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Applications of a new methacrylate-based anion stationary phase for the separation of inorganic anions

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Abstract

A new methacrylate-based packing with quaternary amine functional groups for the analysis of inorganic anions by ion chromatography is described. Columns packed with this new material work for both suppressor-based and single-column ion chromatography methods. A variety of eluents such as carbonate–bicarbonate, phthalic acid and *p*-hydroxybenzoic acid are compatible with this column. The packing is stable under severe conditions such as switching from high pH to low pH eluents, or vice versa. The hydrophilic nature of the packing provides excellent peak shape for all common inorganic anions, including hydrophobic anions such as nitrate and iodide. The performance of the column with different eluents is demonstrated along with the applications using both single-column and suppressor-based ion chromatography systems.

Keywords: Stationary phases, LC; Inorganic anions

1. Introduction

Over the past two decades, ion chromatography (IC) has blossomed into an excellent tool for the analysis of inorganic ions. IC is the best technique available for inorganic anion analysis. In IC, as in any other branch of liquid chromatography, columns are the heart of the system, because that is where the chemistry of separation takes place. Packings for anion analysis should have low ion-exchange capacity, so low ionic strength eluents, required with both single-column and suppressor-based IC methods, may be used. These ion-exchangers should also have good ion-exchange kinetics and selectivity with various eluents, so they can be used with different IC methods.

Over the past several years, many stationary phases have been developed for anion analysis. They can be classified as either silica-based or polymer-based anion exchangers. Silica-based anion exchangers [1,2] are rigid, but can only be used between pH 2 and 6 and are therefore used for single column (SC) IC methods only. Strongly acidic or strongly basic eluents will dissolve the silica packing. Silica-based packings react with fluoride ions and consequently cannot be used for fluoride-containing samples.

Polymer-based columns are more commonly used in anion chromatography because of their resistance to high pH eluents. This feature broadens the compatibility of the column with both types of ion chromatography methods (suppressor-based or single-column IC). Two types of polymer resins are commonly used for anion separation by IC: microporous agglomerated pellicular anion exchangers [3] and macroporous poly(styrene–divinylbenzene) (PS–DVB) copolymer [4,5] anion exchangers. The agglomerated pellicular anion exchangers were de-

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veloped for suppressor-based IC systems. They offer excellent mass-transfer characteristics for rapid separations of a large number of anions. The macroporus, PS–DVB based anion exchangers are pH stable and can be used with both single-column and suppressor-based IC methods. Some of these anion exchangers are very hydrophobic and produce poor peak shape for hydrophobic anions due to the reversed-phase interactions. Microporous polymethacrylate anion-exchange resins [6] are also popular for anion analysis by IC. But this packing is very fragile and can only be operated under 1000 p.s.i. (1 p.s.i.= 6894.76 Pa). A macroporous, hydroxyethylmethacrylate [7] (HEMA)-based anion exchanger was also developed in the past. This anion exchanger is hydrophilic in nature and showed excellent compatibility with both types of IC methods.

The stationary phase described in this paper (Allsep) is a macroporous, methacrylate-based anion exchanger that is rigid, chemically stable and shows good ion-exchange characteristics with a wide range of eluents. The packing is stable at pressures up to 3000 p.s.i. Common IC eluents such as carbonate–bicarbonate, phthalic acid, *p*-hydroxybenzoic acid and succinic acid are compatible with this stationary phase. The advantages of the methacrylate-based stationary phase include high efficiency, short retention times and exceptional peak shape.

2. Experimental

2.1. Instrumentation

Ion chromatography was performed using an Alltech Odyssey Ion Chromatography System (Alltech Associates, Deerfield, IL, USA) that includes the model 526 HPLC pump, ERIS 1000 HP Autosuppressor, model 570 Autosampler, model 530 column heater and model 550 conductivity detector. A PE Nelson Turbochrom EL datasystem (Perkin–Elmer, San Jose, CA, USA) was used to record all data.

