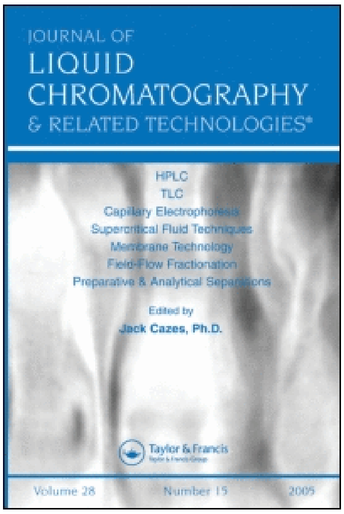

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HYDROPHILIC ANIONS ANALYSIS USING REVERSED PHASE CHROMATOGRAPHY COUPLED WITH EVAPORATIVE LIGHT-SCATTERING DETECTION

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ABSTRACT

A Liquid Chromatography (LC) method using volatile ion pairing agents and Evaporative Light Scattering Detector (ELSD) was developed. The separation of a series of three common inorganic anions (Cl^-, NO_3^- and SO_4^{2-}) and three organo-phosphonic anions (Methyl, ethyl and propylphosphonic acids) was achieved on a reversed-phase C_{18} column with pentylammonium or heptylammonium formiate as ion pairing agent.

This method provides good linearity of the calibration curve and enables a limit of detection to be reached of 4 mg/L and 3.12 mg/L for alkylphosphonic anions and inorganic anions respectively.

The separation for methyl and ethylphosphonic acids, which are poorly resolved in the LC systems commonly used, was successfully achieved.

INTRODUCTION

Analytical methods for the determination of inorganic ions based on ion-interactions in reversed-phased LC published before 1997 in the literature have been exhaustively reviewed by Gennaro and Angelino.[1] Ion-pair reversed-phase chromatography is defined as a technique which uses a reversed-phase column with an aqueous - organic mixture for the mobile phase, to which an ion-pairing agent is added. An ion pairing agent is added to the mobile phase to form, with the ionic analyte, an ion-pair, which according to its hydrophobicity, will be retained on the reversed stationary phase. An indirect / direct UV and conductivity detector constitutes 95 % of detection mode for the analysis of organic and inorganic anions[1] by ion pair chromatography.

The use of alkylamines was described by Marengo et al.[2] for the separation of nitrite and nitrate. Hexylammonium or octylammoniun salts (0.8 mM and 5 mM respectively) formed with orthophosphoric acid allow the resolution of nitrite and nitrate with a long retention time (20 and 45 minutes).

The retention of anions increases with an increase in the alkyl chain length of the ion-pairing reagent.[3] Therefore, pentylammonium and heptylammonium ion pair reagents should give a shorter retention time.

Formic acid was used to form an ion-pair agent with alkylamines (pentyl and heptylammonium formiate) instead of orthophosphoric acid. This choice is required by the evaporative light scattering detection where the mobile phase must be volatile. This detection was chosen instead of the usual detection for ions such as indirect/direct UV absorbance or conductivity.

ELSD is a universal detection method, since the analyte has a lower volatility than the mobile phase and it requires the complete volatilization of the mobile phase. The principle of the detection is based on (i) nebulization of the chromatographic effluent; (ii) evaporation of the chromatographic eluent; (iii) the light is scattered by the residual particles ideally constituting the analytes.[4,5]

Usually used for the detection of non UV absorbent solutes such as carbohydrates[6,7] and cyclodextrins,[8,9,10] only R. Neimi et al.[11] have described an LC-ELSD method for organic anions (clodronate and partial ester derivatives). The original used butylamine acetate as ion-pairing agent and allowed retention of these compounds.

EXPERIMENTAL

Chemicals

HPLC-grade acetonitrile and formic acid were obtained from J. T. Baker (Noisy Le Sec, France), pentylamine and heptylamine were purchased from Fluka (St. Quentin Fallavier, France). Chloride, nitrate, and sulfate were prepared with sodium salt from Sigma standards (St. Quentin Fallavier, France) and methylphosphonic acid (MPA), ethylphosphonic acid (EPA) and propylphosphonic acid (PPA) were purchased from Aldrich (St. Quentin Fallavier, France). All standard mixtures of analytes were prepared in purified water (HPLC-quality) obtained with Elgastat UHQ II System (Villeurbanne, France).

