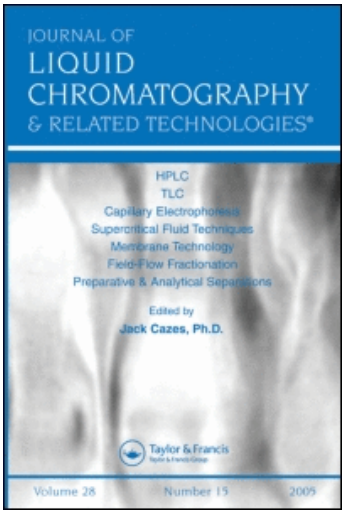

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Separation of Organic Analyte Cations on a Low-Capacity Cation Exchange Column Using Indirect UV Detection

Thomas A. Walker[a]
[a] The NutraSweet Company, Illinois

To cite this Article Walker, Thomas A.(1988) 'The Separation of Organic Analyte Cations on a Low-Capacity Cation Exchange Column Using Indirect UV Detection', Journal of Liquid Chromatography & Related Technologies, 11: 7, 1513 — 1530
To link to this Article: DOI: 10.1080/01483918808067190
URL: http://dx.doi.org/10.1080/01483918808067190

JOURNAL OF LIQUID CHROMATOGRAPHY, 11(7), 1513-1530 (1988)

THE SEPARATION OF ORGANIC ANALYTE CATIONS ON A LOW-CAPACITY CATION EXCHANGE COLUMN USING INDIRECT UV DETECTION

Thomas A. Walker
The NutraSweet Company
601 E. Kensington Road
Mt. Prospect, Illinois 60056

ABSTRACT

The retention of organic analyte cations on a low-capacity cation exchange column using indirect UV detection was studied. It was found that a combination of cation exchange/reversed-phase interactions affected the retention of organic analyte cations provided the analytes have both a cationic charge site and a hydrophobic center. The factors that influenced the organic analyte cation retention were: concentration of organic modifier, concentration of UV absorbing analyte, pH, and mobile phase ionic strength. Elution orders for several of the organic analytes studied on the low-capacity cation exchange column were different than those observed on silica-based strong cation exchange columns.

INTRODUCTION

Indirect photometric or "vacancy" chromatography (IPC) is an analytical method where cations and anions are separated on an ion exchange column and are then

detected through a photometric process (1-4). IPC, which was originally introduced by Small and Miller in 1982 (1), consists of an UV-absorbing counterion in the mobile phase that competes with UV-transparent, injected analytes for the ion exchange sites. As the UV-transparent analyte elutes off the column, it replaces the UV-absorbing counter-ion in the effluent which leads to a decrease in absorbance at the detector and produces a negative peak. Several advantages of IPC are apparent and include; conventional HPLC instrumentation and columns are used, sensitivity is greater when compared to refractive index and conductometric detection (3,4), standardless quantitation (2,3,5,6), and versatility.

IPC has become a commonly used method for the analysis of inorganic and organic UV-transparent analyte ions where a strong cation or anion exchange column is used (7-15). Indirect UV-detection has also been extended to reversed-phase (16,17) and ion-interaction chromatographic separations (18-23). One area of liquid chromatography where little research has been done are separations where low-capacity polymeric ion exchange columns are used in conjunction with indirect UV-detection. Low-capacity ion exchange columns are composed of a high surface area, macroporous polystyrenedivinylbenzene copolymer that is lightly sulfonated. These packings offer the advantages of having both ion exchange and reversed-phase properties, and are stable from pH 1 to 13. Cantwell et al. (24-26) has shown that retention of an organic analyte ion on a low-capacity ion exchange column is due primarily to two interactions; 1) adsorption of the organic analyte ion onto the nonpolar polymeric backbone, provided that the organic analyte has a hydrophobic center, and 2) ion exchange of the organic analyte ion in the diffuse part of the electrical layer resulting from the ion exchange

site and its counterion. These types of ion exchangers have been used for the separation of both inorganic and organic analytes (24-29).