2.2. Columns

The anions were separated on Allsep Anion Columns (100×4.6 mm and 50×4.6 mm sizes) (Alltech Associates). These columns were packed

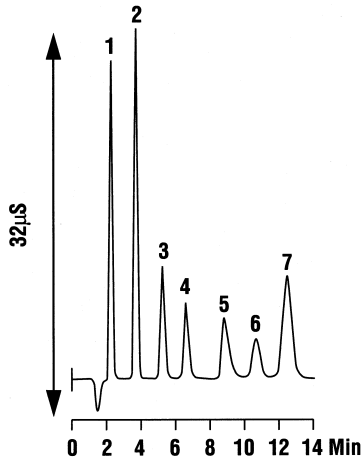

Fig. 1. Anions with bicarbonate–carbonate eluent. Column: Allsep Anion column, 100×4.6 mm, eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate, flow-rate: 1.2 ml/min, peak identification: 1=fluoride (2 ppm), 2=chloride (4 ppm), 3=nitrite (4 ppm), 4=bromide (4 ppm), 5=nitrate (4 ppm), 6=phosphate (6 ppm), 7=sulfate (6 ppm).

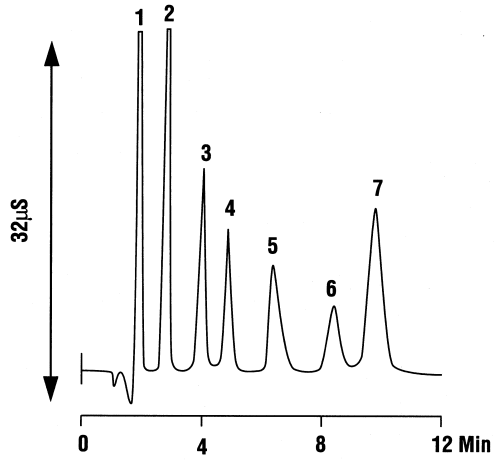

Fig. 2. Fast and sensitive separation of inorganic anions in reagent water. Column: Allsep Anion column, 50×4.6 mm, eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate, flow-rate: 1.1 ml/min, peak identification: 1=fluoride (2 ppm), 2=chloride (4 ppm), 3=nitrite (4 ppm), 4=bromide (4 ppm), 5=nitrate (4 ppm), 6=phosphate (6 ppm), 7=sulfate (6 ppm).

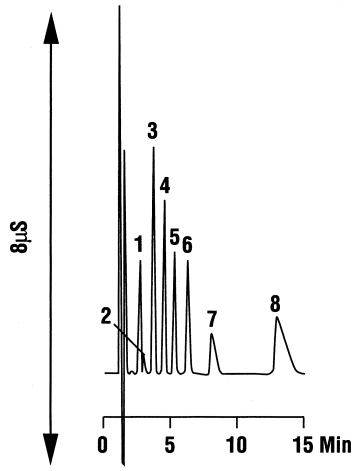

Fig. 3. Anions with *p*-hydroxybenzoic acid eluent. Column: Allsep Anion column, 100×4.6 mm, eluent: 4 m*M* *p*HBA (pH 7.5 w/LiOH), flow-rate: 1.0 ml/min, detector: conductivity, peak identification: 1=fluoride (10 ppm), 2=carbonate, 3=chloride (20 ppm), 4=nitrite (20 ppm), 5=bromide (20 ppm), 6=nitrate (20 ppm), 7=phosphate (30 ppm), 8=sulfate (30 ppm).

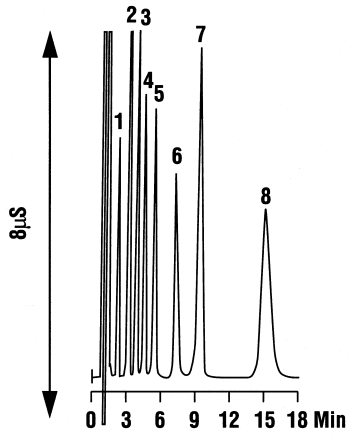

Fig. 4. Anions with phthalic acid eluent. Column: Allsep anion column, 100×4.6 mm, eluent: 4 m*M* phthalic acid (pH 4.2 w/LiOH), flow-rate: 1.0 ml/min, detector: conductivity, peak identification: 1=fluoride (9 ppm), 2=chloride (18 ppm), 3=nitrite (18 ppm), 4=bromide (18 ppm), 5=nitrate (18 ppm), 6=oxalate (50 ppm), 7=sulfate (27 ppm), 8=thiosulfate (50 ppm).

with 7-μm methacrylate-based anion-exchange particles.

