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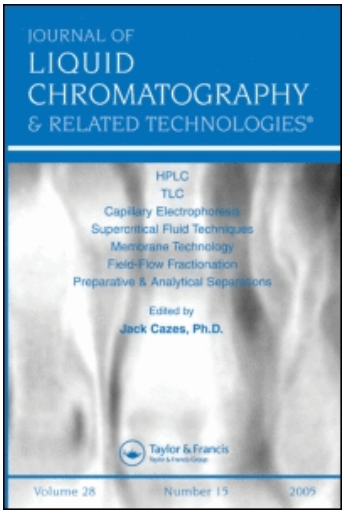

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
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To cite this Article Walker, Thomas A. , Ho, Thanh V. and Akbari, Nisar(1991) 'The Isocratic Separation and Indirect Uv Detection of Inorganic Anions and Mono- and Di-Carboxylic Acids On A Low-Capacity Anion Exchange Column', Journal of Liquid Chromatography & Related Technologies, 14: 7, 1351 — 1366
To link to this Article: DOI: 10.1080/01483919108049325
URL: http://dx.doi.org/10.1080/01483919108049325

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JOURNAL OF LIQUID CHROMATOGRAPHY, 14(7), 1351-1366 (1991)

THE ISOCRATIC SEPARATION AND INDIRECT UV DETECTION OF INORGANIC ANIONS AND MONO- AND DI-CARBOXYLIC ACIDS ON A LOW-CAPACITY ANION EXCHANGE COLUMN

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ABSTRACT

The isocratic separation of inorganic anions, and mono- and di-carboxylic acids on a low-capacity anion exchange column using indirect UV detection was studied. Retention of organic analyte anions on low-capacity anion exchange columns has been attributed to anion exchange, adsorption, or a combination of the two. Previous studies (1) were used to provide the mobile phase conditions for this study. It was found that the mobile phase pH, under the conditions used, had a major affect on the separation and resolution of the different analyte anions studied. Isocratic separations for a complex mixture of inorganic anions, and mono- and di-carboxylic acids at different mobile phase pH's are shown.

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INTRODUCTION

The separation and detection of inorganic and organic analyte ions is an area of analytical chemistry that has received a significant amount of attention over the last several years. An original publication by Small, Stevens and Bauman in 1975 (2) described the separation of inorganic anions and cations. This new separation scheme was named ion chromatography (IC). This separation method consisted of two columns attached in series where the first column was used for separating the analytes of interest (separator column) and the second column was used to chemically suppress the background conductance (suppressor column). The analytes were then detected using a conductance detector.

A single column IC system was developed by Fritz and co-workers (3-5) where a low-capacity ion exchange column was used for the separation of analyte ions in association with low-conducting eluents. Separations and detection limits were found to be comparable between the single and dual column ion chromatographic systems.

Several different separation methods have been used for organic analyte ions. These separation methods include: 1) ion chromatography- where both strong ion exchange columns (6-11) and low-capacity ion exchange columns were used (1,12-14), 2) ion-interaction chromatography (15-21), 3) ion exclusion chromatography (22,23), and 4) ion suppression chromatography (24). Separation schemes have recently focused on complex mixtures of inorganic and/or organic analyte ions. Both isocratic (1,12,25-27) and gradient methodologies (28-31) have been used for these separation.

This paper describes an isocratic separation of a complex mixture that contains inorganic anions, and mono- and di-carboxylic acids. A low-capacity polymer-based anion exchange column was used for the separation and the analyte anions were visualised by indirect UV detection. It was found that the mobile phase pH played a major role in the resolution of the di-

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carboxylic acids. Column length also had an effect on analyte anion resolution.

EXPERIMENTAL

Apparatus

The liquid chromatographic system used in this study consisted of an HP 1090M system with a photo diode array detector. The stationary phases used in this study were a 4.1 x 150 mm and a 4.1 x 250 mm PRP-X100 low-capacity anion exchange columns available from Hamilton Company (Reno, NV, U.S.A.). The PRP-X100 column is a spherical, 10 μm poly(styrenedivinylbenzene)-based anion exchange column with an anion exchange capacity of 200 μEq/g. Flow rates of 2.0 mL/min were used unless noted. Analyte samples of approximately 1 mg/mL were used with injection volumes of 10-25 μL. Inlet pressures of 500-600 psi were observed.

