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Comparison of different electroosmotic flow modifiers in the analysis of inorganic anions by capillary electrophoresis

M.T. Galceran*, L. Puignou, M. Diez

Departament de Química Analítica, Facultat de Química, Universitat de Barcelona, Avenida Diagonal, 647, 08028 Barcelona, Spain

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Abstract

The determination of inorganic anions using capillary electrophoresis with non-treated silica capillaries requires reversal or elimination of the cathodic electroosmotic flow (EOF). The behaviour of different monocharged [tetrabutylammonium (TBA), cetyltrimethylammonium (CTA) and tetradecyltrimethylammonium (TTA)] or multicharged [hexamethonium (HM) and hexadimethrine (HDM)] alkylammonium quaternary ions as electroosmotic flow modifiers has been compared. Indirect spectrophotometric detection using chromate as carrier ion has been used. The effect of EOF modifiers and chromate concentration, pH and applied potential on the separation of bromide, chloride, nitrite, nitrate, sulfate and phosphate has been studied. Optimal conditions for the separation of these anions have been established and resolution has been compared. Reproducibility in areas (R.S.D. 1.8–8.5%) and in migration times (R.S.D. 2.3–7.5%), and detection limits (200–1000 $\mu\text{g l}^{-1}$) were similar for all the EOF modifiers. For HDM very low concentrations, down to 0.0001%, can be used successfully.

Keywords: Electroosmotic flow; Buffer composition; Inorganic anions

1. Introduction

Capillary electrophoresis (CE) is an efficient technique for the separation of small inorganic anions and cations. Conductivity [1,2], ion-selective electrodes [3], indirect UV detection [4–6], indirect fluorescence [7,8] and mass-spectrometric [9] detection have been used for the CE determination of low-molecular-mass anions.

The separation mechanism in CE is based on differences between the electrophoretic mobilities of charged species in the presence of an electroosmotic flow (EOF). In a conventional CE system the EOF is towards the cathode; anions with an electrophoretic

mobility higher than the electroosmotic mobility of the bulk electrolyte cannot reach the detector and the polarity of the potential applied must be reversed in order to detect these anions. However, under these conditions anions with mobilities lower than electroosmotic mobility would never reach the detector. Therefore, for most anion separations it is also necessary to eliminate the EOF or reverse its direction.

Different ways of modifying the EOF for the determination of low-molecular-mass anions have been reported. These included pH control [10], addition of organic solvents [11] or other compounds [4] to the electrolyte solution, and also the use of modified capillaries with bonded phases [12]. The most common method is the addition of a cationic

*Corresponding author.

surfactant, which is adsorbed on the capillary wall and produces a net positive charge on the surface. Under these conditions and using a negative power supply, all anions can be detected at the anode.

Some authors have used a long-chain cationic surfactants, such as cetyltrimethylammonium bromide [12–15], dodecyltrimethylammonium bromide [12,16] or tetradecyltrimethylammonium bromide [12,17,18]. Several soluble polydisperse ionic polymers (polyelectrolytes) have also been used to reverse the direction of the EOF and to induce changes in analyte electrophoretic mobility, separation selectivity and resolution [19–21].

This paper reports the study of the parameters that influence the separation efficiency for common inorganic anions using capillary electrophoresis. Indirect detection was applied using sodium chromate as a carrier electrolyte, and the effect of this concentration was studied. Special attention has been focused on the electroosmotic flow modifier by studying the capacity of the single- and double-charged alkylammonium ions of different chain lengths, and a polymeric ion. The effects of modifier concentration, pH and applied voltage on the separation efficiency have also been studied.

2. Experimental

2.1. Instrumentation

The capillary electrophoresis (CE) system was an Applied Biosystems (Foster City, CA, USA) Model 270 A with spectrophotometric detection. Electrophoretic data were processed with a Merck-Hitachi Model 2500 integrator. A fused-silica capillary (Supelco, Bellefonte, PA, USA), 50 μm I.D. and 72 cm length with a separation length of 50 cm, was used. The temperature was held at 30°C. Hydro-

dynamic (by vacuum, 16.9 kPa) injection mode was applied for 3 s. Indirect UV detection was performed at 254 nm.