Instrumentation

Isocratic elutions were performed with a Thermo Separation Products (Les Ullis, France) HPLC system consisting of a Spectra SERIES P100 equipped with a manual Rheodyne valve (7010). The injection loop has an internal volume of 20 µL.

A Sedex 55 Evaporative Light-Scattering Detector (Sedere, Alforville, France) was used. The detector temperature was set at 40°C, the gain 8 and the pressure of nebulizing gas (dried and filtered nitrogen) at 2.2 bar.

The analytical column was a C_{18} Hypersil Hypurity™ Elite (Hypersil, Eragny-sur-Oise, France), (250 x 2.1 mm I.D., 5 µm).

The pH of each solution was checked on a Beckman pH meter, model ϕ10 (Gagny, France).

The conductivity was checked on a Vydac conductivity (Wescan Instruments, Santa Clara, USA) model 6000 CD. Data were processed using Shimadzu (Kyoto, Japan) model CR 5 A integrator.

Chromatography

The mobile phase for isocratic elution was a mixture of alkylamine (pentyl or heptylamine) and formic acid. The equilibration time is checked by conductivity detection and measurement of the breakthrough allows the amount (µmol.L^{-1}) of ion pairing agent adsorbed on the C_{18} reversed stationary phase to be calculated.

The mobile phase was delivered at a flow rate of 0.4 mL/min (140 bar) by the pump system during equilibration and separation. Standard solutions were injected at a concentration of 100 mg/L during the measurement of the k': capacity factors.

RESULTS AND DISCUSSION

Adsorption Isotherms

The nature (type and chain length of the hydrophobic group) of the ion-pairing is considered to be an important parameter in the retention of anionic solutes in reversed-phase ion-pair chromatography. The adsorption isotherms of pentylammonium formiate and heptylammonium formiate were determined by measuring the breakthrough with conductivity detection. Initially, the reversed-phase system (C_{18} Hypurity^{TM} Elite) is firstly washed with 15 mL of water - tetrahydrofuran (50-50) mixture, then, with 15 mL of water - acetonitrile (50-50) mixture and finally with 15 mL of water.

This step of clean-up and regeneration of the stationary phase has been accomplished at concentration modification or change of the ion-pairing agent.

Figure 1 shows the adsorption isotherms with both surfactants. At a given concentration of ion-pairing agent, the amount of adsorbed compound is greater for heptylammonium formiate than for pentylammonium formiate, indicating as expected,[1] a stronger hydrophobic adsorption of the C_7 alkyl chain rather than the C_5 alkyl chain on a C_{18} reversed stationary phase.

Moreover, the concentration of ion-pairing agent adsorbed on the stationary phase has been plotted versus the percentage of organic solvent (acetonitrile) added to the mobile phase. The logarithm of adsorbed ion-pairing concentration (Figure 2) decreases linearly with an increasing in acetonitrile amount.

