PERFORMANCE CHARACTERISTICS OF SOME COMMERCIALLY AVAILABLE LOW-CAPACITY ANION-EXCHANGE COLUMNS SUITABLE FOR NON-SUPPRESSED ION CHROMATOGRAPHY

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SUMMARY

Five commercial low-capacity anion-exchange columns (Vydac 302 IC 4.6, Interaction ION-100, Hamilton PRP-X100, Bio-Gel TSK IC-Anion-PW and Waters IC Pak A) were compared in terms of their performance characteristics when used for non-suppressed ion chromatography with conductivity detection. Chromatograms were obtained with the manufacturer's recommended eluent and a phthalate eluent designed to give optimal chromatographic performance within the constraints of reasonable run times and acceptable peak shapes for later eluting peaks. These chromatograms were used to provide data on column efficiency, peak asymmetry and resolution of three critical solute pairs. With the exception of the Interaction column, all columns gave acceptable resolution of a standard mixture of seven anions. Choice between columns should be based on such factors as the types and relative concentrations of sample anions, the sample and eluent pH, the percentage of organic modifier to be used in the eluent and the cost of the columns.

INTRODUCTION

Early anion-exchange columns employed in high-performance liquid chromatographic (HPLC) applications were of relatively high ion-exchange capacity (*e.g.* 1 mequiv./g) and were generally used for the separation of organic ionic species[1]. These columns have proved to be unsuitable for application to inorganic anions due to the fact that their high ion-exchange capacities necessitated use of eluents with ionic strengths sufficiently high to prohibit conductivity detection being employed. For this reason, a number of low-capacity anion-exchange materials were developed for ion chromatography of inorganic anions with conductivity detection.

The rationale used was that these columns could be employed with dilute eluents of low background conductivity, which in turn would permit the use of conductivity detection. Gjerde *et al.*[2,3] synthesised a number of low-capacity anion exchangers which when used with eluents comprising a dilute solution of an aromatic acid such as benzoate, provided the first example of single-column anion chromato-

TABLE I

DESCRIPTION AND SPECIFICATIONS OF THE LOW-CAPACITY ANION-EXCHANGE COLUMNS USED IN THIS STUDY

Column	Dimensions (mm)	pH range	Upper pressure limit (p.s.i.)	Maximum flow-rate (ml/min)	Maximum organic modifier (%)	Ion-exchange capacity (μequiv./g)	Particle size (μm)	Type of packing material
Vydac 302 IC 4.6	250 × 4.6	2–6	5000	–*	100	100	20	Spherical silica with bonded quaternary groups
Interaction ION-100	50 × 3.2	0–14	1400	1.0	10	100	10	Neutral hydrophilic macro-porous resin with covalently bound quaternary ammonium groups.
Hamilton PRP-X100	150 × 4.1	1–13	5000	8.0	100	200	10	Highly cross-linked poly-styrene–divinylbenzene coated with quaternary ammonium groups
Bio-Gel TSK Anion PW	50 × 4.6	1–12	–*	2.0	20	30	10	Polymethacrylate gel coated with quaternary ammonium groups
Waters IC Pak A	50 × 4.6	1–12	1000	1.2	20	30	10	Polymethacrylate gel coated with quaternary ammonium groups

* No data available.

graphy. These resins however exhibited poor chromatographic efficiencies, but nevertheless illustrated the potential of this approach.

Several alternative anion-exchange materials have since become commercially available and in this paper, the chromatographic performance of the most commonly used columns is assessed in terms of efficiency, resolution, susceptibility to overloading, selectivity and their ability to perform a separation of seven standard anions. A variety of eluents was used for this study, but in all cases, conductivity detection was employed.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model M590 pump, Model U6K injector, Model M430 conductivity detector and Model M730 data module.