2.2. Chemicals

Reagents used for the preparation of buffer solutions were analytical grade; sodium chromate tetrahydrate 99%, was obtained from Aldrich (Milwaukee, WI, USA). The EOF modifiers studied and their structure are given in Table 1. Tetrabutylammonium bromide, tetradecyltrimethylammonium bromide, hexamethonium bromide and hexadimethrine bromide were obtained from Fluka (Ronkonkoma, NY, USA). Cetyl-N,N,N-trimethylammonium bromide, sodium hydroxide and acetic acid were obtained from Merck (Darmstadt, Germany). Anion standards were prepared from analytical grade sodium salts. A Dowex 2X8 anion-exchange resin in the chloride form was obtained from Fluka. Water was purified using a Culligan (Barcelona, Spain) system. All solutions were passed through a 0.45- μm nylon filter before use.

2.3. Procedure

The electrolyte solution was prepared using 3–10 mM chromate as carrier ion and different EOF modifiers. A 10 mM solution of each alkylammonium bromide was prepared and converted to the hydroxide form in order to use it as electroosmotic flow modifier. Conversion was accomplished using a Dowex 2X8 ion-exchange resin. Different amounts of these stock solutions were used to prepare the running electrolyte containing concentrations lower than 1 mM of each EOF modifier. Acetic acid or NaOH (100 mM each) were used for adjusting the pH of electrolyte solution to 7.5–9.0.

All standard anion solutions were prepared by

Table 1
Abbreviation, name and structure of the different ammonium quaternary ions

Abbreviation	Name	Structure
TBA	Tetrabutylammonium	$(\text{C}_4\text{H}_9)_4\text{N}^+$
TTA	Tetradecyltrimethylammonium	$[\text{CH}_3(\text{CH}_2)_{13}\text{N}(\text{CH}_3)_3]^+$
CTA	Cetyltrimethylammonium	$[\text{CH}_3(\text{CH}_2)_{15}\text{N}(\text{CH}_3)_3]^+$
HM	Hexamethonium	$[(\text{CH}_3)_3\text{N}-(\text{CH}_2)_6\text{N}(\text{CH}_3)_3]^{2+}$
HDM	Hexadimethrine	$[-\text{N}(\text{CH}_3)_2-(\text{CH}_2)_6-\text{N}(\text{CH}_3)_2-(\text{CH}_2)_3-]_n^{2+}$

dilution of a 1000 mg l^{-1} stock solution of each anion. Mixed anion standards were prepared daily at a concentration of 10 mg l^{-1} .

Before use, the capillary was pretreated with 0.1 M NaOH for 60 min, then rinsed with ultrapure water. The capillary was conditioned with the running electrolytes for 60 min before the first run and for 2 min in-between runs. For chromate–hexadimethrine a conditioning time of 120 min was necessary to equilibrate the capillary and to obtain reproducible results. When the electrolyte system was changed, capillaries were flushed with ultrapure water (5 min) followed by 0.1 M NaOH (20 min) for all the electrolytes, except chromate–hexadimethrine, for which 1 M NaOH was necessary to eliminate completely the adsorbed EOF modifier.

3. Results and discussion

3.1. Effect of the alkylammonium ion on the electroosmotic flow

The effect of different alkylammonium ions on the electroosmotic flow was studied at constant elec-

trolyte solution, pH and applied potential (5 mM sodium chromate, pH 8, 20 kV). The electroosmotic mobility was determined by measuring the migration time of the water peak as a neutral marker. Tetradecyltrimethylammonium (TTA), hexadimethrine (HDM) and cetyltrimethylammonium (CTA) reversed the EOF, but only a reduction was observed for hexamethonium, and tetrabutylammonium increased its value. The variation of the EOF with the concentration of the modifier is given in Fig. 1.