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Chemicals

HPLC grade acetonitrile was obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). Potassium hydrogen phthalate, inorganic salts, mono- and di-carboxylic acids were purchased from The Aldrich Chemical Company (Milwaukee, WI, U.S.A.). All chemicals were reagent grade. HPLC grade water was obtained by passing de-ionized water through a Nanopure water purification unit.

RESULTS AND DISCUSSION

Low-capacity polymeric-based anion exchangers have been shown to be useful for the separation of both organic and inorganic anions (1,32-36). These low-capacity anion exchangers have a dual retention mechanism of anion exchange and adsorption for organic analyte anions that contain both a fixed charge site and a hydrophobic center (1,32-36). In order for the dual retention mechanism to be present, the stationary phase must be nonpolar, have a high surface area, and provide

relatively few anion exchange sites. Adsorption of an organic analyte anion is dependent on the number of ion exchange sites present on the surface while anion exchange is independent of the number of anion exchange sites. The two mechanisms, ion exchange (IE) and adsorption (Ads), can be represented by the following equations:

$$A\text{-}NMe_3^+C^- + R\text{-}X^- + M^+ \underset{}{\overset{IE}{\rightleftharpoons}} A\text{-}NMe_3^+ {}^-X\text{-}R + C^- + M^+ \quad (1)$$

$$A\text{-}NMe_3^+C^- + R\text{-}X^- + M^+ \underset{}{\overset{Ads}{\rightleftharpoons}} M^+ {}^-X\text{-}R\cdots A\text{-}NMe_3^+C^- \quad (2)$$

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

In this study, a UV-active counteranion was added to the mobile phase in order to indirectly detect the analyte anions of interest. The UV-active counteranion has the dual role of 1) displacing the analyte of interest from the anion exchange column and 2) in the indirect detection of an UV-transparent analyte anion as a dip or trough in the baseline absorbance. The UV-active counteranion will only be involved in the indirect UV detection of analyte anions that are retained by an adsorption.

The focus of this study was to develop an isocratic method for the separation of a complex mixture of inorganic anions, and mono- and di-carboxylic acids on a low-capacity anion exchange column. The effect of the various mobile and stationary phase parameters on the retention and resolution of inorganic and organic analyte anions was discussed previously (1). It was found that ionic strength (from buffers, added inert electrolytes and/or UV-active counteranion), mobile phase pH and the concentration of organic modifier would affect the separation of the organic analyte anions.

Table I shows the retention of several inorganic and organic analyte anions at different mobile phase pH's. Several

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TABLE I

Effect of pH on the Retention of Inorganic and Organic Analyte Anions

	Capacity Factor, k'			
	pH[a]			
Analyte	3.80	4.50	4.75	5.00
Acetate	2.60	2.80	2.37	2.60
Formate	4.60	4.80	3.24	2.60
Bromide	10.6	11.8	7.10	5.40
Nitrate	11.8	14.7	8.51	7.20
Succinic Acid	5.60	9.53	10.3	11.0
Malonic Acid	5.60	11.4	11.2	12.6
Glutaric Acid	6.50	10.2	13.1	14.6
Adipic Acid	8.00	14.0	14.6	17.0
Maleic Acid	9.70	17.0	15.8	21.4
Pimelic Acid	17.3	19.4	20.6	23.4
Fumaric Acid	39.0	38.7	31.6	39.0
Oxalic Acid	39.0	41.7	31.6	>100.
Suberic Acid	47.0	-	39.7	>100.

a. A mobile phase containing 0.001 M KHP, 5:95 CH_3CN:H_2O, with a 2.0 mL/min flowrate. The pH was adjusted using NaOH.

trends can be observed from this data. The retention of the inorganic anions decreased as the mobile phase pH was raised. This indicates that competition for the anion exchange sites was increasing due to the increased ionization of KHP. The ionization of KHP changes from being a mono-anion to a di-anion (pKa's of 2.89 and 5.51) and this leads to more competition for the anion exchange sites. This increased competition leads to lower retention for the analyte anions that are retained by anion

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TABLE II

Acid Dissociation Constants[1]

Analyte	pK1	pK2
Acetic Acid	4.75	-
Adipic Acid	4.43	5.41
Formic Acid	3.75	-
Fumaric Acid	3.03	4.44
Glutaric Acid	4.31	5.41
Maleic Acid	1.83	6.07
Malonic Acid	2.83	5.69
Oxalic Acid	1.23	4.19
Pimelic Acid	4.71	-
Suberic Acid	4.52	-
Succinic Acid	4.16	5.61

1) CRC Handbook of Chemistry, 66th Edition, CRC Press, Inc., Boca Raton, FL, 1985.