Columns

The columns used in this study were as follows: (1) Vydac 302 IC 4.6 anion chromatography column, 250 × 4.6 mm I.D. (Separations Group, Hesperia, CA, U.S.A.); serial number 831205.4. (2) Interaction ION-100 column, 50 × 3.2 mm I.D. (Interaction Chemicals, Mountain View, CA, U.S.A.); serial number 15-149-3. (3) Hamilton PRP-X100 ion chromatography column, 150 × 4.1 mm I.D. (Hamilton Company, Reno, NV, U.S.A.); serial number 79434. (4) Bio-Gel TSK IC-Anion-PW column, 50 × 4.6 mm I.D. (Bio-Rad labs., Richmond, CA, U.S.A.); serial number 10533. (5) Waters IC Pak-A anion column, 50 × 4.6 mm I.D. (Waters Assoc.); Serial number T42431 21.

Reagents and procedures

All reagents were of the highest available purity and standard solutions of the inorganic anions were prepared by dissolving weighed amounts of the sodium salts in water purified with a Millipore (Bedford, MA, U.S.A.) Milli Q water purification system.

The mobile phases were prepared using analytical grade reagents, chromatographic grade organic solvents and pure water. Mobile phases were filtered through a 0.45-μm filter and degassed in an ultrasonic bath before use.

RESULTS

Column description

The physical dimensions, operating pH range, flow or pressure limitations, permissible percentage of organic modifier, ion-exchange capacity, particle size and a description of the packing material for each column are listed in Table I. One of the columns (Vydac) was packed with a silica-based material, whereas the remainder were resin-based ion exchangers. Stainless steel was used as the column construction material in all cases except the Bio-Gel TSK column which was constructed of PTFE.

The columns used for this study were all newly purchased and each was supplied with a test chromatogram, with the exception of the Waters column which was

TABLE II

DATA FOR TEST CHROMATOGRAMS SUPPLIED BY COLUMN MANUFACTURERS

Column	Anions used in test mixture	Eluent	Flow-rate (ml/min)	Efficiency* (theoretical plates)
Vydac	Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}	2 mM Phthalate, pH 5.0	2.0	4934 (NO_3^-)
Interaction	F^-, Cl^-, NO_2^-, Br^-, NO_3^- SO_4^{2-}	1.2 mM Phthalate, pH 5.0	1.0	No value given
Hamilton	F^-, Cl^-, NO_2^-, Br^-, NO_3^-	2.0 mM Benzoate	2.0	3450 (NO_3^-)
Bio-Gel TSK	F^-, Cl^-, NO_2^-, Br^-, NO_3^- PO_4^{3-}, SO_4^{2-}	1.3 mM Tetraborate, 5.8 mM boric acid, 1.3 mM gluconate, 5 g/l glycerin, 120 ml/l acetonitrile, 30 ml/l n-butanol, pH 8.5	1.2	944 (SO_4^{2-})

* The half-height method was used to calculate efficiency and the solute used for this calculation is shown in brackets.

provided with a guaranteed minimum value of efficiency (900 theoretical plates). Data from the test chromatograms are summarised in Table II and the actual chromatograms are shown in Fig. 1. These test chromatograms were repeated using the manufacturer's recommended eluents and the instrumentation described in the experimental section. In all cases, measured efficiencies were in close agreement with the supplied values listed in Table II.

Performance characteristics

A standard mixture containing 100 ppm each of fluoride, chloride, nitrite, bromide, nitrate, sulphate and iodide was selected for the evaluation of column performance. Two sample injection volumes were used; 10 μl and 100 μl. The first of these injection volumes corresponded to 7 μg of sample and represented a typical working range for the columns, whereas the larger injection volume corresponded to 70 μg of sample, which was expected to cause overloading of some of the columns. The onset of column overloading is an important factor in the analysis of samples which contain ions at widely varying concentrations. The chromatograms obtained with the above standard mixture using the manufacturer's recommended eluent (see Table II) were used to calculate the column efficiency (at capacity factors of 5 and 10), the resolution of three critical peak pairs and the peak asymmetry at two levels of sample loading for each column. The results are given in Table III.