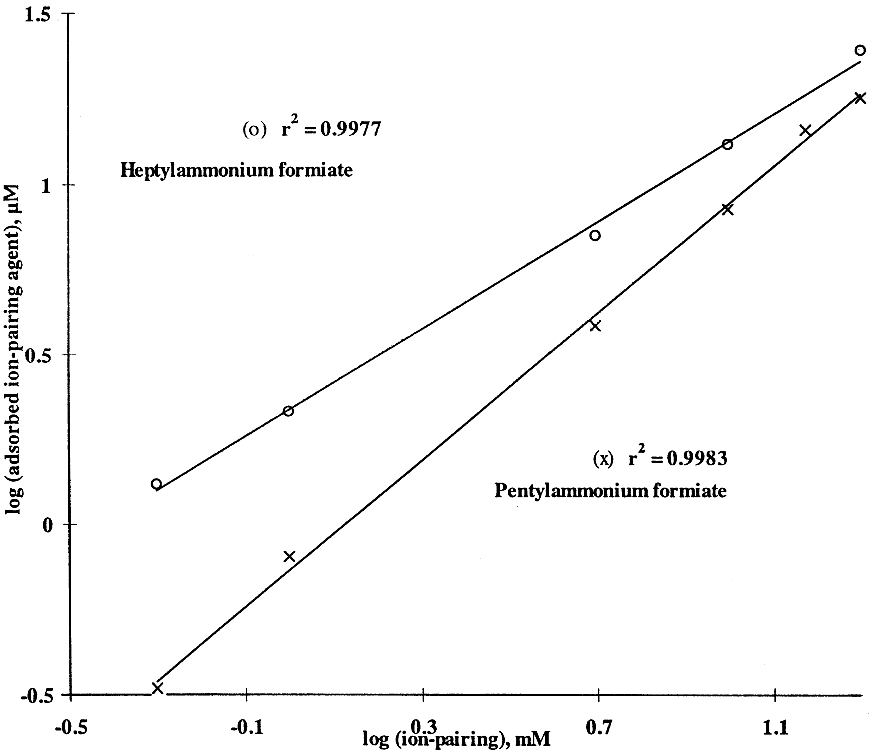


Figure 1. Log of adsorbed ion-pairing amount versus log ion-pairing concentration.

Then, equilibration times are fast. Indeed, equilibration time increases both with a decrease of alkylamine concentration and also with an increase of the alkyl chain length. For example, with pentylammonium formiate, only five minutes are needed to reach the equilibrium (Figure 3) with 10 mM of alkylammonium salt, whereas, ten minutes are necessary for heptylammonium salt (10 minutes for 0.5 mM, 1 mM and 5 minutes for 10 and 20 mM), which corresponds to about five and ten times the dead volume.

Variation of the Capacity Factor k' and the Selectivity α as a Function of Eluent Composition

The capacity factors of six anions have been determined at different ion-pairing agent concentrations (pentylammonium or heptylammonium) as reported in Table 1.

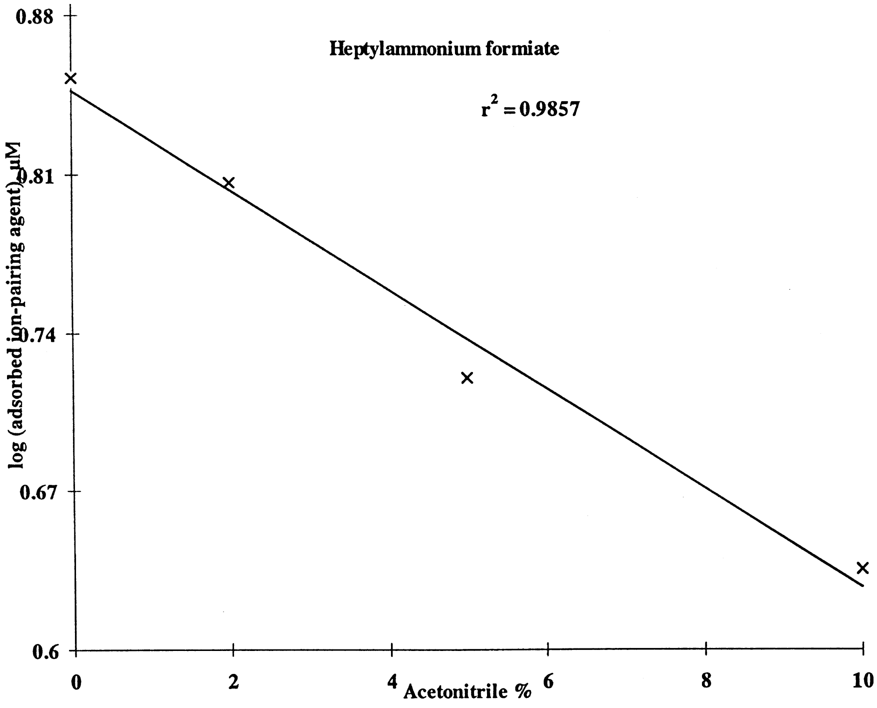


Figure 2. Log of adsorbed ion-pairing amount versus acetonitrile percentage.

With pentylammonium formiate, the retention highly increases versus the concentration of ion-pairing at low concentration (< at 1 mM) and reaches a maximum at 10 mM then slightly decreases above this concentration. Concerning heptylammonium formiate, k' increases at low concentration (< at 1 mM) but decreases slightly above 1 mM.

Figure 4a shows the variation of selectivity versus pentylammonium formiate concentration. Selectivity slightly increases for Cl^-/NO_3^- and greatly for NO_3^-/SO_4^{2-} with an increase in ion pair agent concentration. The selectivity between Cl^- and NO_3^- with heptylammonium as ion-pairing agent (Figure 4b) was greater than with the pentylammonium ion-pairing agent but in these conditions sulfate was not easily eluted (k' > 40, Table 1). Adding acetonitrile to the mobile phase induces a decreasing of the sulfate retention (k' = 10.6) but also a comigration of Cl^- and NO_3^- anions.