Among the single alkylammonium ions which reversed the EOF, CTA is a more effective modifier than TTA and allowed inversion at concentrations 10 times lower. This is important because hydrophobic alkylammonium salts have limited solubility and may form insoluble pairs with some electrolyte components [3]. Therefore, lowering concentration of modifier in the electrolyte solution improves its stability. The anomalous behaviour of TBA, increasing the EOF, may be related to its bulky nature which prevented effective adsorption onto the capillary wall. Moreover, an increase in the EOF was observed when the concentration of TBA increased, which may be related to the higher concentration of non-attached cations in the electrolyte solution.

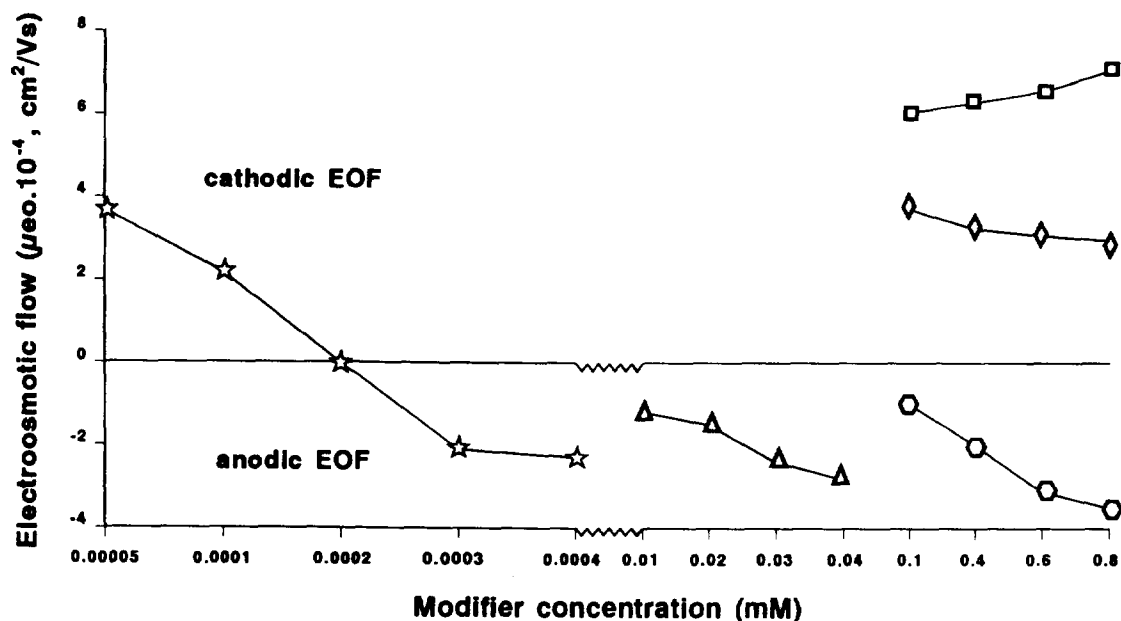


Fig. 1. Variation of electroosmotic flow with electroosmotic flow modifier concentration. (☆) HDM; (△) CTA; (□) TBA; (◇) HM; (○) TTA.

Hexamethonium (HM) is an alkylammonium ion with double charge which is smaller than CTA and TTA. In contrast to the effect of CTA and TTA, the direction of the EOF was not changed by HM in the interval of concentrations studied, although it was significantly reduced. This may be related to the lower adsorption of HM onto the capillary wall due to its lower molecular size. Reduction of EOF with this modifier has also been observed using HM and phosphate as carrier electrolyte [22]. Harrold et al. [23], using pyromellitic acid as carrier ion, reported that HM was very effective in reversing the EOF. This discrepancy between our results and those of Harrold could be explained if the adsorption of the HM ion to the capillary wall was more effective in the presence of the pyromellitic rather than the chromate ion. The fully dissociated tetracarboxylic acid used as carrier ion by Harrold may form a stable ion-pair association with HM, which could be adsorbed onto the silanol groups of the capillary wall, thus reversing the sign of the zeta potential.