2.3. Reagents

Four different eluents were tested with the methacrylate-based anion column: bicarbonate–carbonate, *p*-hydroxybenzoic acid, phthalic acid and succinic acid. All eluents were prepared from EZ-Lute™ buffer concentrate (Alltech Associates) by diluting with deionized water. The standards were prepared from Alltech certified IC standards (Alltech Associates).

3. Results and discussion

The methacrylate-based anion exchanger described in this paper is developed by quaternizing the base resin with trimethyl amine to provide anion-exchange capabilities. The packing is 7 μm in size and stable up to 3000 p.s.i. pressure. Suitability of this stationary phase for anion separation by IC using different eluents and its performance characteristics are the focus of this study.

The eluents used in anion exchange chromatography depend on the detection schemes used for the application [6]. They are classified into two groups: (1) Eluents for conductivity detection with chemical suppression of the background conductance (Suppressor-based IC eluents). (2) Eluents for the conductivity detection with electronic compensation of the background conductivity ((SC) IC eluents). The common eluents used for suppressor-based anion analysis are high pH bicarbonate–carbonate, sodium hydroxide or sodium tetraborate. Low concentrations of carboxylic acids such as phthalic acid, *p*-hydroxybenzoic acid and succinic acid are commonly used for single-column anion analysis. In this study, ionic strength and pH for several common (SC) IC and suppressor-based eluents were optimized to produce maximum resolution and minimum run times.

4. Suppressor-based IC

Suppressor-based IC is more popular than (SC) IC because it provides lower detection limits. The method uses a post-column suppressor device to reduce eluent conductivity and increase analyte

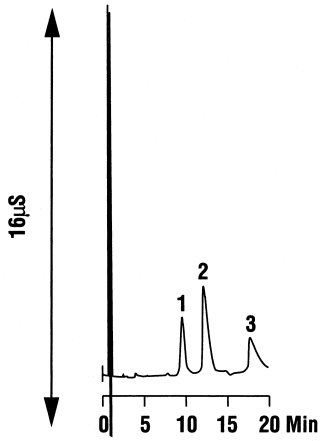

Fig. 5. Polarizable anions. Column: Allsep Anion column, 100× 4.6 mm, eluent: 4 m*M* phthalic acid (pH 4.2 w/LiOH), flow-rate: 1.2 ml/min, detector: conductivity, peak identification: 1=iodide (50 ppm), 2=thiosulfate (50 ppm), 3=thiocyanate (50 ppm).

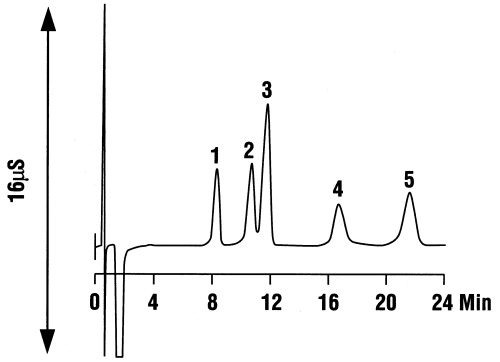

Fig. 6. Anions with succinic acid eluent. Allsep Anion column, 100×4.6 mm, eluent: 20 m*M* succinic acid (pH 3.0 w/LiOH), flow-rate: 1.5 ml/min, detector: conductivity, peak identification: 1=phosphate (10 ppm), 2=phosphite (10 ppm), 3=hypophosphite (10 ppm), 4=chloride (5 ppm), 5=nitrite (8 ppm).

conductivity, improving signal-to-noise ratios. In anion analysis, high pH eluents such as a mixture of sodium bicarbonate and sodium carbonate or sodium hydroxide are commonly used.

4.1. Bicarbonate–carbonate

Sodium bicarbonate–carbonate eluents are widely used because anion selectivity may be adjusted by varying the ratio of carbonate to bicarbonate. Fig. 1 shows the separation of seven common anions in reagent water on the methacrylate-based column using the bicarbonate–carbonate eluent. All seven anions are separated within 15 min. This new stationary phase completely resolves fluoride from the water dip making fluoride quantification easy. Many commercially available columns lack this feature making the quantification of fluoride extremely difficult. For shorter retention times, a 50× 4.6 mm column can be used as shown in Fig. 2.