This paper describes the mobile phase parameters that affect the separation, indirect UV detection, and quantitation of tetraalkylammonium salts, alkylamines, and amino acids on a low-capacity polymeric cation exchange column.

EXPERIMENTAL

Chemicals.

HPLC grade acetonitrile was obtained from Fisher Scientific, Fairlawn, NJ. HPLC grade water was obtained by passing de-ionized water through a Millipore water purification unit. Benzyltrimethylammonium chloride, tetraalkylammonium salts, and alkylamines were obtained from The Aldrich Chemical Company, Milwaukee, WI. L-Lysine, L-arginine, L-ornithine and 2,4-diaminobutyric acid were obtained from Sigma Chemical Company, St. Louis, MO. Glacial acetic acid and concentrated phosphoric acid were obtained from Mallinckrodt, Paris, KY. All chemicals were reagent grade.

Instrumentation.

The liquid chromatographic apparatus used consisted of a WISP Model 710B Autosampler, Waters Model 590 pump, Kratos Model 783 variable wavelength detector, Linear Model 500 strip chart recorder. The column used in this study was a 4.1 x 150 mm Hamilton PRP-X200 low-capacity cation exchange column available from Hamilton Company, Reno, NV. The PRP-X200 column is a spherical, 10 µm poly(styrenedivinylbenzene)sulfonate packing with a cation exchange capacity of 35 µEq/g. Flow rates of 1.0 mL/min were used unless otherwise noted. Aqueous analyte samples of approximately 1 mg/mL were used. Sample

aliquots of 10 µl were used, except for quantitation where 50-µl aliquots were used. Inlet pressures of 500-600 psi were observed.

RESULTS AND DISCUSSION

Low-capacity ion exchange columns have been shown to have a dual retention mechanism of ion exchange and adsorption for organic analyte ions that contain both a fixed charge site and a hydrophobic center (25-27). For this dual mechanism to be present, the stationary phase must be nonpolar, have a high surface area and provide relatively few ion exchange sites. Cantwell et al. (29) has shown that adsorption of an organic analyte ion is dependent on the electrical potential of the surface (due to the number of ion exchange sites) while ion exchange is independent of electrical potential. The two mechanisms, adsorption (Ads) and ion exchange (IE), can be represented by the following equations:

$$A\text{-}SO_3^-C^+ + R\text{-}X^+ + M^- \overset{\text{Ads}}{\rightleftharpoons} M^-X^+\text{-}R\cdots A\text{-}SO_3^-C^+ \quad (1)$$

$$A\text{-}SO_3^-C^+ + R\text{-}X^+ + M^- \overset{\text{IE}}{\rightleftharpoons} A\text{-}SO3^{-+}X\text{-}R + C^+ + M^- \quad (2)$$

where A represents the copolymeric matrix, C^+ is the countercation, $R\text{-}X^+$ is an analyte with a cationic site X^+ and a hydrophobic center R, and M^- is the mobile phase counteranion.

Retention of an organic analyte by an adsorption process is dependent on the following mobile and stationary phase parameters: 1) concentration of organic modifier in the mobile phase, 2) ionic strength, 3) pH, 4) hydrophobicity of the organic analyte ion, and 5) hydrophobicity of the stationary phase. Increasing the concentration of organic modifier in the mobile phase

will lead to a decrease in organic analyte retention. The pH of the mobile phase will affect the charge of the analyte and its hydrophobicity. Adjustments in ionic strength will produce a change in analyte retention. The hydrophobicity of the stationary phase is dependent on the number of ion exchange sites present on the packing. As the number of ion exchange sites are increased, adsorption of an analyte will decrease. In this study, the effect of the number of ion exchange sites present was not studied but has been reported elsewhere (15-20).

