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ANION CHROMATOGRAPHY OF CARBOXYLIC ACIDS AND KETO ACIDS USING A HOLLOW-FIBRE SUPPRESSOR

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SUMMARY

Anion-exchange chromatography of carboxylic acids and keto acids was carried out on an ion chromatograph equipped with a hollow-fibre ion-exchange membrane suppressor and a dual detector system comprised of a UV and a conductivity detector. Low-molecular-weight monocarboxylic acids were separated with a sodium tetraborate solution as eluent, and dicarboxylic acids and corresponding keto acids with a carbonate buffer as eluent. The sensitivity of the conductivity detector to carboxylic acids was a function of the pK values of the acids. The detection limit for succinic acid was less than 0.5 nmol.

INTRODUCTION

Since its introduction by Small *et al.*[1] ion chromatography has been developed to provide a rapid and sensitive analytical means for ions in aqueous solution, especially for the determination of inorganic anions.

Ion chromatography differs from conventional ion-exchange chromatography in that a low-capacity ion-exchange column and eluents of low concentrations are used and a suppressor column is employed. The suppressor column is effective in neutralizing the eluent and suppressing the background, thus enabling sensitive detection, however, it also has many drawbacks. Therefore efforts to determine ions without the use of a suppressor column have been made by several workers[2–5].

Recently, Stevens *et al.*[6] developed a new suppressor system using a hollow-fibre ion-exchange membrane made from sulphonated polyethylene. Naphion tubing is also an excellent material for the suppressor[7,8]. This system solves the problems caused by the large-volume and high-capacity suppressor column.

Many reports on the separation of organic anions of carboxylic acids by conventional ion-exchange chromatography have been published. The conductivity detector is more convenient and sensitive than the post- or precolumn derivatization methods employed for colorimetric detection or titrimetric determination of organic acids. Most of these studies on carboxylic acid analysis were carried out using ion-exclusion chromatography[9]. Despite the importance of the analysis of organic acids, only a few papers deal with the ion-exchange chromatographic behaviour of these

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compounds[10,11]. The present paper reports a sensitive and simple method for carboxylic acid and keto acid analysis by ion chromatography on an anion-exchange resin column.

MATERIALS AND METHODS

The instrument used was a preproduction prototype of Ion Chromatographic Analyzer Model IC 100 (Yokogawa Electric Co., Tokyo, Japan). The separation column was 250 × 4.6 mm I.D. A low-capacity anion-exchange resin was specially prepared by Yokogawa Electric Co. by gluing very fine particles of a strong anion-exchange resin onto the surface of polystyrene–divinylbenzene copolymer beads (mean diameter 10 μm) whose ion-exchange capacity was 35 μequiv./g. The same material was used in a 50 × 4.6 mm precolumn. The suppressor was constructed from drawn Nafion 811 X tubing (hollow-fibre perfluorosulphonic acid cation-exchange membrane) (0.4 mm I.D.) from DuPont which was inserted coaxially in a PTFE tubing of 1.0 mm I.D. The eluent was passed through the inside of the membrane tubing and on the outside a 0.05 *M* solution of dodecylbenzenesulphonic acid was pumped at a flow-rate of 2 ml/min in the counter-current direction as a scavenger. The whole system including a conductivity detector cell was thermostatted at 40°C. A UVIDEC II UV monitor (JASCO, Japan Spectroscopic Co., Tokyo, Japan) was connected between the analytical column and the suppressor. Its wavelength was tuned to 210 nm in accord with a dual-detector system. Peak areas were determined with a Chromatopak CR1-A integrator (Shimadzu, Kyoto, Japan).

Dodecylbenzenesulphonic acid was obtained from Tokyo Chemical Industries (Tokyo, Japan). Carboxylic and keto acids and other chemicals were purchased from Nakarai Chemicals Ltd. (Kyoto, Japan). The acids were dissolved in an eluent or water to an appropriate concentration and 2–20 μl of the solution were applied on the column.

RESULTS AND DISCUSSION

A typical chromatogram of an inorganic anion mixture recorded using a dual-detection system is shown in Fig. 1. The eluent was 4 m*M* sodium carbonate and 4 m*M* sodium bicarbonate and was pumped at a flow-rate of 2 ml/min. With the UV detector, nitrite and nitrate ion peaks appeared and a bromine peak was also recognizable. A large negative water peak, not depicted fully in the figure, was observed at the position indicated by the arrow.