5. Single-column IC

In (SC) IC, low concentrations of carboxylic acids are used as eluents for anion analysis. The eluent should have lower equivalent conductance and high affinity towards the stationary phase to promote effective elution of sample ions [8]. We studied the compatibility of three different (SC) IC eluents: *p*-hydroxybenzoic acid, phthalic acid and succinic acid.

5.1. p-Hydroxybenzoic acid

p-Hydroxybenzoic acid (*p*HBA) [9] is the most common eluent used for (SC) IC anion separations. It is most useful in the pH range of 7.5–8.5, where

Table 1
Column efficiency with various mobile phases

Mobile phase	Efficiency[a] (plates/m) (100×4.6 mm column)
0.85 m*M* Sodium bicarbonate–0.9 m*M* sodium carbonate	40 291
4 m*M* *p*-Hydroxybenzoic acid (pH 7.5) W/LiOH	37 323
4 m*M* Phthalic acid (pH 4.5) W/LiOH	42 615

[a] Calculated at half-height using nitrate peak.

Table 2
Anion exchange capacity factors of anions on the Allsep column

Anion	k'	
	$NaHCO_3$–Na_2CO_3	*p*HBA (pH 7.5)
Fluoride	1.56[a]	1.20
Chloride	2.99	2.15
Nitrite	4.73	2.83
Bromide	5.99	3.42
Nitrate	8.32	4.26
Phosphate	11.24	6.00
Sulfate	13.45	9.46

[a] The higher k' value for the fluoride peak results in longer retention time, providing excellent resolution of fluoride from the water dip.

one carboxylate group is completely ionized (pK_1 = 4.5) and the hydroxyl group is only partially ionized (pK_2=9.3). In the pH range of 7.5–8.5, *p*HBA is a mixture of 1- and 2-driving ions, making it possible to separate both monovalent and divalent anions in one run. Fig. 3 shows the separation of seven common anions plus carbonate using *p*HBA eluent. Good peak shape is achieved for all anions, including the hydrophobic nitrate ion. This eluent is also suitable for carbonate analysis.

5.2. Phthalic acid

Phthalic acid [10] eluents are used in the pH 3.8–4.5 range. In this pH range a mixture of 1- and 2-phthalate anions is the driving ion. This eluent is not recommended for phosphate analysis because in this pH range phosphate exists as 1-charge and elutes in the void volume. Carbonate also elutes in the void, making this an excellent eluent for analyzing samples containing high concentrations of carbonate. Fig. 4 shows the separation of six common anions plus oxalate and thiosulfate. Phthalic acid is an excellent eluent for the separation of polarizable anions such as iodide, thiosulfate and thiocyanate as shown in Fig. 5. By adding a small amount of methanol the peak tailing of thiocyanate due to adsorption effects can be reduced.

Table 3
Retention reproducibility of chloride, nitrate and sulfate

Column serial number	Retention time (min)		
	Chloride	Nitrate	Sulfate
97020016	4.38	10.21	15.78
97020014	4.31	9.98	15.33
97011573	4.36	10.23	15.49
96111466	4.22	9.89	15.33
97011870	4.29	10.02	15.41

Table 4
Method detection limits (MDL) for anions

Anions	Suppressor-based IC (ppb)[a]	(SC) IC (ppb)[b]
Fluoride	1.40	30
Chloride	1.53	20
Nitrite-N	2.00	60
Bromide	2.10	50
Nitrate-N	2.00	40
Phosphate-P	2.50	108
Sulfate	3.50	50

[a] Detection limits at 3× signal-to-noise level for 200 μl injection volume; eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate; flow-rate: 1.2 ml/min; detector: suppressed conductivity.
[b] Detection limits at 3× signal-to-noise level for 100 μl injection volume; eluent: 4 m*M* *p*HBA (pH 7.5). Flow-rate: 1.0 ml/min; detector: conductivity.

5.3. Succinic acid

Succinic acid is a weak eluent and can be used for the separation of monovalent anions that are weakly retained. Separation of phosphate, phosphite, hypophosphite, chloride and nitrite on the methacrylate-based stationary phase is shown in Fig. 6.