Retention of an organic analyte ion by an ion exchange process will be influenced by: 1) ionic strength, 2) concentration of countercation, 3) mobile phase pH, and 4) stationary phase cation exchange capacity. According to eq. 2, as the mobile phase ionic strength is increased, analyte retention will decrease due to increased competition for the ion exchange sites. Mobile phase pH will affect the ionization of a weak base. Its retention due to ion exchange, therefore, will be dependent on its degree of ionization. In this study both the buffer and the added UV-absorbing countercation will influence the mobile phase ionic strength and pH. The effect of analyte retention due to the number of cation exchange sites was not studied here but has been reported elsewhere (24-29).

In Indirect Photometric Chromatography (IPC), the UV-absorbing countercation has a dual role: 1) displacement of an analyte cation from the cation exchange column and 2) detection of an UV-transparent analyte cation as a dip or trough in the baseline absorbance. When a low-capacity cation exchange column is used for separating organic analyte cations, the UV-absorbing countercation will be involved in the detection of the organic analyte cation and will compete for the cationic exchange sites. If the UV-transparent

analyte is charged, the UV-absorbing countercation will compete with the charged analyte for the cation exchange sites and will also participate in the indirect detection of the analyte cation. However, if retention of the UV-transparent analyte is predominantly by adsorption, then the UV-absorbing countercations major role is in the indirect detection of the analyte.

The first mobile phase variable studied was the concentration of added organic modifier. It would be expected that as the concentration of organic modifier is increased analytes that are retained predominatly by adsorption should decrease in retention. As shown in Figure 1, the retention of tetrapentylammonium chloride ($TPeA^+Cl^-$) decreased significantly indicating that adsorption is the predominant retention mechanism, while the retention of tetramethylammonium chloride (TMA^+Cl^-) increased indicating that its retention is due primarily to cation exchange. Increasing the mobile phase concentration of acetonitrile leads to a change in the relative polarity of the mobile and stationary phases. As the mobile phase becomes more nonpolar, the stationary phase, with respect to the mobile phase, increases in polarity. Analytes that are cationic and have a small hydrophobic center will tend to be attracted toward the phase that is more polar and will, therefore, be more highly retained on the cation exchange sites as the concentration of acetonitrile increases. Figure 2 shows the separation of the tetraalkylammonium salts (TAA^+) at two different concentrations of acetonitrile. At 70% acetonitrile, tetraethylammonium chloride (TEA^+Cl^-) and TMA^+Cl^- eluted after $TPeA^+Cl^-$, while tetrapropylammonium chloride ($TPrA^+Cl^-$) and tetrabutylammonium chloride (TBA^+Cl^-) were separated but not baseline resolved. When the concentration of acetonitrile was reduced to 60%, $TPeA^+Cl^-$ eluted after TMA^+Cl^- and TEA^+Cl^-, while the

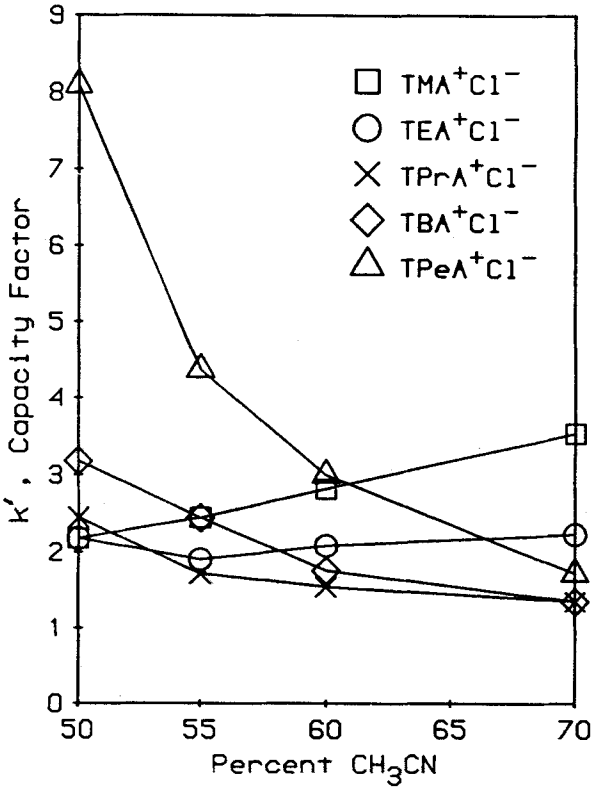

FIGURE 1

The Effect of CH_3CN Concentration on the Retention of TAA^+ Salt Cations.