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exchange. The retention of the di-carboxylic acids, however, increased as the mobile phase pH was raised. At pH 3.8, the acids are either unionized or partially ionized (pKa's are listed in Table II). As the mobile phase pH was raised, ionization of the di-carboxylic acids took place. The ionization of the di-carboxylic acids leads to a significant increase in ionic interactions between the analyte anions and the fixed anion exchange sites This in turn leads to higher retention times for the shorter chain organic acids. When the di-carboxylic acids are unionized or partially ionized, organic analyte retention is due predominantly to adsorption. As the mobile phase pH is raised and the di-carboxylic acids become ionized, the retention mechanism for the acids changes from adsorption to anion exchange or a combination of the two.

Figure 1 shows the separation of a mixture of inorganic anions and mono- and di-carboxylic acids at a mobile phase pH of 3.8. At this pH, several of the di-carboxylic acids are

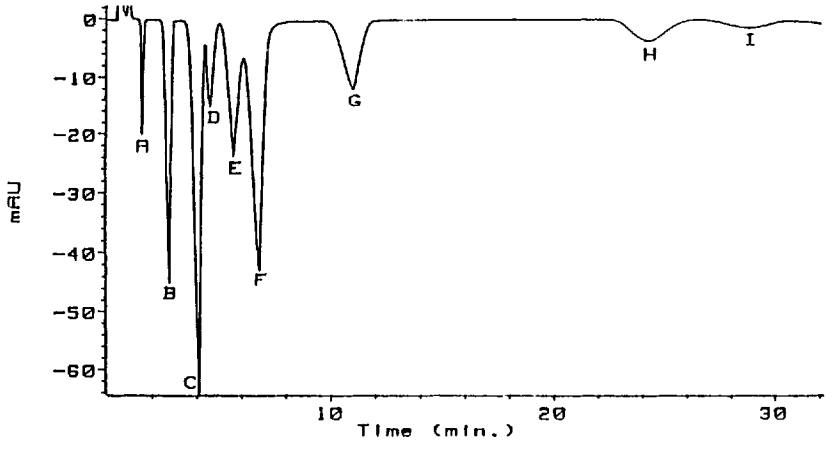

FIGURE 1

The Separation of A) Acetate, B) Formate, C) Chloride, Succinic Acid, Malonic Acid, D) Bromide, Glutaric Acid, E) Nitrate, Adipic Acid, F) Maleic Acid, G) Pimelic Acid, H) Fumaric Acid, Oxalic Acid, I) Suberic Acid, at pH 3.8.

A 0.001 M KHP, pH 3.8, 5:95 CH_3CN:H_2O mobile phase.

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unionized or are partially ionized (Table II) and retention was found to be due primarily to adsorption. Generally, retention increased for the di-carboxylic acids as the pH of the mobile phase was increased. Fumaric, oxalic and suberic acids were found to be highly retained at pH 3.8. The high retention for fumaric acid can be attributed to a combination of anion exchange and adsorption whereas oxalic acid, which is ionized at this pH, is retained exclusively by anion exchange. Suberic acid, which is unionized, is retained by adsorption. Several anions were found to co-elute at this pH; chloride, succinic acid, and malonic acid; bromide and glutaric acid; nitrate and adipic acid.