Experience in this laboratory has indicated that phthalate eluents gave optimal separations with most low-capacity anion-exchange columns. Furthermore, use of a common eluent would facilitate comparison of the columns. The eluent concentration and pH was adjusted for each column so that the total time taken for elution of the mixture was approximately equal. In addition, every attempt was made to maximise the resolution of earlier eluting peaks, whilst at the same time ensuring that adequate peak shape was obtained for late eluting species. The chromatograms obtained are
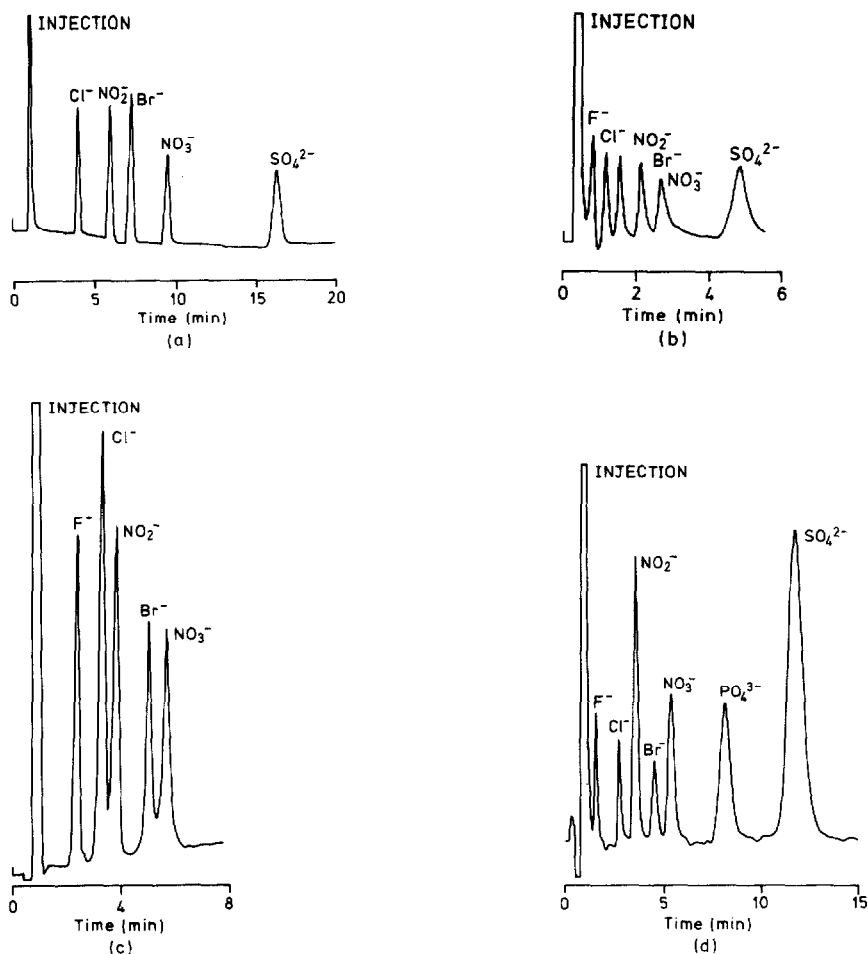
Fig. 1. Manufacturer's test chromatograms. (a) Vydac 302 IC 4.6, (b) Interaction ION-100, (c) Hamilton PRP-X100, (d) Bio-Gel TSK-lC-Anion PW. Conditions: Eluents and flow-rates are given in Table II. Conductivity detection was used in all cases.

given in Fig. 2 and efficiency and resolution data calculated from these chromatograms are listed in Table IV.

DISCUSSION

Type of packing material

The column packing materials may be broadly divided into silica-based and resin-based types, with the majority of columns tested falling into the latter category. Use of a silica material provides excellent rigidity of the packing and hence tolerance of high back-pressures and flow-rates, but creates problems with eluent pH limitations and a tendency to adsorb strongly fluoride ion, making it difficult to determine this ion.

TABLE III

PERFORMANCE CHARACTERISTICS FOR SEPARATION OF A STANDARD ANION MIXTURE USING TEST CONDITIONS RECOMMENDED BY COLUMN MANUFACTURERS

Chromatographic conditions are listed in Table II, except for the Waters column for which an eluent containing 1.5 mM tetraborate, 5.8 mM boric acid, 1.3 mM gluconate, 5 g/l glycerin and 120 ml/l acetonitrile was used.