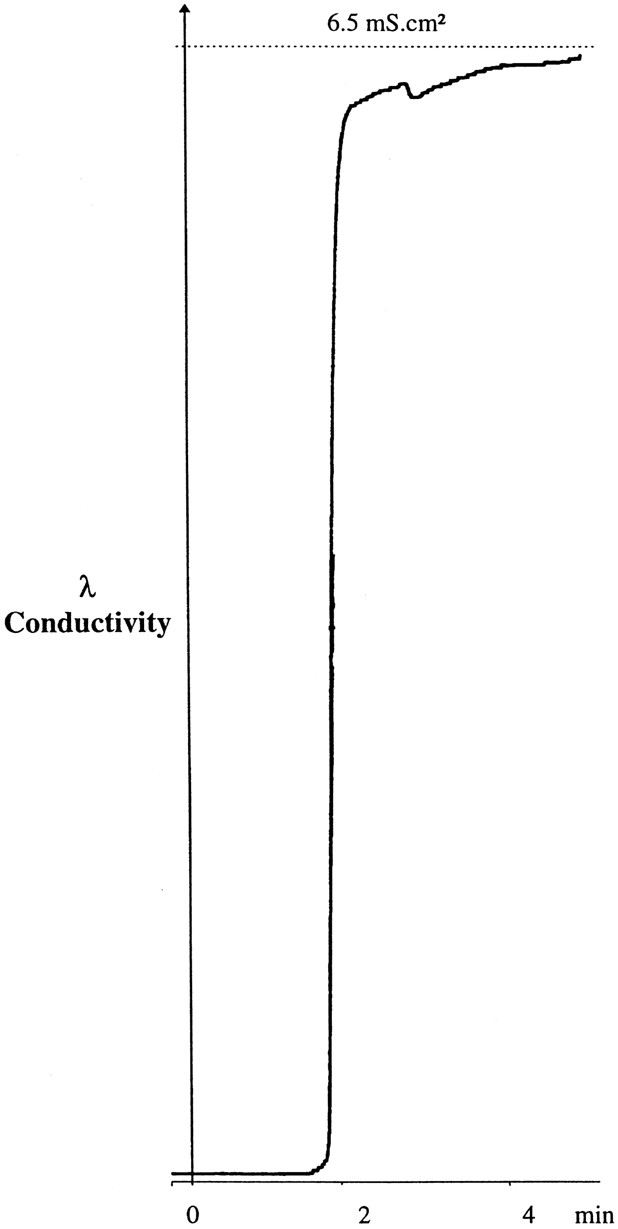
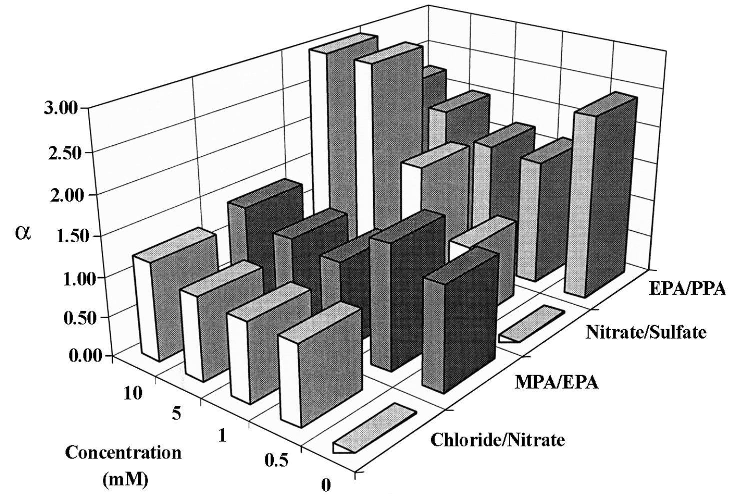


Figure 3. Adsorption curve of 10 mM pentylammonium formiate.