Figure 2 shows the separation for the same mixture where the mobile phase pH was increased to 4.5. Resolution of the

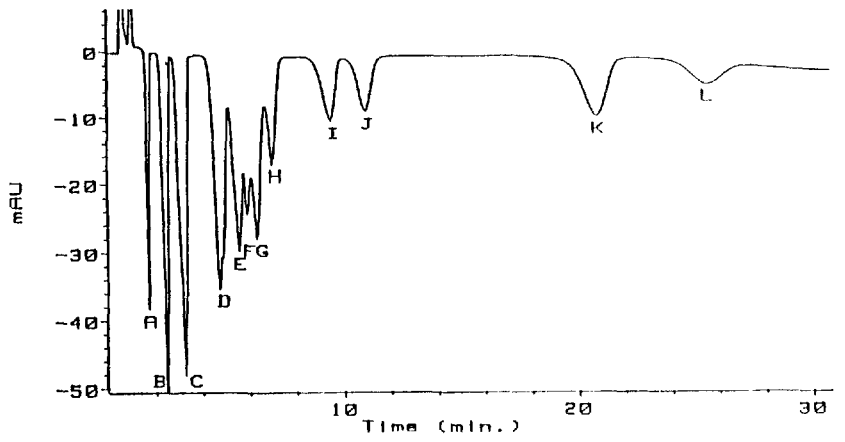

FIGURE 2

The Separation of A) Acetate, B) Formate, C) Chloride, D) Succinic Acid, E) Bromide, F) Glutaric Acid, G) Nitrate, Malonic Acid, H) Adipic Acid, I) Maleic Acid, J) Pimelic Acid, K) Fumaric Acid, Oxalic Acid, L) Suberic Acid, at pH 4.50.

A 0.001 M KHP, pH 4.50, 5:95 CH_3CN:H_2O mobile phase.

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analyte anions improved significantly over that found for the pH 3.8 mobile phase. At pH 4.5, several of the smaller chain acids are anionic and interact with the fixed anion exchange sites. This leads to higher retention times than when retention was due to just adsorption. Only nitrate and malonic acid, and oxalic acid and fumaric acid were found to co-elute at this pH. Resolution for several of the analytes, however, was still unacceptable.

The separation for the same mixture of inorganic and organic anions at a mobile phase pH 5.0 is shown in Figure 3. None of the analyte anions co-eluted, however, oxalic and suberic acids were found to have retention times greater than 120 minutes. Oxalic acid is a di-anion at this pH and would be

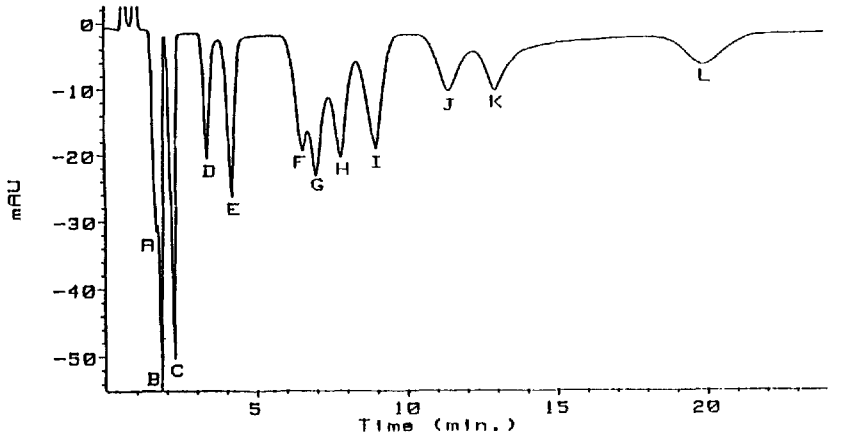

FIGURE 3

The Separation of A) Acetate, B) Formate, C) Chloride, D) Bromide, E) Nitrate, F) Succinic Acid, G) Malonic Acid, H) Glutaric Acid, I) Adipic Acid, J) Maleic Acid, K) Pimelic Acid, L) Fumaric Acid, at pH 5.0.

A 0.001 M KHP, pH 5.0, 5:95 CH_3CN:H_2O mobile phase.

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expected to be more highly retained than when it is a mono-anion. The higher retention for suberic acid can be attributed to a combination of anion exchange and adsorption since the mobile phase pH is above its pK_a.