HDM is an alkylammonium polymer composed by hexadimethonium units (25–50), and was more effective in reversing the EOF at lower concentrations than the other alkylammonium ions studied. This behaviour could be related to the polymeric nature of this modifier, which allowed a more effective adsorption of the polymer cation on the capillary at low concentrations providing a net positive charge to the wall capillary, which suggested that there was a change in both the magnitude and the sign of the zeta potential.

3.2. Influence of the modifier concentration

The effect of alkylammonium ion concentration on the separation of bromide, chloride, sulfate, nitrite, nitrate and phosphate was evaluated. The alkylammonium ions which reversed or significantly reduced the electroosmotic flow were studied using 5 mM sodium chromate as electrolyte solution, pH 8 and 20 kV. The concentration range studied for each modifier was selected in order to obtain the best efficiency of the separation, i.e., short analysis time and good resolution. Values of migration times for the fastest and slowest ions (Br^- and HPO_4^{2-}) and resolution between sulfate and nitrite are given in Table 2 and Table 3.

Generally, when the modifier concentration was increased the apparent mobility of all anions also increased, which was related to the EOF variation. Moreover, when the modifier concentration was changed over the range studied, the separation between sulfate and nitrite was affected. For all the modifiers a decrease in the resolution between sulfate and nitrite was observed at high concentrations. This finding may be due to a higher stability constant for the ion-association equilibria of sulfate and the alkylammonium ion, which resulted in a lower apparent mobility of sulfate.

Using CTA as modifier at concentrations between 0.01 and 0.8 mM the migration order of chloride and bromide was also affected. The apparent mobility of each anion normalized to that of nitrate is represented vs. CTA concentration (Fig. 2). When the

Table 2
Migration times (min) of bromide and phosphate with several electroosmotic flow modifiers at different concentration levels

Modifier	Electroosmotic flow modifier concentration (mM)							
	0.2		0.4		0.6		0.8	
	Br^-	HPO_4^{2-}	Br^-	HPO_4^{2-}	Br^-	HPO_4^{2-}	Br^-	HPO_4^{2-}
TTA	3.70	4.89	3.55	4.63	2.90	3.64	2.77	3.45
HM	6.30	13.16	6.18	12.27	6.00	12.16	5.89	11.63
CTA	0.01		0.02		0.03		0.05	
	3.30	4.28	3.00	3.84	2.92	3.70	2.81	3.50
HDM	$0.5 \cdot 10^{-4}$		$1 \cdot 10^{-4}$		$2 \cdot 10^{-4}$		$4 \cdot 10^{-4}$	
	8.98	16.21	4.60	7.69	3.08	3.96	3.02	3.86

Experimental conditions: 5 mM Na_2CrO_4 , pH 8.0, 20 kV

Table 3

Resolution between sulfate and nitrite with several electroosmotic flow modifiers at different EOF modifier concentration, chromate concentration and pH

Modifier	Modifier concentration (mM)				Chromate concentration (mM)				pH			
	0.2	0.4	0.6	0.8	3	5	7	10	7.5	8.0	8.5	9.0
HM	1.2	1.1	0.9	0.6	1.4	1.1	– ^a	1.2	1.1	1.1	1.1	1.1
TTA	1.0	0.7	0.5	0.3	0.9	1.0	– ^a	– ^a	0.9	1.0	1.0	1.0
CTA	0.01	0.02	0.03	0.05	1.3	1.1	– ^a	– ^a	1.0	1.1	1.1	1.1
	1.2	1.1	0.9	0.9								
HDM	0.5·10 ^{−4}	1·10 ^{−4}	2·10 ^{−4}	4·10 ^{−4}	1.3	1.1	–	0.2	0.9	1.0	0.9	0.9
	1.9	1.2	1.1	0.8								