a **Pentylammonium formiate**



b **Heptylammonium formiate**

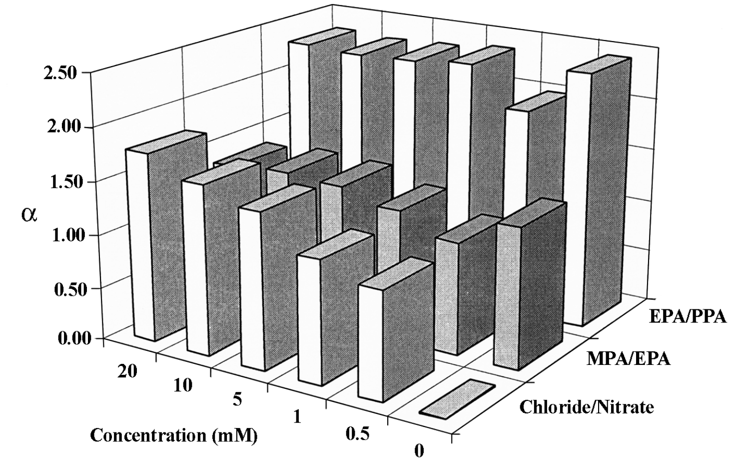


Figure 4. Variation of the selectivity according to ion-pairing concentration.

Table 1

Calculated k' According to the Eluent Studied

Salt	Conc. m(M)	pH	Adsorbed Ion-Pairing Agent (μm)	Cl^-	NO_3^-	SO_4^{2-}	MPA	EPA	PPA
Pentyl-ammonium formate	20	5.6	18.12	1.0	1.3	3.1	1.0	1.5	3.7
	15	5.13	14.50	1.0	1.4	4.3	1.1	1.5	3.6
	10	3.89	6.5	1.1	1.4	4.3	1.1	1.6	3.5
	5	4.26	3.84	1.1	1.2	3.7	1.1	1.4	2.8
	1	5.33	0.80	0.7	0.7	1.8	1.1	1.2	2.0
	0.5	5.55	0.33	0.7	0.7	0.7	0.7	1.1	1.7
Heptyl-ammonium formate	20	3.92	24.80	3.0	5.4	>40	2.6	3.6	8.2
	10	3.91	13.10	3.3	5.3	>40	3.2	4.5	10.4
	5	3.91	7.12	3.6	5.3	>40	3.5	4.8	11.0
	1	6.8	2.16	4.1	4.7	>40	4.1	5.1	12.0
	0.5	6.2	1.31	4.6	4.6	>40	4.5	4.8	9.4

10 mM of pentylammonium formiate was therefore selected as the optimum concentration for the separation of chloride, nitrate, and sulfate (Figure 5a). The three anions were correctly resolved with good selectivity and a short run time.

Hydrophilic organophosphonic anions have been resolved by adding water/acetonitrile mixture (98/2) to 5 mM of heptylammonium formiate (Figure 5b). With heptylammonium formiate, the selectivity was slightly greater than with pentylammonium formiate, particularly with EPA/PPA. So, adding 2 % of acetonitrile to the mobile phase decreases the retention without decreasing resolution and selectivity. Such a resolution (Rs = 2) has never been obtained for the separation of methyl and ethylphosphonic acids.[12,13]

At last, we have compared the influence of formic and acetic acid (Table 2). Whatever the alkylammonium salt concentration, 5 mM for heptylammonium and 10 mM for pentylammonium, the k' slightly decreases with acetate salt for inorganic anions and increases for hydrophilic organic anions. This phenomenon shows the complex retention mechanism with this stationary phase, where hydrophobic and electronic interactions are in competition.