The concentration of added UV-active counteranion will have an affect on the retention of the analyte anions. If the concentration of counteranion is decreased, analyte anion retention will increase. Figure 4 shows the separation of the analyte anions where the mobile phase pH was 4.5 and the concentration of KHP was decreased to 0.5 mM. The separation is similar to that shown in Figure 2. The only difference is that the analyte anions, as expected, are more highly retained. The

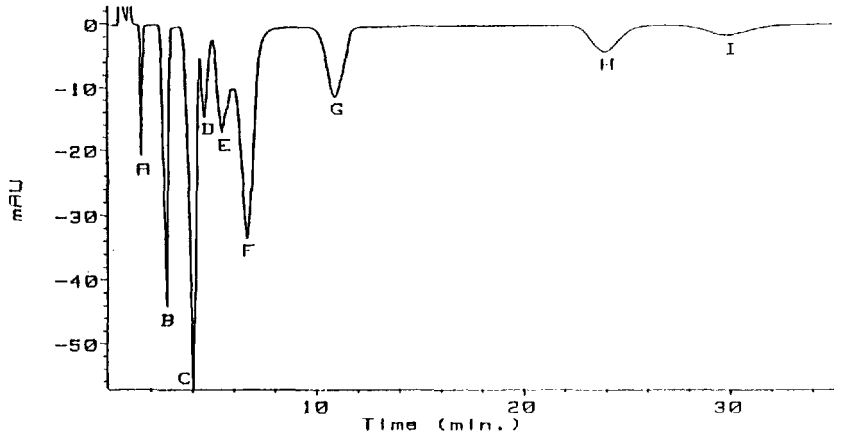

FIGURE 4

The Separation of A) Acetate, B) Formate, C) Chloride, Succinic Acid, D) Bromide, Glutaric Acid, E) Nitrate, Malonic Acid, F) Adipic Acid, G) Pimelic Acid, H) Fumaric Acid, Oxalic Acid, I) Suberic Acid, at 0.0005 M KHP.

A 0.0005 M KHP, pH 4.50, 5:95 CH_3CN: H_2O mobile phase.

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same analyte anions were found to co-elute at both 1.0 mM and 0.5 mM KHP.

At pH 4.5, several analyte anions co-eluted, especially the shorter chain di-carboxylic acids and the inorganic anions. At pH 5.0, the inorganic anions and the di-carboxylic acids did not co-elute, however, oxalic acid and suberic acid were very highly retained and did not elute for several hours. Neither pH 4.5 or pH 5.0 mobile phases provided a desirable separation. Each mobile phase, however, did provide separations of particular groups of analyte anions that did meet the requirements for an isocratic separation. Therefore, a mobile phase with a pH of 4.75 was tried in order to determine if an acceptable separation would result. Figure 5 shows the separation that was obtained

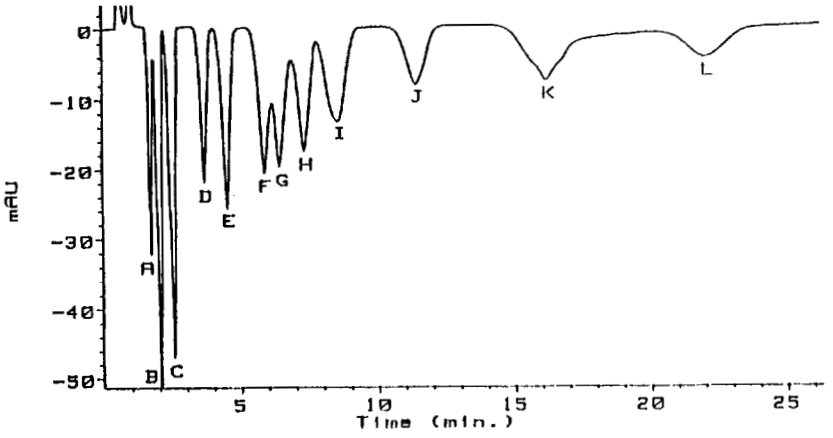

FIGURE 5

The Separation of A) Acetate, B) Formate, C) Chloride, D) Bromide, E) Nitrate, F) Succinic Acid, G) Glutaric Acid, Malonic Acid, H) Adipic Acid, I) Maleic Acid, J) Pimelic Acid, K) Fumaric Acid, Oxalic Acid, L) Suberic Acid, at pH 4.75 and using a 15 cm PRP-X100 column.

A 0.001 M KHP, pH 4.75, 5:95 CH_3CN:H_2O mobile phase.