^a Coelution.

concentration of CTA was above 0.6 mM, chloride migrated faster than bromide; in contrast, below 0.1 mM a reversal in the migration order was observed, and between 0.1–0.6 mM both anions showed the same apparent mobility. This behaviour may also be related to the ion association equilibria of the chloride and bromide ions with the modifier. A higher stability constant for bromide could provide lower apparent mobility. This has also been observed with other modifiers, which always produce a low increase in the migration time of the bromide with concentration, which suggests that at higher concentrations a reversion of the migration order of bromide and chloride could be obtained. This was observed using 0.02 mM HDM [19].

All the modifiers can be used for the separation of the studied anions giving relatively short analysis

times except for HM, for which EOF was not reversed (Table 2 and Table 3). Taking into account resolution between the anions and analysis time the optimum value for each modifier was 0.4 mM for HM, 0.2 mM for TTA, 0.02 mM for CTA and 2·10^{−4} mM (0.0001%) for HDM.

3.3. Carrier electrolyte

The use of UV detection in capillary electrophoresis for the analysis of the non-absorbing inorganic anions requires a running electrolyte which allows indirect detection. In general, absorbing species in the electrolyte also act as carriers, then, selection of a suitable absorbing ion must include not only the consideration of high sensitivity but also of separation efficiency. The chromate ion was chosen, following Cousins et al. [6].

When cationic surfactants are used the effective electrophoretic mobilities of inorganic anions depend on the concentration of the carrier. This behaviour may be related to the competition between chromate and the sample anions for the positive charge of the cationic surfactant.

Separation between bromide, chloride, sulfate, nitrite, nitrate and phosphate was studied at different concentrations of the carrier ion (3–10 mM). Using HM, TTA, CTA and HDM as modifiers the concentration of the modifiers was kept constant at the optimum value established previously.

The apparent mobilities are practically constant for all the anions in the concentration range studied,

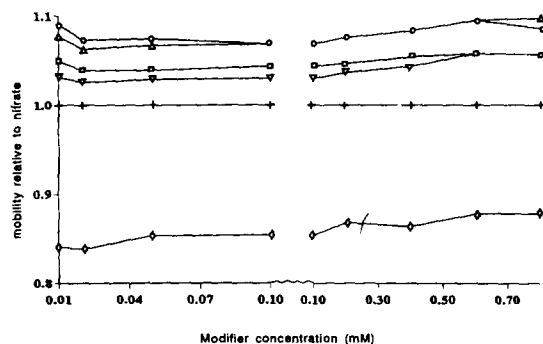


Fig. 2. Influence of CTA concentration on the mobility of the anions relative to nitrate. (○) Br[−]; (△) Cl[−]; (□) SO₄^{2−}; (▽) NO₂[−]+NO₃[−]; (◇) HPO₄^{2−}.

except for sulfate, which showed a decrease between 3 and 7 mM of chromate. This phenomenon was observed with all the modifiers, probably due to the stronger affinity of the sulfate for the modifier compared to the other anions. In all cases coelution between nitrite and sulfate at 7 mM chromate was observed, at higher concentrations the nitrite eluted first.

Resolution between sulfate and nitrite is a critical parameter in the separation of the anions for all the modifiers studied. The best results for these anions were obtained at 3 mM (Table 3). For HM good resolution can also be obtained at 10 mM. Resolution between bromide and chloride also depended on chromate concentration. Good separation can be obtained with HM and HDM at all the concentrations between 3 and 10 mM. In contrast when CTA and TTA were used, not enough resolution was obtained at low concentrations (3 mM). Phosphate gave tailing peaks at low concentrations of chromate for all the modifiers; thus a solution 5 mM was chosen as the optimum value to obtain the best separation efficiency for all the anions.