With this eluent or even with a weaker carbonate buffer, monocarboxylic acids such as formic acid, acetic acid, and oxalacetic acid, eluted in a narrow range between fluoride and chloride ions, were not separated. The behaviour of these compounds was then examined with 2 m*M* sodium tetraborate which is a much weaker eluent than carbonate buffer. As shown in Fig. 2, the retention of these compounds was increased and several monocarboxylic acids were separated. However, the pairs acetic and lactic acid and formic and propionic acid were not resolved.

For higher-molecular-weight monocarboxylic acids, 4 m*M* sodium carbonate and 4 m*M* sodium bicarbonate were employed as eluent. Fig. 3 depicts the analyses of butyric and valeric acids and corresponding keto acids, the latter acids being eluted after the former.

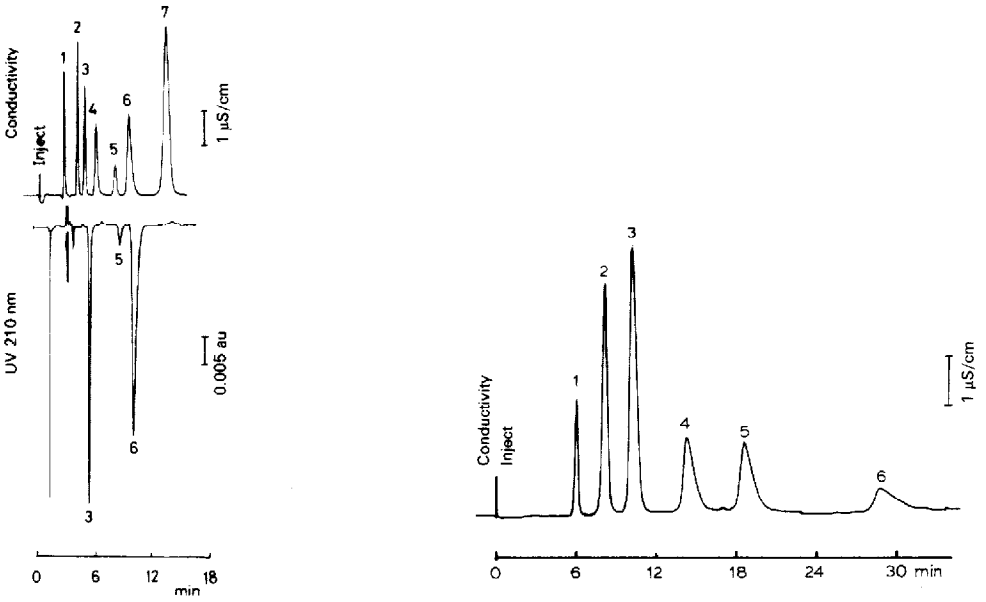

Fig. 1. Chromatogram of inorganic anions recorded with a dual-detection system. Eluent: 4 m*M* sodium carbonate and 4 m*M* sodium bicarbonate; flow-rate, 2 ml/min. Temperature: 40°C. Sample size: 20 µl. Peaks: 1 = F^- (5 ppm); 2 = Cl^- (10 ppm); 3 = NO_2^- (15 ppm); 4 = PO_4^{3-} (30 ppm); 5 = Br^- (10 ppm); 6 = NO_3^- (30 ppm); 7 = SO_4^{2-} (60 ppm).

Fig. 2. Chromatogram of monocarboxylic acids with 2 m*M* sodium tetraborate solution. Peaks: 1 = F^-; 2 = lactic acid (64 nmol); 3 = formic acid (64 nmol); 4 = glyceric acid (32 nmol); 5 = oxalacetic acid (64 nmol); 6 = Cl^-.

Dicarboxylic acids and corresponding keto acids were also separated under the same conditions, as shown in Fig. 4. Malonic acid was eluted very close to glutaric acid; however, corresponding keto acids were more strongly retained and well separated, α-ketoglutaric acid being eluted before α-ketomalonic acid. The separation of geometrical and structural isomers of unsaturated dicarboxylic acids, citraconic, itaconic and mesaconic acids, is shown in Fig. 5. The sensitivity of the UV detector to these compounds was much higher than to saturated acids such as malic acid.