6. Column efficiency with various eluents

Table 1 shows the efficiencies of nitrate on the column, using three different eluents. The efficiencies are expressed as number of theoretical plates per meter, calculated by the half height method (N=5.54 $(t_R/W_{1/2})^2$×1000/column length in mm). These efficiencies are comparable to the efficiencies reported for many commercially available columns [11].

7. Selectivity of anions on the methacrylate-based anion stationary phase

Table 2 summarizes the selectivity of seven

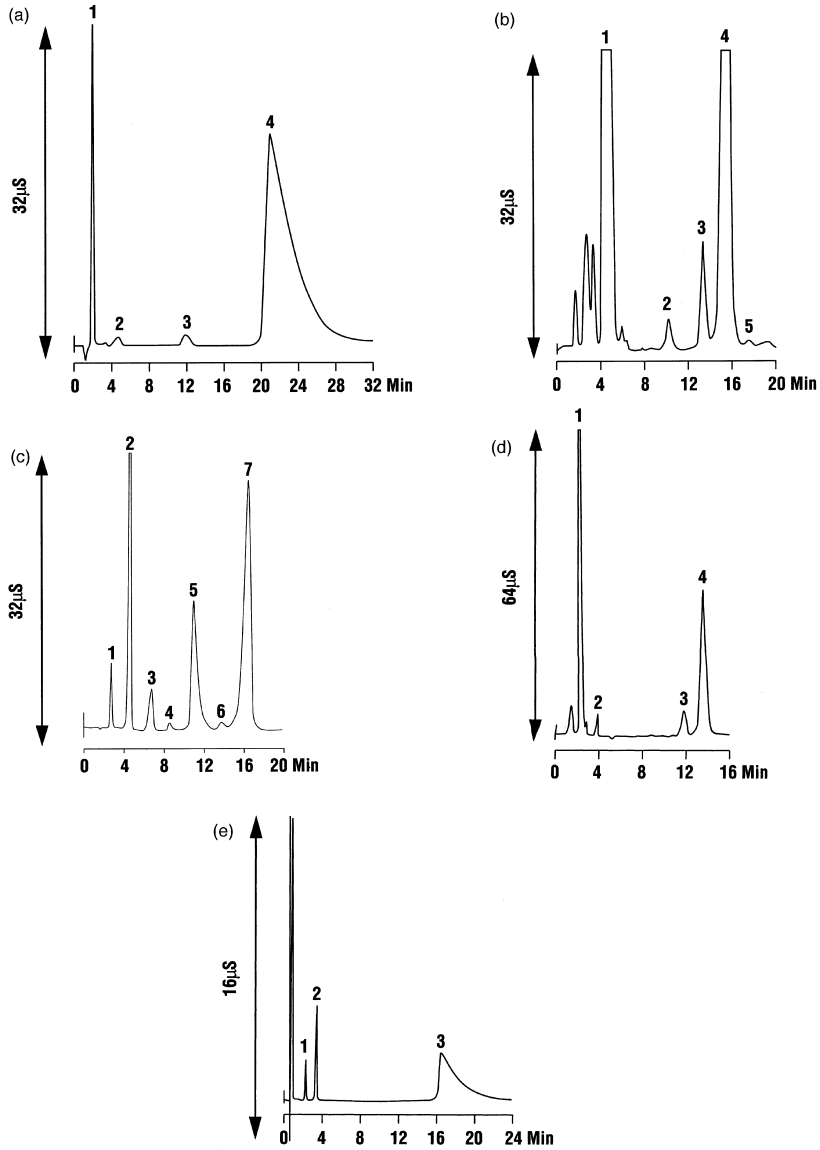

Fig. 7. (a) Chromate plating bath. Column: Allsep Anion column,100×4.6 mm, eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate, flow-rate: 1.2 ml/min, detector: suppressed conductivity, peak identification: 1=hexafluorosilicate, 2=nitrite, 3=sulfate, 4=chromate. (b) Anions in urine. Column: Allsep Anion column, 100×4.6 mm, eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate, flow-rate: 1.2 ml/min, detector: suppressed conductivity, peak identification: 1=chloride, 2=nitrate, 3=phosphate, 4=sulfate, 5=oxalate. (c) Inorganic anions in water per EPA method 300.0 (A). Column: Allsep Anion column, 100×4.6 mm, eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate, flow-rate: 1.2 ml/min, detector: suppressed conductivity, peak identification: 1=fluoride (2 ppm), 2=chloride (20 ppm), 3=nitrite-N (2 ppm), 4=bromide (2 ppm), 5=nitrate-N (10 ppm), 6=phosphate-P (2 ppm), 7=sulfate (60 ppm). (d) Anions in toothpaste. Column: Allsep anion column, 100×4.6 mm, eluent: 0.85 m*M* sodium bicarbonate–0.9 m*M* sodium carbonate, flow-rate: 1.2 ml/min, detector: suppressed conductivity, peak identification: 1=fluoride, 2=chloride, 3=phosphate, 4=monofluorophosphate (MFP). (e) Azide and perchlorate. Column: Allsep anion column, 100×4.6 mm, eluent: 4 m*M* phthalic acid (pH 4.2) w/LiOH, flow-rate: 1.0 ml/min, detector: conductivity, peak identification: 1=azide (15 ppm) 2=nitrate (15 ppm) 3=perchlorate (100 ppm).

common anions using bicarbonate–carbonate and *p*-hydroxybenzoic acid eluents. All anions show high k' values resulting in excellent selectivity for all anions. The high k' value for fluoride provides complete resolution from the water dip, improving fluoride quantification.

8. Reproducibility and column life

The column to column reproducibility was studied on five different columns using bicarbonate–carbonate as the eluent. Table 3 demonstrates the retention times of chloride, nitrate and sulfate ions. This study showed excellent column to column reproducibility. Since the capacity of the column is very low, this column is very sensitive to changes in eluent pH. Eluents should be prepared daily to avoid this problem. The column life time study was performed by continuously injecting anion standards under optimized conditions. These experiments proved that after 1000 injections, the column still maintained the same resolving power as the initial injection.

