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## Note

# High-performance liquid chromatography of non-UV-absorbing anions using indirect photometric chromatography and an amino column

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High-performance liquid chromatographic (HPLC) analysis of anions that absorb in the ultraviolet (UV) region has been accomplished by derivatization (to organic species)[1], using ion-pair formation[2–4] or by direct UV detection after separation on amino bonded silica based columns[5,6]. Those anions which do not absorb in the UV region can be determined by conductimetric detection[7] and more recently by a technique known as indirect photometric chromatography (IPC), in which a UV-absorbing counterion is added to the eluent, and the decrease in absorbance obtained when an anion elutes is measured.

IPC was reported in detail by Small and Miller[8] and later by Cochrane and Hillmann[9].

The purpose of our present work is to demonstrate the increased versatility and utility of indirect photometric chromatography when coupled with the amino bonded silica based column used in the weak base ion-exchange mode.

## EXPERIMENTAL*

### *Chemicals*

The inorganic chemicals used were reagent grade available from J. T. Baker (Philipsburg, NJ, U.S.A.). The phthalic acid used was reagent grade, available from Aldrich (Milwaukee, WI, U.S.A.). Eluents were prepared with deionized water and filtered through a 0.45-$\mu$m membrane filter.

### *HPLC conditions*

The liquid chromatograph consisted of an Altex Model 110 A pump, a Rheodyne Model 7125 injection valve equipped with a 50-$\mu$l loop, an LC 55 (Perkin-Elmer) variable wavelength UV detector and a Sargent Model SRG recorder.

The column used throughout this work was a 250 × 4.6 mm I.D. Zorbax® $NH_2$ (DuPont, Wilmington, DE, U.S.A.).

The eluent consisted of phthalic acid in water, adjusted to various pH values with 5 *M* sodium hydroxide solution. Flow-rate was 2.0 ml/min. Detection was by

* The methods described in this publication are the subject of pending patents which are licensed to Dionex Corporation for commercial use.

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UV at 290–310 nm. Column pressure was 1500 p.s.i.g. and operating temperature was 24°C.

*Column conversion to ion exchange mode*

Amino bonded columns which are commercially available are usually stored in non-polar (normal-phase) eluents. In order to obtain good performance, it is important to convert the columns completely to the ion-exchange mode. We have found that best results are obtained by washing the column with approximately 100 ml each

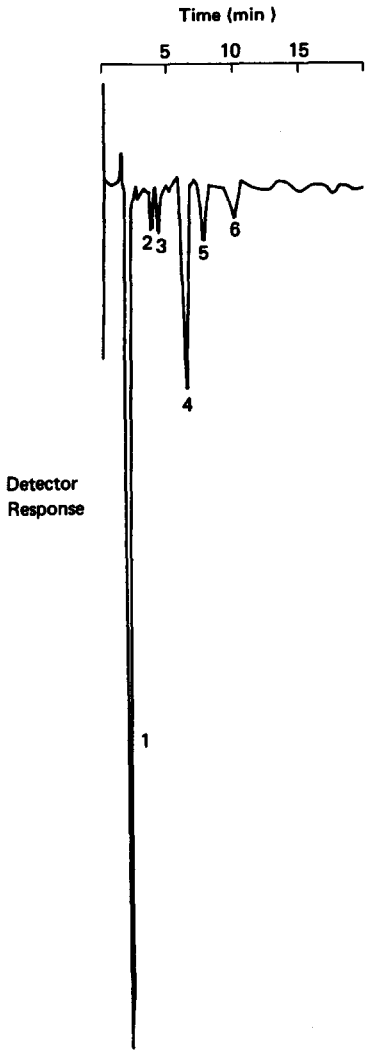



Fig. 1. Separation of fluoride (1), iodide (2), nitrate (3), chloride (4), phosphate (5) and sulfate (6). Column, Zorbax $NH_2$ (250 × 4.6 mm I.D.); mobile phase, 0.02 *M* phthalic acid, pH adjusted to 2.9 with sodium hydroxide; flow-rate, 2.0 ml/min; temperature, 24°C; recorder, 5 mV; detection, UV at 300 nm; pressure 1500 p.s.i.g. Injected sample was prepared to give concentrations of 25–100 μg/ml in eluent.