| Column | Retention times (min) | | | | | | | Efficiency* (theoretical plates per column) | | Theoretical plates per meter ($k' = 5$) | Peak asymmetry** for nitrate | | Resolution (R_s) | | |
	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	SO_4^{2-}	I^-	$k' = 5$	$k' = 10$		$7-\mu g$ loading	$70-\mu g$ loading	Cl^-/NO_2^-	NO_2^-/Br^-	Br^-/NO_3^-
Vydac	—***	4.33	6.17	6.65	8.55	12.84	20.34	3609	1554	13 084	1.07	0.41	2.24	0.80	2.44
Interaction	0.97	1.30	1.54	2.12	2.55	5.97	10.41	618	373	12 360	1.33	0.42	0.58	0.85	0.96
Hamilton	2.90	4.10	4.68	5.92	6.70	16.51	20.18	3450	3217	22 999	1.68	1.10	0.92	2.13	0.94
Bio-Gel TSK	1.46	2.49	3.12	3.95	4.59	10.18	11.40	934	898	18 680	1.17	0.72	0.90	1.10	0.89
Waters	1.57	2.73	3.39	4.17	4.83	14.20	10.46	1276	1220	25 520	1.02	0.57	1.69	1.89	1.39

* Efficiency was calculated using the 5σ method.
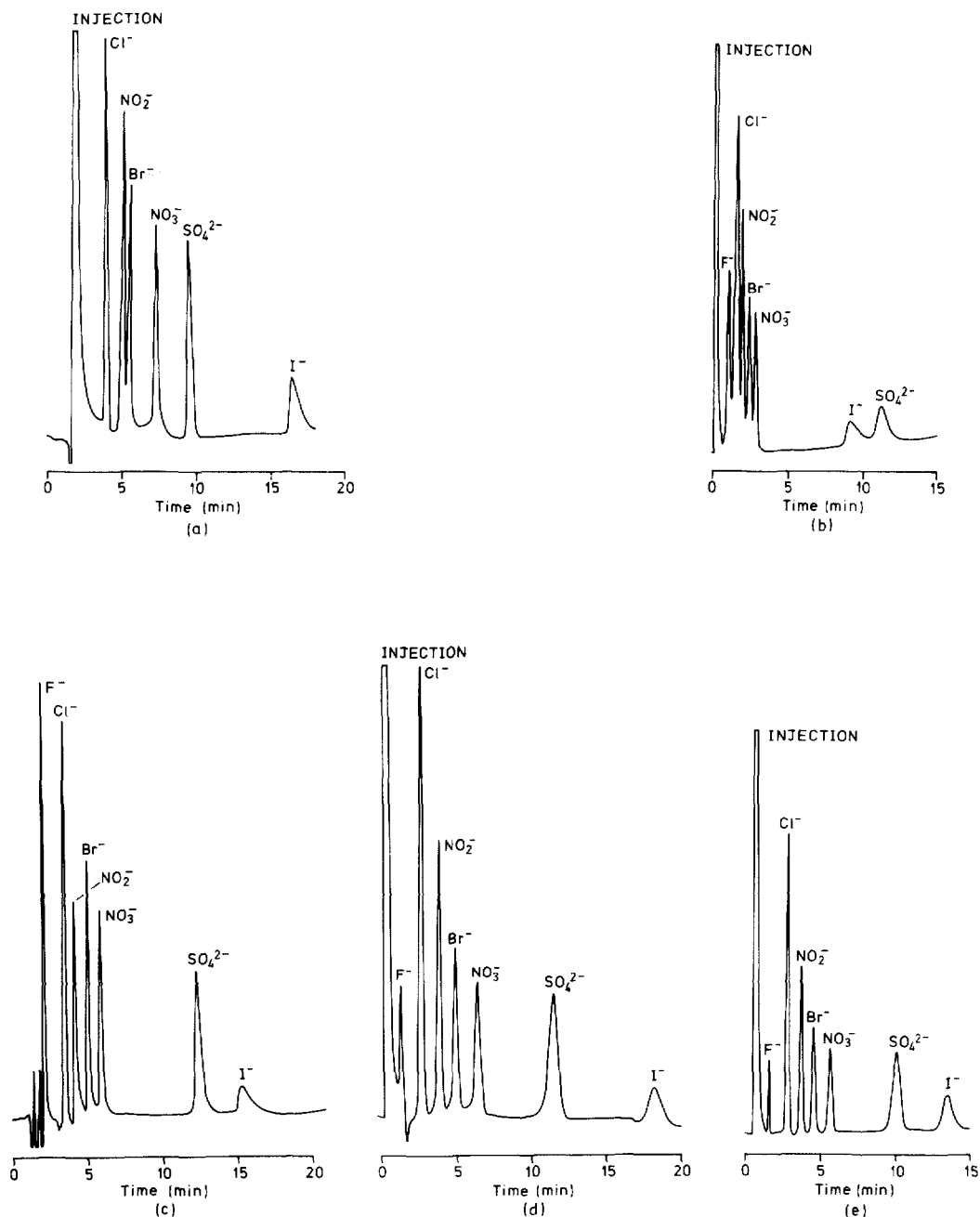** Calculated according to Saunders[7].
*** No peak observed.