Most other IC columns can only be used with one type of eluent within a limited pH range. We switched between several eluents (high and low pH) on a single methacrylate-based anion column. Column performance was not affected and between-eluant equilibration times were short. Thus, a single methacrylate column may be used for many different methods, reducing column inventory and cutting cost.

9. Detection limits

Detection limits for anions using the methacrylate-based column in suppressor-based and single-column IC modes are shown in Table 4. The detection limits are calculated on a 3×signal-to-noise ratio, based on 200-μl injection volume in the suppressor-based mode. The detection limits for seven anions using *p*HBA eluent are calculated based on a 100-μl injection volume. The detection limits are reported in parts per billion (ppb).

10. Applications

Separations of several real world samples on the methacrylate-based anion column were studied. Fig. 7a shows the simultaneous separation of hexafluorosilicate (SiF_6^{2-}), nitrite, sulfate and chromate in chromate plating bath solution. Simple dilution with deionized water (1000×) was the only sample preparation used.

Fig. 7b shows the separation of oxalate and other anions in urine. Urinary oxalate concentration is a diagnostic marker for kidney stones.

Fig. 7c and d show chromatograms of anions in water [per US Environmental Protection Agency (EPA) method 300] and anions in a toothpaste sample. This column is ideal for determining inorganic anions in water per EPA method 300.0 and excellent for fluoride determination, because fluoride elutes far from the water dip. The toothpaste sample was prepared for IC analysis as follows. One gram of toothpaste sample was dispersed in 1 ml deionized water and sonicated for 10 min to release fluoride. This solution was then filtered through a 0.4-μm syringe filter and injected. Determination of anions including azide and perchlorate in air bag effluent is shown in Fig. 7e. This separation is performed by (SC) IC.

The methacrylate-based anion-exchange column is an alternative to many columns available in the market including the agglomerated-pellicular and PS–DVB-based anion exchangers. The column is compatible with various eluents and can be operated in suppressor-based IC or (SC) IC modes.

11. Conclusion

The new methacrylate-based stationary phase is useful for anion separation by suppressor-based IC or (SC) IC methods. Several eluents such as bicarbonate–carbonate, *p*-hydroxybenzoic acid, phthalic acid and succinic acid can be used for these applications. Since the ion-exchange capacity is low, but selectivity is high, fast efficient separations are achieved. The hydrophilic packing provides symmetrical peaks for all anions including hydrophobic anions such as nitrate. High resolution between fluoride and the

water dip make this an excellent choice for fluoride analysis. Switching from one eluent to another will not affect column performance, so a single column can be used for different IC methods.

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