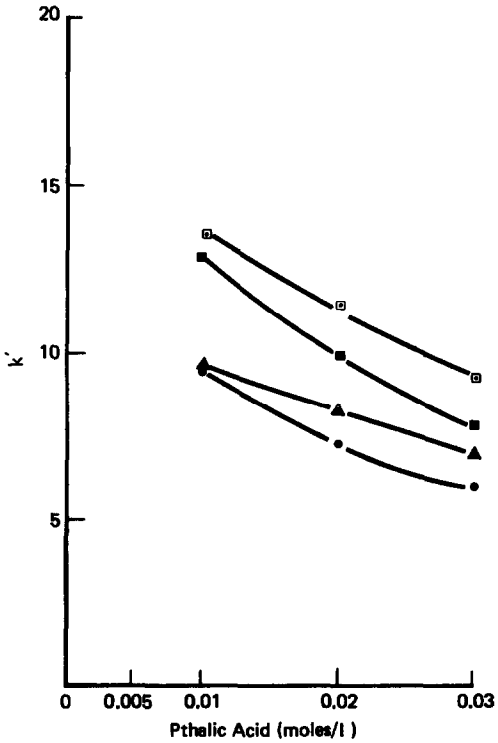



Fig. 2. Dependence of $k'$ of phosphate (⊡), chloride (■), nitrate (△) and iodide (●), on the phthalic acid concentration in the eluent using an amino column in indirect photometric chromatography. Other conditions as in Fig. 1.

of acetonitrile, methanol, water, then the eluent in that order. Initial equilibration of the column usually requires about 200 ml of eluent flow.

## RESULTS AND DISCUSSION

An example of the separations obtained is shown in Fig. 1. The effect of phthalic acid concentration on capacity factor ($k'$) was studied as shown in Fig. 2. The data indicate the expected relationship between $k'$ and counterion concentration.

Since a weak base ion-exchange column was used, ion-exchange capacity (and the $k'$ of the separated ions) is a function of eluent pH[6,10]. Fig. 3 represents a plot of $k'$ *vs.* eluent pH for various anions. The expected decrease in $k'$ with a pH increase is observed.

An example of the best separation obtained for chloride, sulfate and phosphate is shown in Fig. 4. Table I summarizes estimated detection limits obtained under the conditions listed. Detection limits were calculated as three times the baseline random noise level.

Column efficiencies obtained were in the range of 800 effective theoretical plates calculated at a $k'$ value of 3.6, *i.e.* chloride (Fig. 1).