Fig. 2. Chromatograms obtained with optimised phthalate eluents. (a) Vydac 302 IC 4.6, (b) Interaction ION-100, (c) Hamilton PRP-X100, (d) Bio-Gel TSK IC-Anion-PW, (e) Waters IC Pak A. Conditions: Eluents, potassium hydrogen phthalate solutions at the following concentrations and pH values: (a) 3 mM, pH 5.3, (b) 1 mM, pH 4.1, (c) 1 mM, pH 5.5, (d) 1 mM, pH 5.3, (e) 1 mM, pH 7.0. Flow-rates (ml/min): (a) 2.0, (b) 1.0, (c) 2.0, (d) 1.2, (e) 1.2. Sample: 10 μl of a mixture containing 100 ppm each of the indicated anions.

TABLE IV

EFFICIENCY AND RESOLUTION DATA FOR OPTIMISED PHTHALATE ELUENTS

Chromatographic conditions and actual chromatograms used for data calculations are given in Fig. 2.

Column	Efficiency*		Resolution (R_s)		
	Theoretical plates per column	Theoretical plates per meter	Cl^-/NO_2^-	NO_2^-/Br^-	Br^-/NO_3^-
Vydac	4163	16 652	1.97	0.73	2.09
Interaction	605	12 100	0.75	0.91	0.87
Hamilton	2356	15 706	1.28	1.76	1.62
Bio-Gel TSK	959	19 180	1.92	1.62	2.03
Waters	1306	26 120	2.37	2.05	2.64

* Calculated for nitrate ion using the 5σ method.

The pH limitation towards alkaline eluents is imposed by the solubility of the silica material and represents a serious drawback to the use of silica-based columns in anion chromatography. In the first place, the use of eluents with pH values greater than 6 is often necessary for the elimination of interfering "system" peaks[4]. Such system peaks are especially prevalent when phthalate eluents are employed with indirect UV absorption detection. In addition, some of the eluents known to be useful for anion chromatography are quite strongly alkaline, for example, 0.5 mM potassium hydroxide[5] and the gluconate–borate eluent described in Table II. Secondly, it is often advantageous, and in some cases essential, to operate at high eluent pH in order to obtain satisfactory peaks for species which are subject to protolytic equilibria. For example, bicarbonate ion can be determined only with eluents of pH greater than approximately 6.5 and useful selectivity effects can be achieved for phosphate, oxalate etc. using eluent pH changes. Any restriction on the range of eluent pH values must necessarily inhibit exploitation of these effects. Resin-based columns therefore offer considerable advantages in this respect. It should be stressed here that the wide pH limits for resin-based columns shown in Table I may be quite temperature sensitive. For example, experience in our laboratory with the Hamilton column has shown that when eluents were used at temperatures exceeding 30°C, column lifetime was dramatically reduced if eluent pH values above 8 were employed.

Whilst resin-based columns offer advantages in terms of eluent pH, they lack the packing rigidity of silica-based materials. Examination of Table I indicates that with the exception of the Hamilton column, severe restrictions exist regarding the maximum pressures and flow-rates which can be used with the resin-based columns. These restrictions in turn lead to the necessity for short columns to be used. The polystyrene–divinylbenzene resin employed in the Hamilton column appears to have good pressure stability and may be used at relatively high flow-rates.