A 0.003 M $BTMA^+Cl^-$, 0.01 M CH_3COOH, CH_3CN:H_2O Mobile phase.

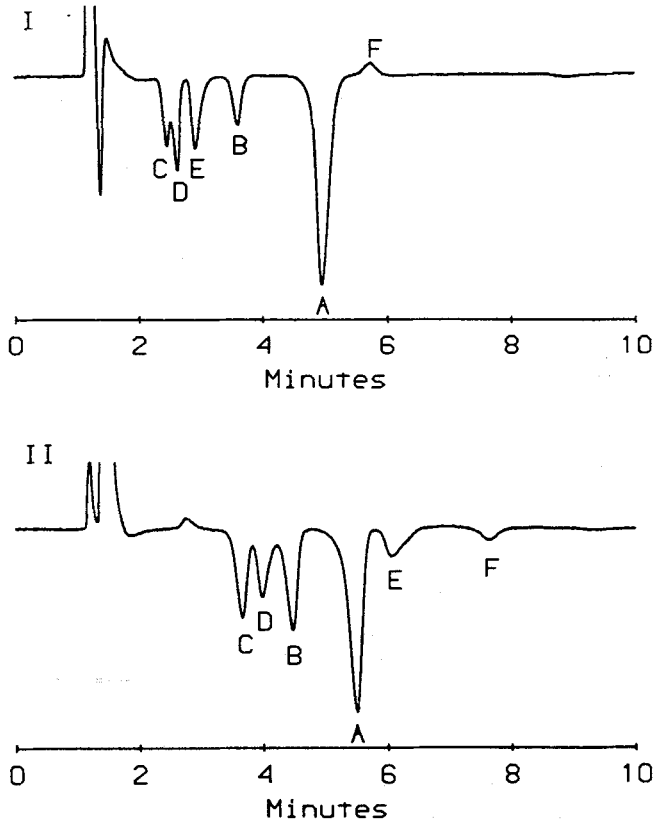

FIGURE 2

The Separation of A) TMA^+Cl^-, B) TEA^+Br^-, C) $TPrA^+Br^-$, D) TBA^+Cl^-, E) $TPeA^+Cl^-$, F) System Peak, at Different Concentrations of CH_3CN.

I) A 7:3 $CH_3CN:H_2O$, 0.003 M $BTMA^+Cl^-$, 0.01 M CH_3COOH mobile phase.

II) A 6:4 $CH_3CN:H_2O$, 0.003 M $BTMA^+Cl^-$, 0.01 M CH_3COOH mobile phase.

resolution between $TPrA^+Cl^-$ and TBA^+Cl^- improved. The elution order of the TAA^+ salts were found to be different on the low-capacity cation exchange column (PRP-X200) than that observed on a silica-based strong cation exchange column (12). By adjusting the mobile phase concentration of acetonitrile, elution orders on the PRP-X200 column can be changed.

The mobile phase ionic strength plays a major role in the retention of an organic analyte cation on a cation exchange column. An increase in the mobile phase ionic strength leads to increased competition for the cation exchange sites and lower analyte cation retention. Figure 3 shows that the retention of mono-, di-, and tributylamine on the PRP-X200 column as a function of mobile phase ionic strength. The mobile phase ionic strength in this study was controlled by the addition of NaCl but is also a function of the amount of UV-absorbing countercation present in the mobile phase. The retention of several TAA^+ salts with respect to the amount of benzyltrimethylammonium chloride ($BTMA^+Cl^-$) added to the mobile phase is shown in Figure 4. The role of $BTMA^+Cl^-$ in the separation and detection of the organic analyte cations is two-fold; 1) to visualize an UV-transparent analyte and 2) to compete for the cation exchange sites. As the concentration of $BTMA^+Cl^-$ was increased a corresponding decrease in the retention of the TAA^+ salt was observed. The concentration of $BTMA^+Cl^-$ also influences the intensity of the signal for an analyte as it elutes from the column. Therefore, the concentration of $BTMA^+Cl^-$ must be determined so that the best separation and the optimum sensitivity are obtained.