3.4. Effect of pH and potential applied

The pH is an important parameter in CE separation when fused-silica capillaries are used because it governs the electroosmotic flow by changing the charge of the capillary surface. The pH also governs the ionization of the compounds, but for some anions this effect is not important because they are totally ionized.

The separation between the anions was studied using 5 mM chromate at the optimum electroosmotic modifier concentration established before for each modifier and the pH was changed from 7.5 to 9.0. The influence of the pH of the electrolyte is very similar for the different modifiers studied. The migration times of the anions were not affected by electrolyte changes. Only phosphate showed peak tailing which increased when the pH decreased. This fact could be related to the interaction with the silica surface of the capillary wall [24].

Migration times of anions and resolution were studied, as a function of the applied potential between 15 to 30 kV, using the established conditions for the other parameters. Low applied voltages resulted in lower mobilities giving a long analysis

time, with the best results being obtained at voltages higher than 25 kV.

3.5. Optimal conditions and quality parameters

Fig. 3 shows the electropherograms obtained under the optimal conditions for the different electrolyte compositions. As can be seen these optimal conditions were similar for TTA, HM and CTA, except for the concentration of the alkylammonium ion. Resolution between the anions was similar for all the EOF modifiers. Moreover, when the high-molecular-mass polyelectrolyte HDM was used good separation was achieved at very low concentrations. Although high concentrations of EOF modifier have been used in some of the applications [14] the corresponding salts have limited solubility and may form insoluble pairs with some electrolyte or sample components. Therefore, a lower concentration of modifier may be beneficial for the analysis of samples, as has been demonstrated in our laboratory for the routine analysis of inorganic anions in waste waters.

The detection limits, expressed as $\mu\text{g l}^{-1}$ of each inorganic anion, based on a signal-to-noise ratio of 3, are given in Table 4 and were similar for all electrolytes studied. Using HM the detection limit for phosphate was considerably higher. Six replicate determinations of 5 mg l^{-1} standard solution of each compound were carried out under the optimum conditions to determine run-to-run reproducibilities. Relative standard deviations (R.S.D.) based on the peak area in the range of 1.2 to 3.0% were obtained, except for phosphate with HM. In order to test the day-to-day reproducibility based on both peak area and migration times six replicate analysis of the same standard solution were carried out on six different days. The results for areas range from 1.8 to 8.5%. Slightly better results were obtained for HDM, showing the suitability of this high-molecular-mass polyelectrolyte as electroosmotic flow modifier. Good results for reproducibility of migration times were also obtained, ranging from 2.4 to 7.5%.

4. Conclusions

A different behaviour was observed for the alkylammonium quaternary ions, TTA, CTA, HM