Fig. 6 shows the chromatogram of citric acid and iodide eluted with 29 m*M* sodium carbonate and 20 m*M* sodium bicarbonate buffer. With a conductivity detector, unresolved mono- and dicarboxylic acids were observed at the left-hand side of the chromatogram, on the other hand, the UV detector revealed two major peaks of the isomers of the unsaturated maleic and fumaric acids, besides an iodide peak.

In Fig. 7, the logarithm of the capacity factor, k', for various mono- and dicarboxylic acids is plotted against the eluent carbonate concentration. Within this concentration range, monocarboxylic acids were barely retained on the column, so that a weaker eluent was required to separate these compounds. However, the pH of the diluted carbonate buffer was unstable and retention times fluctuated, therefore these compounds were examined with a borate eluent whose selectivity is lower than of carbonate. The ln k' values of the dicarboxylic acids were dependent on the carbonate concentration in the eluent; at lower concentrations, the compounds were

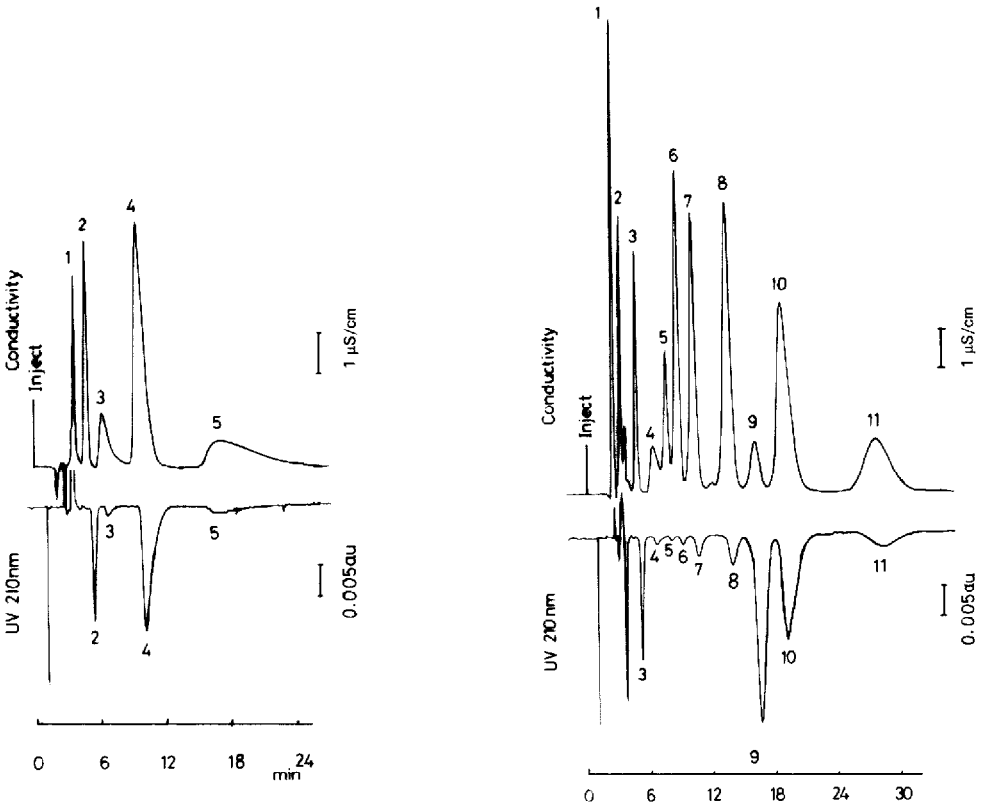

Fig. 3. Chromatogram of higher-molecular-weight monocarboxylic acids and corresponding keto acids. Peaks: 1 = butyric acid (40 nmol); 2 = α-ketobutyric acid (20 nmol); 3 = valeric acid (120 nmol); 4 = α-ketovaleric acid (40 nmol); 5 = caproic acid (350 nmol). Conditions as in Fig. 1.