The addition of organic modifiers to the aqueous mobile phases typically used in anion chromatography may be desirable. The possible benefits which could arise are selectivity effects due to the influence of organic modifier solvation effects on the ion-exchange equilibria, or prevention of the adsorption of non-polar sample components on the column packing material. This latter effect may result in column

poisoning and we have found that the Hamilton column is particularly susceptible in this regard, undoubtedly due to the reversed-phase nature of the polystyrene–divinylbenzene material used[6]. The tolerance of the anion-exchange resins to organic modifiers is therefore of some importance, and Table I shows that a considerable range exists for this parameter.

Ion-exchange capacity

In single-column (non-suppressed) ion chromatography, the sensitivity of detection is usually determined by the background conductivity of the eluent since the detector signal is essentially the difference between the conductivities of the eluent anion and the solute anion. The eluent concentration (and hence conductivity) required for efficient separation is related to the total ion-exchange capacity of the column and this is governed by the column dimensions and the exchange capacity of the packing material. When phthalate eluents were used, the eluent strengths (taking into account the eluent pH and concentration) increased in the following column order: Interaction < Bio-Gel TSK < Waters < Hamilton < Vydac. Detection sensitivity was equivalent for the Waters, Bio-Gel TSK and Hamilton columns, with the Vydac and Interaction columns giving reduced sensitivities due to high eluent strength and poor efficiency, respectively.

Column performance characteristics

When the manufacturer's recommended eluent was used for the separation of the standard anion mixture, the results given in Table III were obtained. The longer columns (Vydac and Hamilton) gave the highest numbers of theoretical plates per column, but when efficiency was expressed as theoretical plates per meter, the Waters column gave the highest value. Comparison of the column efficiency values calculated at capacity factors of 5 and 10 shows that the Vydac and Interaction columns exhibited a large decrease in efficiency for longer retained peaks.

The resolution values given in Table III reflect the ability of each column to resolve three closely eluting peak pairs in the standard anion mixture. The selectivity differences which existed between the columns were reflected by the fact that the worst resolved peak pair differed from column to column. For example, nitrite and bromide were poorly resolved on the Vydac column, whereas chloride and nitrite, as well as bromide and nitrate, were poorly resolved with the Hamilton column. The Waters column provided the best overall resolution.

A further selectivity effect was also observed. In general, all of the solutes tested eluted in the same order (Table III), with the exception of iodide and sulphate which showed a reversal of retention order on the Waters column. This effect disappeared when the Waters column was used with a phthalate eluent (see Fig. 2e), but was observed when the Interaction column was used with a phthalate eluent of lower pH than that employed in the manufacturers test chromatogram (see Fig. 2b). However it is fair to say that all of the columns tested showed similar overall selectivity.

The peak asymmetry values shown in Table III illustrate that the columns examined were easily overloaded, with severely fronted peaks observed for a column loading of 70 μg of sample. Despite this peak distortion, the test mixture was still adequately resolved on all columns except the Interaction column.

The chromatograms obtained with optimised phthalate eluents (Fig. 2) provide a convenient means to compare the column performances. It should be remembered that the concentration and pH of each phthalate eluent was selected so that the overall length of each chromatogram was similar, and that acceptable peak shapes were obtained for the later eluting species (iodide and sulphate). The latter condition required that the chromatogram obtained for the Interaction column was somewhat shorter than those for the other columns, leading to a tendency for the early eluting peaks to be poorly resolved. The data given in Table IV show that with the exception of the Hamilton column, the numbers of theoretical plates per column obtained with the optimised phthalate eluents were approximately equal to, or higher than, the values observed using the manufacturer's recommended eluent (see Table III). Comparison of Tables III and IV also shows that the resolution of critical peak pairs was significantly better with the phthalate eluents than with the manufacturer's recommended eluent, for all columns except the Vydac column.

CONCLUSIONS

Of the five columns tested, four (Vydac, Hamilton, Bio-Gel TSK and Waters) gave adequate separation of the seven standard anions. Elution order was almost identical with all columns, showing that the selectivities of the columns were very similar.

The choice of column for a particular separation is dependent on such factors as the types and relative concentrations of sample anions, the sample pH and the desired eluent pH, the percentage of organic modifier required in the eluent and in view of the wide variation in prices for the columns, cost.

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