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on a 15-cm PRP-X100 column. All of the analyte anions were separated except for glutaric acid-malonic acid, and fumaric acid-oxalic acid. Of all the mobile phases studied, the pH 4.75 mobile phase provided the best separation for this mixture of inorganic and organic analyte anions.

One way in which resolution can be improved is by increasing the column length. This will increase the number of adsorption and anion exchange sites with which the analyte anions may interact. Figure 6 shows the separation of the inorganic anions and mono- and di-carboxylic acids on a 25-cm PRP-X100 column. Retention times were increased and resolution improved significantly with only fumaric acid and

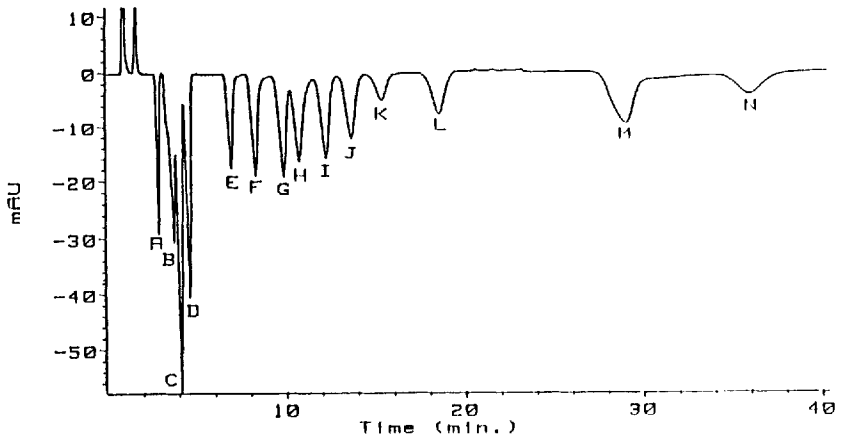

FIGURE 6

The Separation of A) Acetate, B) Propionate, C) Formate, D) Chloride, E) Bromide, F) Nitrate, G) Succinic Acid, H) Malonic Acid, I) Glutaric Acid, J) Adipic Acid, K) Maleic Acid, L) Pimelic Acid, M) Fumaric Acid, Oxalic Acid, N) Suberic Acid, on a 25-cm PRP-X100 column and at pH 4.75.

A 0.001 M KHP, pH 4.75, 5:95 CH_3CN:H_2O mobile phase.

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oxalic acid co-eluting. Propionic acid was added to the mixture and was found to elute between acetate and formate.

On an anion exchange column, the elution order for inorganic anions is: $Cl^- < NO_2^- < Br^- < NO_3^-$. From Figure 6, it was determined that nitrite could also be added to the mixture and should elute from the column after chloride and before bromide. Figure 7 shows this separation. It was observed that all of the analytes in the mixture were separated except for fumaric acid and oxalic acid. The peak shape for these two acids was not as sharp as what was observed for the other analytes. This indicates that the two analytes are partially resolved, however the long retention times lead to band broadening and peak

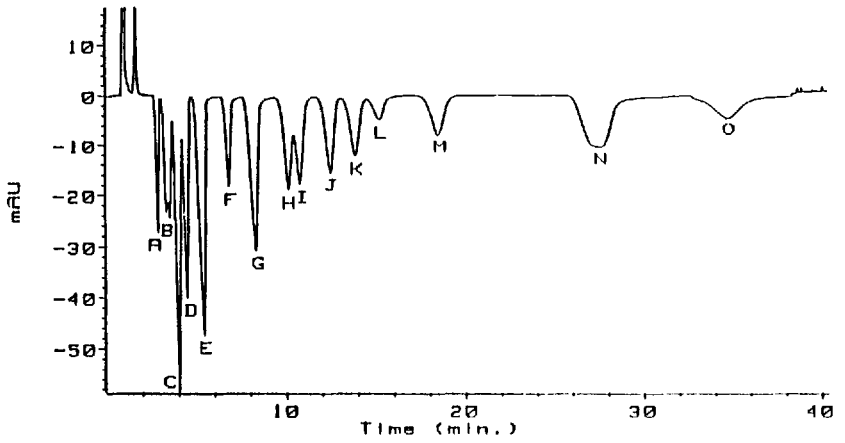

FIGURE 7

The Separation of A) Acetate, B) Propionate, C) Formate, D) Nitrite, E) Chloride, F) Bromide, G) Nitrate, H) Succinic Acid, I) Malonic Acid, J) Glutaric Acid, K) Adipic Acid, L) Maleic Acid, M) Pimelic Acid, N) Fumaric Acid, Oxalic Acid, O) Suberic Acid, on a 25 cm PRP-X100 column and at pH 4.75.