Mobile phase pH plays a major role in the ionization of an organic analyte. For an organic analyte to be retained by a cation exchange mechanism on the PRP-X200 column, the analyte must be cationic. Three basic amino

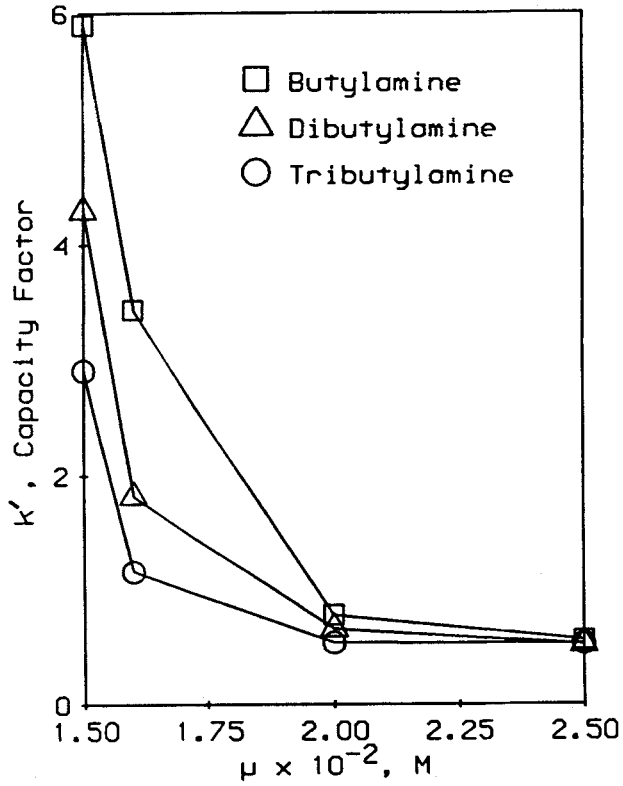

FIGURE 3

The Effect of Mobile Phase Ionic Strength on Mono-, Di-, and Tributylamine Retention.

A 0.005 M $BTMA^+Cl^-$, 0.01 M CH_3COOH, NaCl, 7:3 $CH_3CN:H_2O$ mobile phase.

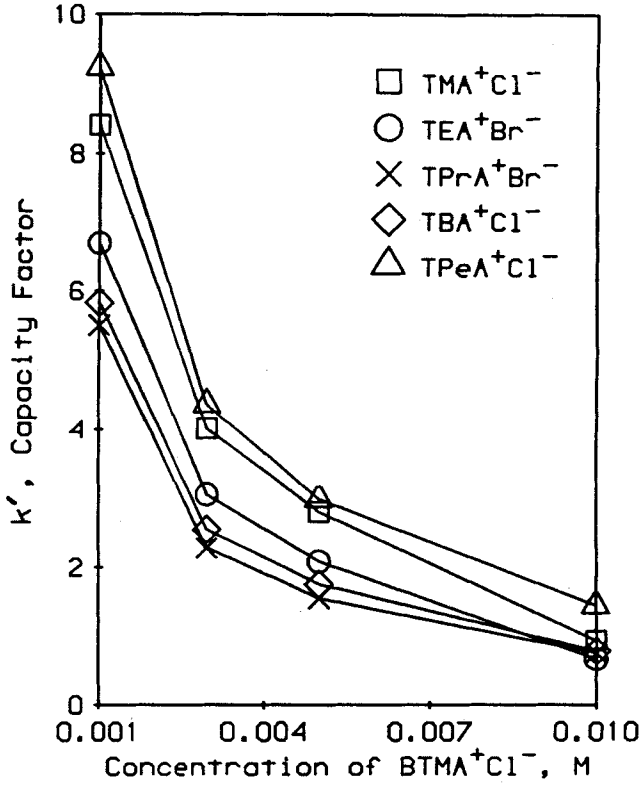

FIGURE 4

The Effect of $BTMA^+Cl^-$ Concentration on TAA^+ Salt Retention.