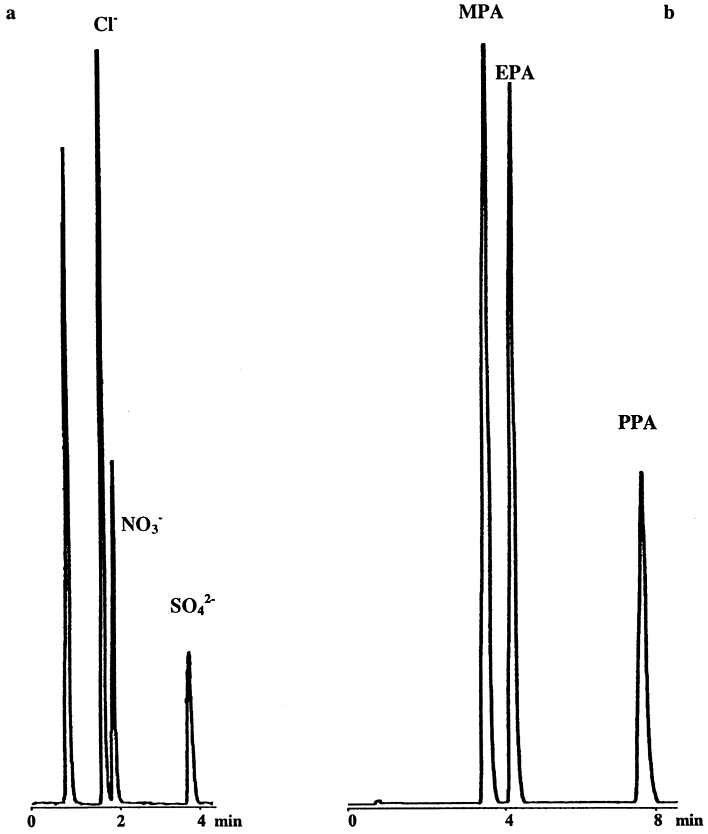


Figure 5. Separation of inorganic anions (a) and hydrophilic inorganic anions (b). (Conditions as Cf. 2. 2.).

When the concentration of alkylammonium salt is less than 1mM, the ion-pair reversed phase mechanism seems to be predominant, whereas above 1 mM, the interpretation of interactions becomes very complex. Nevertheless, the use of formic acid instead of acetic acid undergoes a decreasing of the solubility of analytes in the mobile phase, all the more so since analytes are less soluble.

Then, interactions of hydrophilic organic anions increases due to the stronger interactions with the hydrophobic stationary phase. Besides, the

Table 2

Comparison of Capacity Factor' and Selectivity Between Acetic and Formic Acid

Salt	Conc. (mM)		Cl^-	NO_3^-	SO_4^{2-}	MPA	EPA	PPA
Pentyl-ammonium	10	k'	1.1	1.4	4.3	1.1	1.6	3.5
formiate	10	α	1.5		3.09	1.42		2.33
Pentyl-ammonium	10	k'	0.8	1.0	2.2	1.7	1.7	8.0
acetate	10	α	1.27		2.35	1.0		1.93
Heptyl-ammonium	5	k'	3.6	5.3	>40	3.5	4.5	11.0
formiate	5	α	1.47		---	1.29		2.42
Heptyl-ammonium	5	k'	2.8	2.8	3.7	6.9	6.9	12.2
acetate	5	α	1.0		1.35	1.0		1.76

selectivity slightly decreases with the replacement of formic acid by acetic acid, whatever the alkylammonium salt.

Limit of Detection (LOD) Calibration Curve

The LOD was 3.13 mg/L for chloride, nitrate, and sulfate anions by using 10 mM of pentylammonium formiate. For the hydrophilic organic anions (MPA, EPA, and PPA), the LOD was 4 mg/L with 5 mM of heptylammonium formiate in 98%-2% water-acetonitrile mixture. The LOD was calculated for a 3/1 signal-to-noise ratio. This LOD seems very acceptable compared to the values generally obtained with this kind of detector (30-50 mg/L);[11] the injected amounts were 63 µg for inorganic anions (0.65µmol) and 80 µg of organic anions (0.65 µmol). Nevertheless, UV and conductivity detection modes give higher sensitivities.

Calibration curves of inorganic anions (peak area logarithm versus analyte concentration logarithm) were plotted in the 4-125 mg/L concentration range; the equation of the type: $\log A = a.\mathrm{Log}\ C + b$ with rather good correlation coefficients (r^2) where A and C are, respectively, peak area and analyte concentration. Regardless of the nature of the inorganic anions and hydrophilic organic anions, correlation coefficients were generally 0.99 or higher.

CONCLUSION

This study demonstrates, (i) the use of ion pairing chromatography with ELSD detector and volatile mixtures of alkylamine and formic acid as mobile phase; (ii) fast equilibration and desorption steps so that this system can, therefore, be used without any problems in elution gradient which generally induces a severe perturbation of the baseline due to the long equilibration time; and (iii) efficient separation of commonly analyzed anions and hydrophilic anions on C_{18} reversed phase, the use of which avoids using ion-exchange.

Work is in progress to demonstrate the system's ability for elution gradient in order to carry out the simultaneous separation of hydrophilic and hydrophobic anions.

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