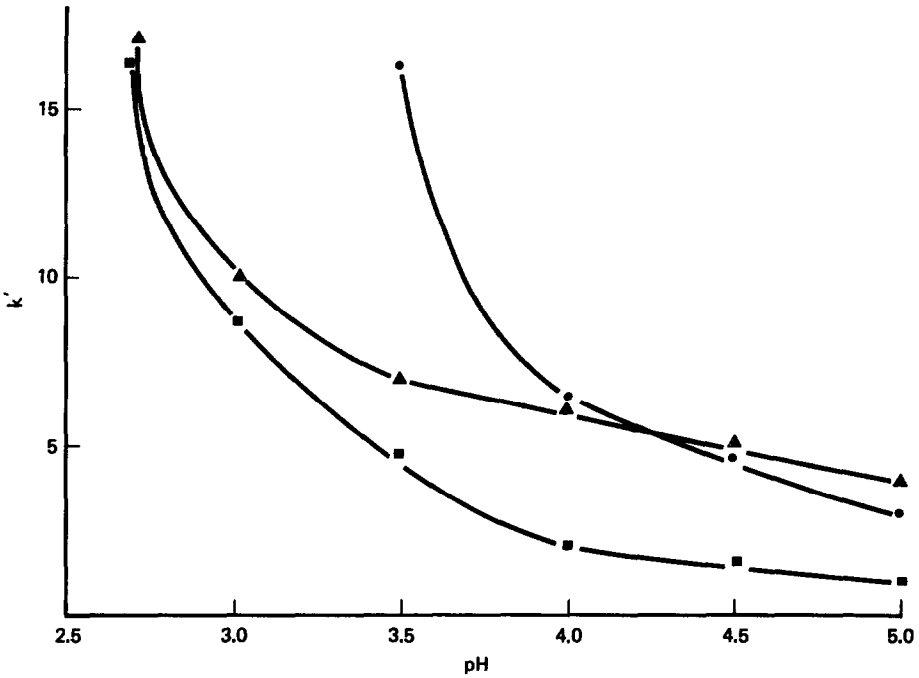



Fig. 3. Dependence of $k'$ of chloride (■), phosphate (△) and sulfate (●) on the eluent pH, using an amino column in indirect photometric chromatography. Other conditions as in Fig. 1, except eluent, 0.01 *M* phthalic acid.

*Sensitivity and efficiency*

An important characteristic of IPC is that sensitivity, on the basis of peak area, is a direct function of the concentration of a UV-absorbing counterion in the eluent[8]. Lower counterion concentrations result in higher sensitivity. When conventional strong base ion-exchange columns are used, lower counterion concentrations result in long analysis time. Shortening such a column would result in normal analysis time with a lower counterion concentration, but also with a decreased theoretical plate number.

Shortening an ion-exchange column to reduce capacity is but one alternative. The column capacity of a weak base ion exchanger is a function of eluent pH (ref. 10). Therefore, experiments were performed with the amino column using an eluent containing ten times less UV-absorbing counterion and an increased eluent pH so that the $k'$ value of injected chloride was the same as with the more concentrated eluent (Figs. 5–7). The chloride concentration in the injected sample was 1,000 μg/ml (Figs. 5 and 6). The chloride peak area in Fig. 6 is about ten times larger than that in Fig. 5, but the "peak height" in Fig. 6 is not ten times larger than that in Fig. 5 because the columns were overloaded by 1000 μg/ml of chloride. The overload arises due to the reduced column capacity resulting from the use of a higher eluent pH. The chloride peak in Fig. 7 does not show the effect of overloading when the concentration of chloride injected was reduced to 100 μg/ml. A comparison of Figs. 5 and 7 indicates that the use of a ten-fold reduction in eluent strength with an amino column