Fig. 4. Chromatogram of dicarboxylic acids and corresponding keto acids. Peaks: 1 = lactic acid (40 nmol) and acetic acid (40 nmol); 2 = oxalacetic acid (40 nmol); 3 = α-ketobutyric acid (20 nmol); 4 = valeric acid (120 nmol); 5 = succinic acid (40 nmol); 6 = malonic acid (40 nmol); 7 = tartaric acid (40 nmol); 8 = oxalic acid (40 nmol); 9 = fumaric acid (20 nmol); 10 = α-ketoglutaric acid (40 nmol); 11 = α-ketomalonic acid (16 nmol). Conditions as in Fig. 1.

more strongly retained and the distance between adjacent peaks became larger; however, this did not necessarily mean an improvement in resolution, due to peak broadening. Phthalic and citric acids were more strongly retained than other carboxylic acids.

In Fig. 8, the ln k' values for n-alkyl mono- and dicarboxylic acids are plotted against carbon number. Monocarboxylic acids were eluted with sodium tetraborate solution and the retention time of formic acid was not as expected from the carbon number. The minimum k' value was obtained with acetic acid (carbon number = 2). Similar results were observed for dicarboxylic acids eluted with a carbonate buffer, in which succinic acid (carbon number = 4) gave the minimum k'.

In anion-exchange chromatography, both ionic interactions between the dissociated carboxylic residue and the functional group in the ion-exchange resin and

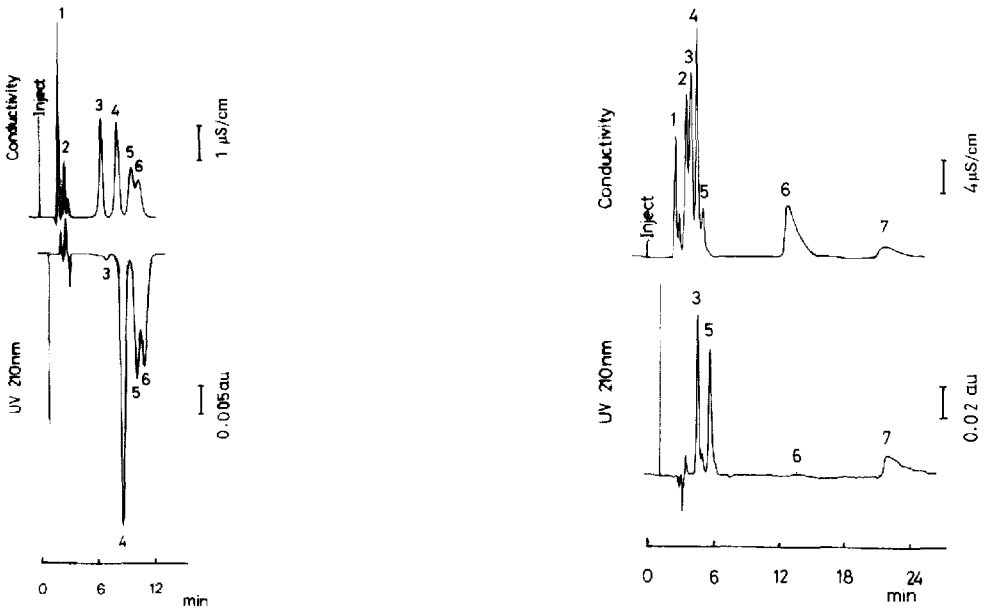

Fig. 5. Chromatogram of unsaturated dicarboxylic acid isomers. Peaks: 1 = gluconic acid; 2 = oxalacetic acid; 3 = malic acid; 4 = citraconic acid; 5 = itaconic acid; 6 = mesaconic acid. Sample amount: 15 nmol of each acid, except mesaconic acid which was 7.5 nmol. Flow-rate: 2.5 ml/min. Other conditions as in Fig. 1.

Fig. 6. Chromatogram of citric acid and iodide. Eluent: 20 m*M* sodium carbonate and 20 m*M* sodium bicarbonate. Peaks: 1 = acetic acid; 2 = adipic acid; 3 = maleic acid; 4 = oxalic acid; 5 = fumaric acid; 6 = citric acid; 7 = I^-. Other conditions as in Fig. 1.