A $BTMA^+Cl^-$, 0.01 M CH_3COOH, 6:4 $CH_3CN:H_2O$ mobile phase.

acids (AA), L-ornithine (L-Orn), L-lysine (L-Lys), and L-arginine (L-Arg) were studied to show the effect of pH on retention. AA were used in this study since they can exist as cations, zwitterions or anions. Figure 5 is a plot of the retention data for the three AA. As the mobile phase pH was raised, retention of the three AA decreased. It should be noted that if the PRP-X200 column did not contain any cationic exchange sites, or if the AA were in a zwitterionic or anionic form, little or no retention would have been observed. If the pH was lowered to 2.0, the three AA would be di-cations and their retention would be significantly higher (30,31). Therefore, for an organic analyte to be retained by cation exchange, the mobile phase pH must be low enough to assure that the analytes will be in a cationic form.

Figure 6 shows the separation of 2,4-diaminobutyric acid (DABA), L-Orn, L-Lys and L-Arg. As the length of the side chain for DABA, L-Orn, and L-Lys increases, the analytes become more hydrophobic and their retention increases. The enhanced retention is attributed to cation exchange and adsorption contributions that provide better selectivity with increasing chain length.

Figure 7 shows the separation of mono-, di-, and tributylamine. The elution order of the three alkylamines can be reversed by decreasing the concentration of CH_3CN in the mobile phase. It was also found that at lower concentrations of CH_3CN, the elution order of the alkylamines were different than that observed on a silica-based strong cation exchange column (11).

Analyte standards were prepared for the di- and trialkylamines, and $TPrA^+Cl^-$ and $TPeA^+Cl^-$. Linear calibration curves of peak area versus ppm of analyte were obtained for a 50-µL injection over the range of 5.0 to 800 ppm. The correlation coefficients obtained were greater than 0.995 for both groups of standards.

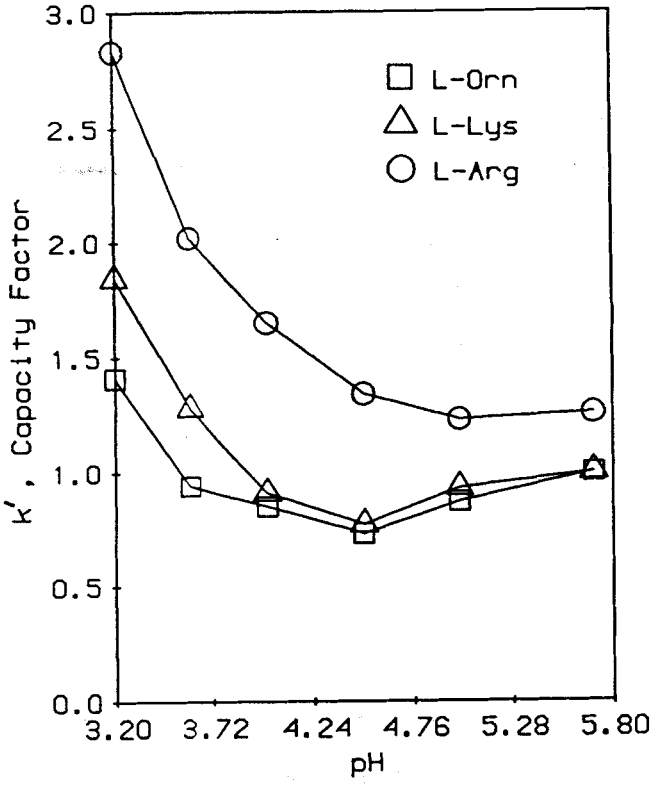

FIGURE 5

The Effect of Mobile Phase pH on Amino Acid Retention.