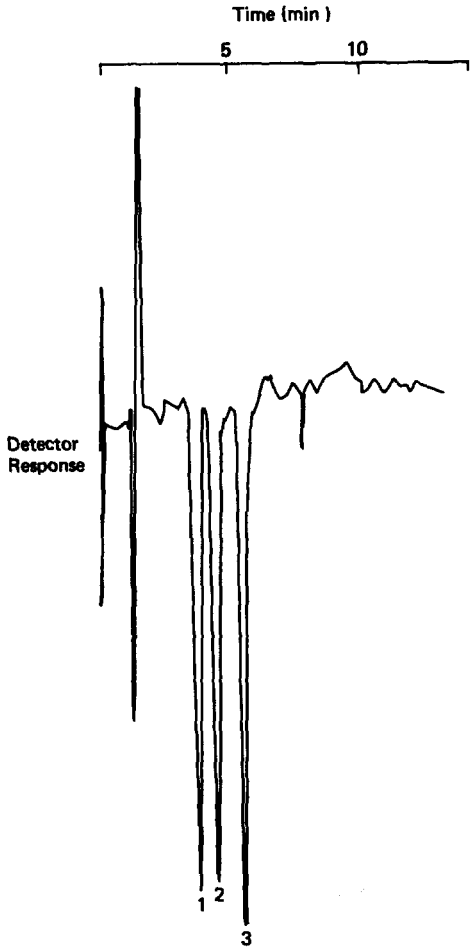



Fig. 4. Separation of chloride (1), phosphate (2), sulfate (3). Injected sample was prepared in water to give concentrations of 50 μg/ml of each component. Other conditions as in Fig. 1, except detector wavelength, 302 nm; recorder, 1 mV full scale; eluent, 0.001 *M* phthalic acid at pH 3.9.

results in a ten-fold increase in sensitivity without excessive analysis time or significantly reduced theoretical plate count. This would not have occurred if a conventional strong base ion-exchange column had been reduced in length. If such a column gave 800 theoretical plates for a 25 cm length, 80 theoretical plates would be the expected plate count for a 2.5 cm long column.

The problem of column overloading as shown in Fig. 6 is not serious, since it can be solved by simply diluting the sample or by using a smaller injection volume.

*Theoretical explanation of the data*

When the pH of the eluent is more than two units greater than the $pK_a$ of the conjugate acid of the weak base functionality of the stationary phase of a weak base column, column ion-exchange capacity would be expected to decrease ten fold for

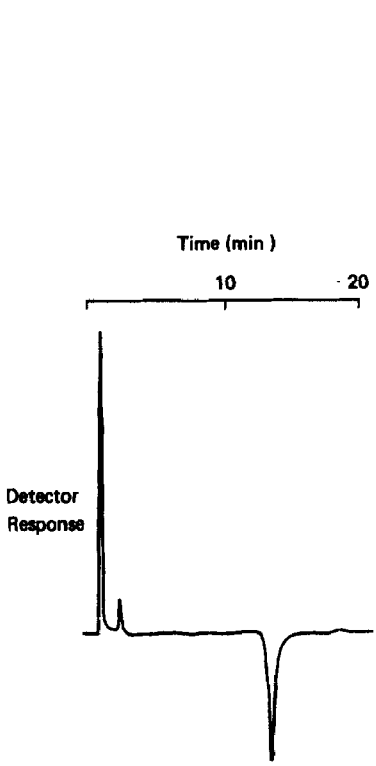
TABLE I

RETENTION TIMES AND DETECTION LIMITS OF INORGANIC ANIONS

Column: Zorbax $NH_2$ (250 × 4.6 mm I.D.); eluent: 0.02 *M* phthalic acid, pH 2.9; flow-rate: 2.0 ml/min. Calculations were performed using chromatograms obtained at a 5 mV full-scale setting.

| *Anion* | *Retention time (min)* | *Calculated detection limit (μg/ml)** |
|---|---|---|
| Sulfate | 3.4 | 6.1 |
| Phosphate | 4.0 | 6.3 |
| Chloride | 5.2 | 5.7 |
| Iodide | 2.0 | 10.0 |
| Nitrate | 2.3 | 10.0 |
| Fluoride | 1.2 | 0.5 |

* Calculated as 3 times random noise level.