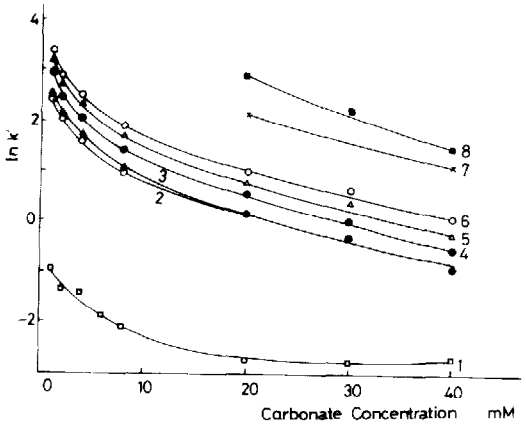

Fig. 7. Relationship between ln k' of carboxylic acids and the carbonate concentration in the eluent. Carboxylic acids: 1 = acetic acid, glycolic acid, lactic acid and glyoxylic acid; 2 = succinic acid; 3 = malic acid and glutaric acid; 4 = maleic acid; 5 = oxalic acid; 6 = fumaric acid; 7 = phthalic acid; 8 = citric acid.

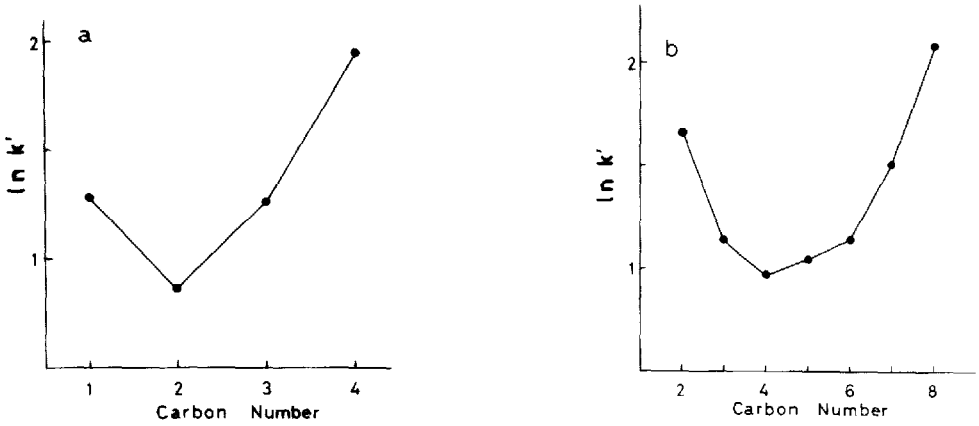

Fig. 8. Relationship between ln k' and solute carbon number: a, monocarboxylic acids eluted with 2 mM sodium tetraborate; b, dicarboxylic acids eluted with 4 mM sodium carbonate and 4 mM sodium bicarbonate buffer.

hydrophobic interactions between the solute alkyl chain and the matrix of the resin occur as previously shown in cation-exchange chromatography of amines[12]. The presence of minima in Fig. 8 implies that inhibition of the ionic interaction owing to the hydrophobic effect of the carbon atom adjacent to the dissociated carboxylic residue also plays an important rôle in solute retention.

Calibration curves for oxalic acid and succinic acid were constructed by two methods, by computing the peak areas with an integrator and by measuring the peak heights on the chart paper. As shown in Fig. 9, linear relations were obtained between

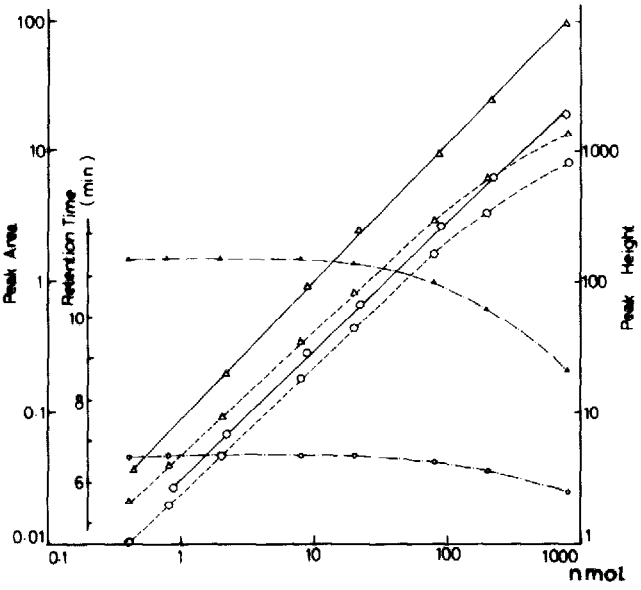

Fig. 9. Relationships between peak area (———), peak height (– –) and retention time (·····) of oxalic acid (○) and succinic acid (△) and sample amount applied on the column.