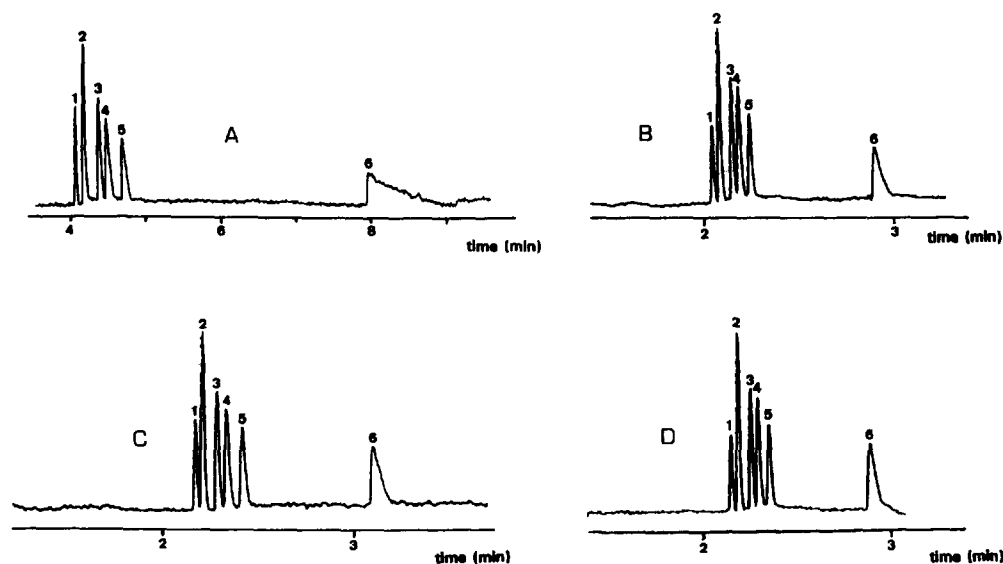


Fig. 3. Electropherograms obtained at the optimal conditions for the different electrolyte compositions. (A) 5 mM sodium chromate, 0.4 mM HM, pH 8, 30 kV. (B) 5 mM sodium chromate, 0.2 mM TTA, pH 8, 30 kV. (C) 5 mM sodium chromate, 0.02 mM CTA, pH 8, 30 kV. (D) 5 mM sodium chromate, $2 \cdot 10^{-4}$ mM HDM, pH 8, 25 kV. (1) Br^- ; (2) Cl^- ; (3) SO_4^{2-} ; (4) NO_2^- ; (5) NO_3^- ; (6) HPO_4^{2-} .

and HDM, which showed a reduction or reversal of the EOF, although TBA produces a slight increase. In contrast with some published data, HM did not reverse the EOF when chromate was used as electrolyte carrier ion, showing the influence of the carrier on the behaviour of the EOF. Moreover, the size of the alkylammonium ion influenced not only the reduction of EOF, but also its optimal concentration range, which decreased when larger ions were used. Using HDM, a high-molecular-mass polyelectrolyte, good efficiencies were observed at concentrations as low as 0.0001%. This finding may

be beneficial for the analysis of complex samples because the formation of insoluble pairs between alkylammonium ions and both electrolyte and sample components can be avoided.

The optimal conditions for TTA, CTA and HDM were similar except for the concentration of the alkylammonium ion. Moreover, figures of merit for the anion determination including detection limits, reproducibility and analysis time were similar, which indicated that the chemical structure of the modifier had little or no influence, provided that the EOF was reversed.

Table 4
Detection limits, reproducibility run-to-run and day-to-day for different electroosmotic flow modifiers

Anion	Electroosmotic modifier															
	HM			TTA			CTA			HDM						
	LOD	Reproducibility			LOD	Reproducibility			LOD	Reproducibility			LOD	Reproducibility		
	r_A	R_A	R_{tm}		r_A	R_A	R_{tm}		r_A	R_A	R_{tm}		r_A	R_A	R_{tm}	
Br^-	500	1.8	2.4	2.3	500	1.7	2.4	2.5	450	1.5	2.6	3.2	500	1.4	2.3	3.2
Cl^-	200	1.5	2.3	2.4	250	1.5	2.9	2.4	200	1.6	2.0	3.4	250	1.3	1.8	3.5
SO_4^{2-}	300	1.4	2.9	2.5	350	1.3	2.7	2.6	300	1.2	2.6	3.5	300	1.1	2.5	3.2
NO_2^-	380	1.8	2.8	2.7	400	1.7	3.1	2.5	400	1.6	2.6	3.5	400	1.1	2.7	3.1
NO_3^-	400	2.0	3.1	2.8	450	2.1	2.9	2.6	400	1.8	2.9	3.3	400	1.4	2.8	2.5
HPO_4^{2-}	1000	5.4	8.5	7.5	550	3.0	4.6	2.5	500	2.9	4.5	4.4	450	2.7	4.1	3.4

LOD, limit of detection ($\mu\text{g l}^{-1}$); r_A , area reproducibility run-to-run (R.S.D. (%), $n=6$); R_A , area reproducibility day-to-day (R.S.D. (%), $n=6$); R_{tm} , time reproducibility day-to-day (R.S.D. (%), $n=6$).

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