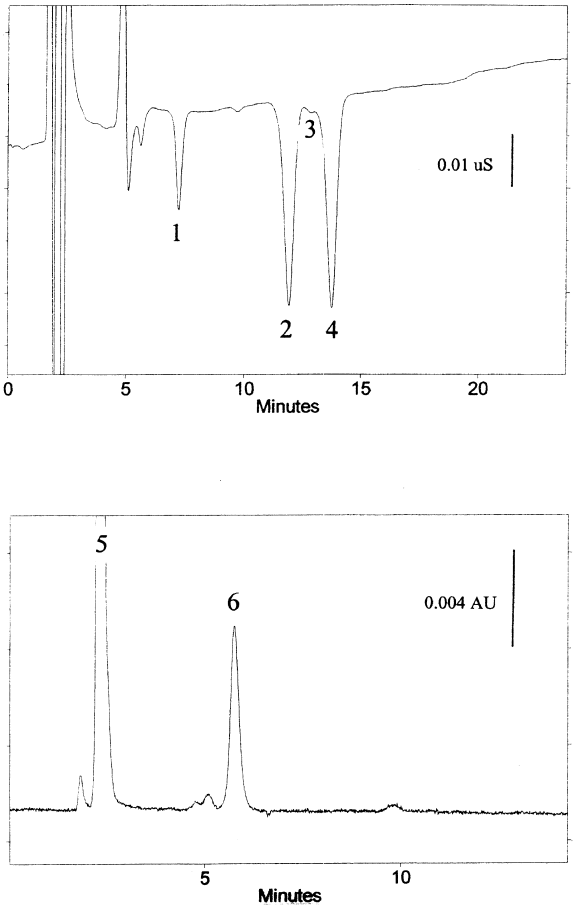

Fig. 7. Chromatogram of aqueous extract of ''One-A-Day'' multivitamin on a strong-acid cation-exchange column. Mobile phase: methanol–water (10:90, v/v), 8 m*M* tartrate, 2.5 m*M* ethylenediammonium, apparent pH 3.4. Detection: top, conductivity; bottom, UV at 254 nm. Analytes: (1) Zn(II), (2) Ca(II), (3) Mn(II), (4) Mg(II), (5) vitamin C, (6) niacin (nicotinamide).

and not to characterize the vitamin, no further work was done to improve this quantitation.

4. Conclusions

The results presented here indicate that the reversed-phase mechanism operates on the strong-acid cation-exchange stationary phase, and that this mechanism and cation-exchange operate essentially independently. Changing the pH has no effect on neutral organic–analyte retention, and changing methanol concentration has little or no effect on metal–ion retention. Linear behavior obtains over about three orders-of-magnitude for both metal cations and neutral organics. It would appear that strong-acid cation-exchange columns can be used not only in the conventional metal–ion determination mode, but also in the reversed-phase mode for organic compounds, even down to 0% organic modifier. Lastly, these two modes can be combined to perform simultaneous cation–neutral organic analyte separations on a silica-based cation-exchange phase. It is likely that similar results can be expected on other silica-based ion-exchange phases as well as on organic polymer-based packings. Despite the relatively modest efficiencies obtained in this and our earlier study [3], it appears that simultaneous mixed-mode separations on ion-exchange phases have a place in the repertoire of the separation scientist.

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