A 0.003 M $BTMA^+Cl^-$, 0.05 M CH_3COOH, 1:9 $CH_3CN:H_2O$ mobile phase.

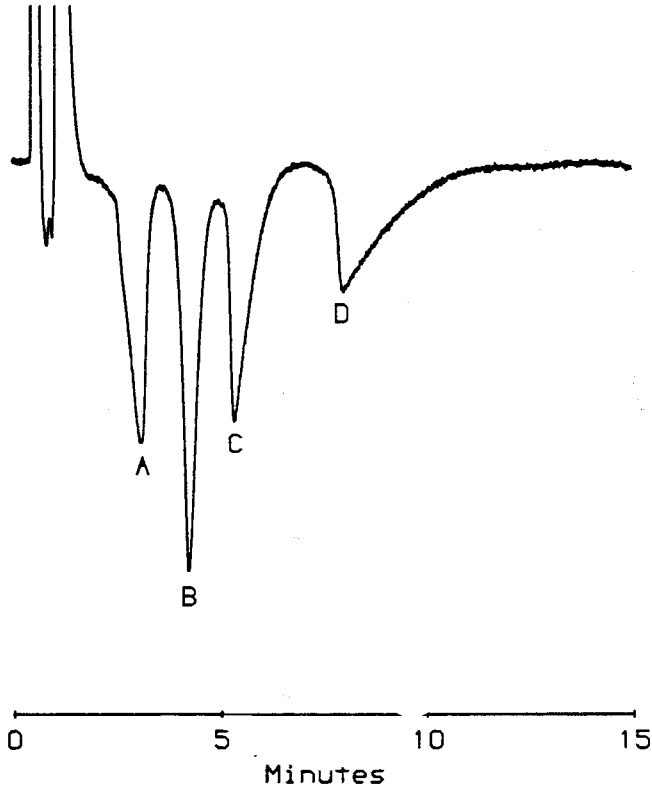

FIGURE 6

The Separation of A) DABA, B) L-Orn, C) L-Lys, D) L-Arg.

A 0.003 M $BTMA^+Cl^-$, 0.05 M CH_3COOH, 3:7 $CH_3CN:H_2O$ mobile phase, 3.0 ml/min.

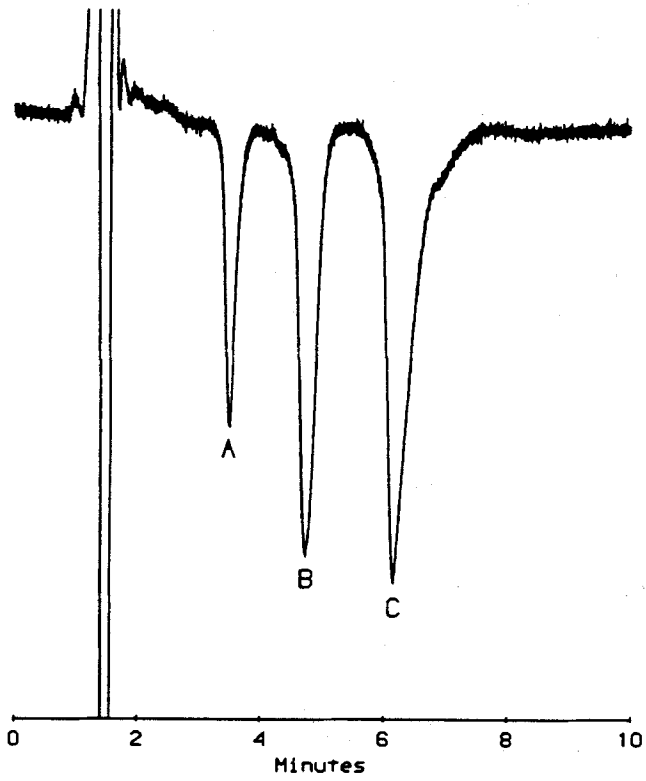

FIGURE 7

The Separation of A) Tributylamine, B) Dibutylamine, C) Butylamine.