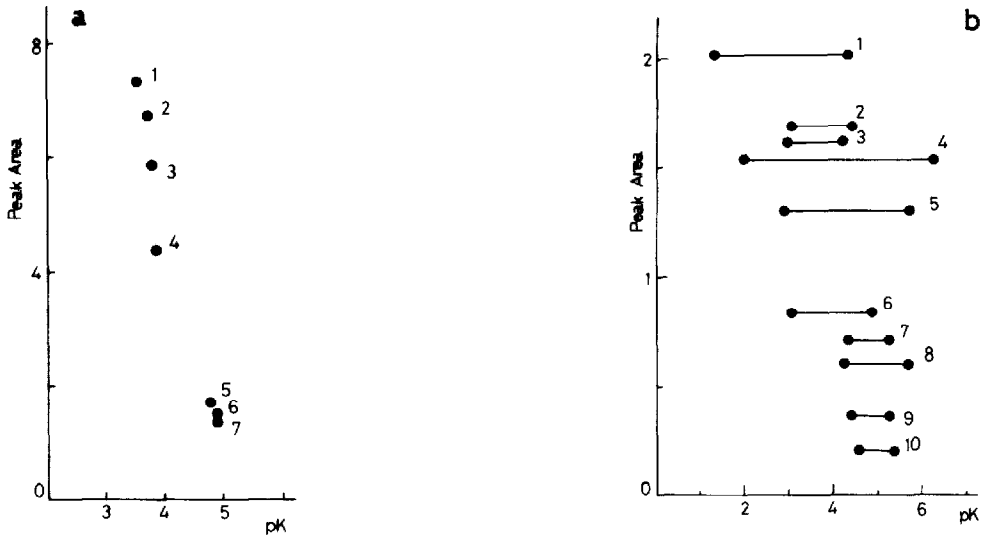

Fig. 10. Relationship between peak area and solute dissociation constant determined with a conductivity detector. a, Monocarboxylic acids eluted with 2 m*M* sodium tetraborate: 1 = glyceric acid; 2 = formic acid; 3 = glycolic acid; 4 = lactic acid; 5 = acetic acid; 6 = butyric acid; 7 = propionic acid. b, Dicarboxylic acids eluted with 4 m*M* sodium carbonate and 4 m*M* sodium bicarbonate buffer: 1 = oxalic acid; 2 = fumaric acid; 3 = tartaric acid; 4 = maleic acid; 5 = malonic acid; 6 = mesaconic acid; 7 = glutaric acid; 8 = succinic acid; 9 = adipic acid; 10 = azelaic acid. The first and second dissociation constants are linked by a bar for each acid.

peak area and the amount of compound injected in the range 0.5 nmol to 0.8 μmol. The relations between peak height and amount were also linear up to 0.1 μmol, however above this amount the plots became curved reflecting the broad and skewed shape of peaks when a large amount of solute was injected. In the latter case the retention time became smaller due to overloading of the the low-capacity ion-exchange column. The dynamic range is approximately two orders of magnitude for these compounds. When less than 0.5 nmol of acid were injected, the peak area had a tendency to be smaller than the expected value, probably due to adsorption of the solute on the surface of the walls of, *e.g.*, glass ware, syringe or instrument components.

The peak areas determined with the conductivity detector for monocarboxylic acids are plotted in Fig. 10a as a function of the acid dissociation constants. A similar relation was observed with dicarboxylic acids; Fig. 10b shows the result obtained by eluting with carbonate buffer. Under both elution conditions, the pH of the eluates measured after the detector was approximately 4.5. The response of the conductivity detector was dependent on the ratio of the dissociated to the undissociated carboxylic acid molecules.

CONCLUSIONS

With a conventional suppressor column containing an ion-exchange resin, weak electrolytes, such as organic acids, give complicated results due to ion exclusion

and band spreading[6]. In the present study, no such problems were observed in the analysis of organic acids, retention times were reproducible and no significant peak broadening during passage through the hollow-fibre suppressor was observed with UV and conductivity detectors placed before and after the suppressor tube respectively. The hollow-fibre suppressor should provide further applications for ion chromatography.

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