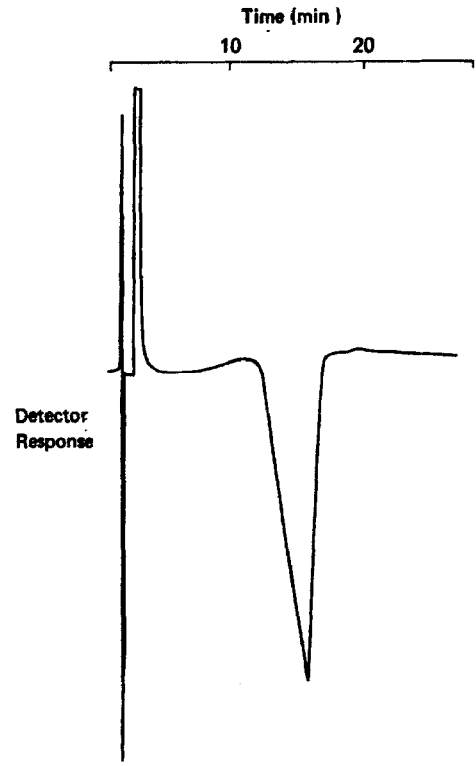




Fig. 5. Response obtained for 1000 ppm chloride. Conditions as in Fig. 1, except eluent, 0.01 *M* phthalic acid; detector wavelength, 302 nm; recorder, 10 mV full scale. Injected sample was prepared to give a concentration of 1000 μg/ml in eluent.

Fig. 6. Response obtained for 1000 ppm chloride. Conditions as in Fig. 5, except eluent, 0.001 *M* phthalic acid adjusted to pH 3.7 with sodium hydroxide; detector wavelength, 302 nm.

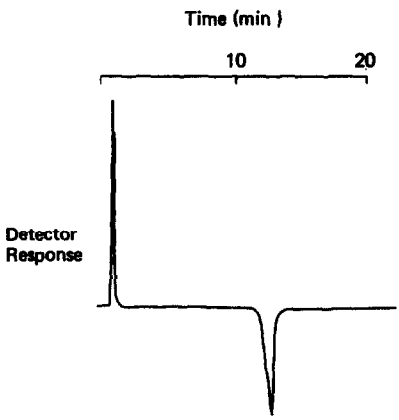



Fig. 7. Response obtained for 100 ppm chloride in low-capacity system. Conditions as in Fig. 6.

each unit increase in eluent pH[11]. When this is the case, the eluent counterion concentration can be reduced ten fold with an expected ten-fold increase in sensitivity at about the same analysis time, for many ions, by increasing eluent pH one unit. The actual eluent counterion concentration is a function not only of the amount weighed into a given volume of carrier but also of the pH of the eluent for eluent counter ion originating from weak acids, such as phthalic acid. Thus, increasing eluent pH decreases column capacity and increases the fraction of weak acid in the active counterion form, and at any given eluent pH, diluting the eluent also increases the fraction of weak acid in the active counterion form. The foregoing discussion explains the experimental observations seen in Figs. 5–7. The shift of elution order of phosphate and sulfate in Fig. 3 with increasing eluent pH is explained as the appearance of significant concentration of divalent phthalate counterion in the eluent at higher eluent pH.

## CONCLUSIONS

The technology for the determination of various anions by Indirect Photometric Chromatography has been expanded by the use of a weak-base ion-exchange column. A decrease in counterion concentration can result in both an increase in sensitivity and maintenance of analytical time without loss of theoretical plate count, simply by adjusting eluent pH.

## REFERENCES

1 G. L. Wheeler and P. F. Lott, *Microchem. J.*, 19 (1974) 390.
2 R. N. Reeve, *J. Chromatogr.*, 177 (1979) 393.
3 N. E. Skelly, *Anal. Chem.*, 54 (1982) 712.
4 I. Molnár, H. Knauer and D. Wilk, *J. Chromatogr.*, 201 (1980) 225.
5 U. Leuenberger, R. Gauch, K. Rieder and E. Baumgartner, *J. Chromatogr.*, 202 (1980) 461.
6 H. J. Cortes, *J. Chromatogr.*, 234 (1982) 517.
7 H. Small, T. S. Stevens and W. C. Bauman, *Anal. Chem.*, 47 (1975) 1801.
8 H. Small and T. E. Miller, *Anal. Chem.*, 54 (1982) 462.
9 R. A. Cochrane and D. E. Hillman, *J. Chromatogr.*, 241 (1982) 392.
10 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, Chichester, 2nd ed., 1979, p. 417.
11 R. Fisher and D. Peters, *Quantitative Chemical Analysis*, W. B. Saunders, Philadelphia, PA, 1969, 181.