A 0.005 M $BTMA^+Cl^-$, 0.01 M CH_3COOH, 7:3 $CH_3CN:H_2O$ mobile phase.

CONCLUSIONS

The separation of UV-transparent organic analyte cations on a low-capacity cation exchange column using indirect UV detection was studied. This separation and detection method has been successfully applied to several different groups of organic analyte cations. Elution orders of the organic analyte cations were found to be different than those observed on silica-based strong cation exchange columns. Elution orders can be changed on the low-capacity cation exchange column by adjusting the concentration of organic modifier. Calibration curves were linear down to 5.0 ppm.

REFERENCES

1) Small, H. and Miller, T.E., Jr., Anal. Chem.. 54, 462, 1982.

2) Small, H. and Miller, T.E., Jr., United States Patent No. 4414842, 1983.

3) Brown, D., Payton, R., and Jenke, D., Anal. Chem., 57, 2264, 1985.

4) Jenke, D., Mitchell, P. and Pagenkopf, G., Anal. Chim. Acta., 55, 249, 1983.

5) Wilson, S. and Yeung, E., Anal. Chim. Acta., 157, 53, 1984.

6) Jenke, D., Anal. Chem., 56, 2468, 1984.

7) Larson, J.R., J. Chromatogr., 356, 379, 1986.

8) Sithole, B.B. and Guy, R.D., Analyst, 111, 395, 1986.

9) Sherman, J.H. and Danielson, N.D., Anal. Chem., 59, 490, 1987.

10) Downey, B.P. and Jenke, D.R., J. Chromatogr. Sci., 25, 519, 1987.

11) Larson, J.R. and Pfeiffer, J. Chromatogr., 259, 519, 1983.

12) Larson, J.R. and Pfeiffer, C.D., Anal. Chem., 55, 393, 1983.

13) Jenke, D.R. and Raghavan, N., J. Chromatogr. Sci., 23, 75, 1985.

14) Gaffney, M.H. and Cooke, M., Anal. Proc., 21, 440, 1984.

15) Iskandarani, Z. and Miller, T.E., Jr., Anal. Chem., 57, 1591, 1985.

16) Benson, G.A. and Lennon, M., J. High Resolution Chromatogr. & Chromatogr. Comm., 10, 109, 1987.

17) Parkin, J.E., J. Chromatogr., 351, 532, 1986.

18) Rigas, P.G. and Pietrzyk, D.J., Anal. Chem., 58, 2226, 1986.

19) Hackzell, L., Rydberg, T. and Schill, G., J. Chromatogr., 282, 179, 1983.

20) Barber, W.E. and Carr, P.W., J. Chromatogr., 260, 89, 1983.

21) Barber, W.E. and Carr, P.W., J. Chromatogr., 301, 25, 1984.

22) Barber, W.E. and Carr, P.W., J. Chromatogr., 316, 211, 1984.

23) Mullins, F.G.P., Analyst, 112, 665, 1987.

24) Cantwell, F.F., "Ion Exchange and Solvent Extraction", Vol. 9, J.A. Marinsky and Y. Marcus Eds., M. Dekker, Inc., N.Y., 1985, 339.

25) Hux, R.A. and Cantwell, F.F., Anal. Chem., 56, 1258, 1984.

26) Afrashtehfar, S. and Cantwell, F.F., Anal. Chem., 54, 2422, 1982.

27) Pietrzyk, D.J., Iskandarani, Z. and Schmitt, G.L., J. Liq. Chromatogr., 9, 2633, 1986.

28) Fritz, J.S., Gjerde, D.T. and Pohlandt, C., "Ion Chromatography", Huthing Verlag, Heidelberg, 1982.

29) Cantwell, F.F. and Puon, S., Anal. Chem., 51, 623, 1979.

30) Walker, T.A. and Pietrzyk, D.J., J. Liq. Chromatogr., 8, 2047, 1985.

31) Walker, T.A. and Pietrzyk, D.J., J. Liq. Chromatogr., 10, 161, 1987.