A 0.001 M KHP, pH 4.75, 5:95 CH_3CN;H_2O mobile phase.

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overlap. The separation between fumaric acid and oxalic acid could be improved by going to an even longer column, however, this was not done since longer analysis times would result. The propionic acid peak was found to split when the pH 4.75 mobile phase was used. This is attributed to the mobile phase pH (4.75) being close to the pK_a of propionic acid (4.87). Studies were done with mobile phases at higher and lower pH values, and propionic acid did not show peak splitting. Overall, this isocratic separation of the complex mixture of 16 inorganic and organic analyte anions was found to be acceptable.

CONCLUSIONS

The isocratic separation of UV-transparent inorganic and organic analyte anions on a low-capacity anion exchange column using indirect UV detection was studied. An isocratic separation was developed for a complex mixture of inorganic and organic analyte anions. Adsorption and ion exchange interactions could be changed by controlling the mobile phase parameters. Careful control of the mobile phase parameters could result in interesting changes in analyte retention times, elution orders, selectivity and resolution.

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ACKNOWLEDGMENTS

We would like to thank Drs. Michael Kellogg, Grant DuBois and Robert Mazur at The NutraSweet Company and Dr. Steven Simmons at Marion Merrell Dow Inc. for their encouragement. Parts of this paper were presented at the Second International Ion Chromatography Forum in Boston, MA, September 1989, paper number 41 and at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy in Chicago, IL, March 1991, paper 74.

REFERENCES

1) Walker, T.A., Ho, T.V., and Akbari, N., J. Liq. Chromatogr., 12, 1213 (1989).

2) Small, H., Stevens, T.S., and Bauman, W.C. Anal.Chem., 47 1801 (1975).

3) Gjerde, D.T., Fritz, J.S., and Schmuckler, G., J. Chromatogr., 186, 509 (1979).

4) Gjerde, D.T., Schmuckler, G., and Fritz, J.S., J. Chromatogr., 187, 35 (1980).

5) Fritz, J.S., Gjerde, D.T., and Becker, R.M., Anal. Chem., 52, 1519 (1980).

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

12) Haddad, P.R. and Heckenberg, A.L., J. Chromatogr., 252, 177 (1982).

13) Chauret, N. and Hubert, J., J. Chromatogr., 469, 329 (1989).

14) Haddad, P.R., Jackson, P.E., and Heckenberg, A.L., J. Chromatogr., 346, 139 (1985).

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

16) Pietrzyk, D.J., Rigas, P.R., and Dongxing, Y., J. Chromatogr. Sci., 27, 485 (1989).

17) Prohlich, D.H., J. High Resolut. Chromatogr./Chromatogr. Commun., 10, 12 (1987).

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

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

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

21) Arvidsson, E., Hacksell, L., Schill, G., and Westerlund, D., Chromatographia, 25, 430 (1988).

22) Rocklin, R.D., Slingsby, R.W., and Pohl, C.A., J. Liq. Chromatogr., 9, 757 (1986).

23) Turkelson, V.T. and Richards, M., Anal. Chem., 50, 1420 (1978).

24) Manning, D.L. and Maskarinec, M.P., J. Liq. Chromatogr., 6, 705 (1983).

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25) Takeuchi, T., Suzuki, E., and Ishii, D., Chromatographia, 25, 480 (1988).

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

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

28) Slingsby, R.W., J. Chromatogr., 371, 373 (1986).

29) Rocklin, R.D., Pohl, C.A. and Schibler, J.A., J. Chromatogr., 411, 107 (1987).

30) Jones, W.R. and Jandik, P., R&D, 30 (9), 92 (1988).

31) Jones, W.R., Jandik, P. and Heckenberg, A.L., Anal. Chem., 60, 1977 (1988).

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

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

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

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

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

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