

# Indirect fluorimetric detection of alkali and alkaline earth metal ions in capillary zone electrophoresis with cerium(III) as carrier electrolyte

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## ABSTRACT

It is shown that cerium(III) sulphate is a useful electrolyte for the determination of ammonium and alkali and alkaline earth metal ions using capillary zone electrophoresis with indirect fluorimetric detection. The limits of detection are in the low micromolar range. The separation can be influenced by adding 18-crown-6 to the electrolyte solution to change the retention behaviour of the analyte ions. Some examples of separations of standard solutions and practical applications are given to demonstrate the possibility of qualitative and quantitative analyses.

## INTRODUCTION

In capillary zone electrophoresis (CZE), different types of detectors have been used for the determination of inorganic ions. The most common methods are conductivity detection [1–3] and indirect UV photometric detection [4–7]. Further, Gross and Yeung [8] demonstrated the determination of alkali and alkaline earth metal ions in the low femtomole range using laser-induced indirect fluorescence detection.

In this paper we present an inexpensive system for indirect fluorescence measurement using a commercial HPLC fluorescence detector, simply modified for on-column detection. For the determination of inorganic cations in CZE with indirect fluorescence detection a fluorescent cation is used as a component of the electrolyte system. Analyte cations displace the fluorescent co-ions in the electrolyte showing negative peaks. Therefore, a high fluorescence inten-

sity of the electrolyte co-ion is necessary in order to allow the measurement of low concentrations of the analyte [9]. For electrophoretic separations with high efficiencies, an additional condition must be fulfilled by the co-ion, as shown in the investigations of Hjertén [10]. He reported that highly symmetrical peak shapes in electrophoretic separations can be achieved if the conductivity difference  $\Delta\kappa$  at the boundary between analyte zone and carrier electrolyte is low. Therefore, the ion mobilities of the electrolyte co-ions must closely match those of the analyte ions as can be seen in the equation

$$\Delta\kappa = \frac{c_s}{\mu_s} (\mu_e - \mu_s)(\mu_c - \mu_s) \quad (1)$$

where  $\mu_s$ ,  $\mu_e$  and  $\mu_c$  are the ionic mobilities of the sample ion, the electrolyte co-ion and the electrolyte counter ion, respectively, and  $c_s$  is the sample ion concentration.

For the determination of highly mobile alkali and alkaline earth metal ions ( $\mu = 4 \cdot 10^{-4} - 8 \cdot 10^{-4} \text{ cm}^2/\text{V} \cdot \text{s}$ ) [11], most of the highly fluorescent ions are organic ions and are not useful because of their low ionic mobilities. A group of fluorescent inorganic

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substances are the lanthanides [12], of which cerium(III) has both the highest mobility [11] and the highest fluorescence intensity. For this reason, a cerium(III) solution was selected as the electrolyte in this work.

Another problem in the electrophoretic separation of inorganic cations is the nearly identical ionic mobilities of some species. For example, potassium and ammonium, which are important ions for many analytical investigations, show co-migration in electrophoretic separations. The separation of these ions has been successfully achieved at  $\text{pH} > 8$  [7], but this method is not possible with a cerium(III) electrolyte because of the formation of cerium hydroxides under these conditions. We solved this separation problem by adding 18-crown-6 to the electrolyte to reduce the mobility of potassium. The mobilities of barium and strontium can be influenced in the same way. This is useful especially if an analysis requires the simultaneous determination of calcium and strontium, which also have similar ion mobilities and are therefore difficult to separate without 18-crown-6 as an additive.

#### EXPERIMENTAL

The experimental CZE equipment used in this work was laboratory built. The separations were carried out using conventional untreated fused-silica capillaries (75  $\mu\text{m}$  I.D.) from Scientific Glass Engineering (Weiterstadt, Germany). The capillary, the outlets of which are placed in two 10-ml electrolyte vials, pass through a Spectroflow 980 fluorescence detector (Applied Biosystems, Weiterstadt, Germany) modified for on-column detection. Two holes were drilled through a laboratory-made detector cell housing that allowed the capillary to be placed at the surface of the  $2\pi$  sr mirror (Fig. 1). With this equipment it is possible to collect 75% of the fluorescence emission light whereas in the classical  $90^\circ$  right-angle design of the detection cell only a part of emission light is measured with the photomultiplier.

The capillary was fixed with two screws after centring the capillary window exactly in the middle of the excitation beam. The excitation wavelength was 251 nm and the fluorescence was isolated from the scattered light with a 345-nm cut-off filter. The high voltage from a  $\pm 30$ -kV high-voltage power

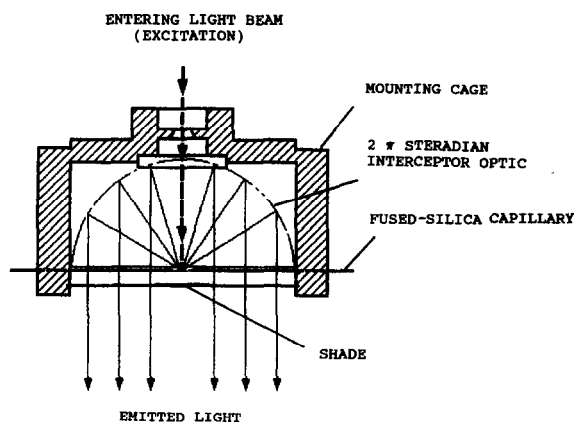


Fig. 1. Modified detector cell for on-column detection.

supply (F.u.G. Elektronik, Rosenheim, Germany), used in the positive voltage mode, was applied between the two ends of the capillary through platinum–iridium electrodes dipped in the electrolyte vials.

All solutions were prepared each day from 10 mM stock solutions, filtered through a 0.22- $\mu\text{m}$  membrane, and were degassed under vacuum for 10 min.

Before each electrophoresis, the capillary was rinsed for 3 min with electrolyte solution by vacuum applied at the capillary outlet side. After the electrolyte container at the capillary inlet had been replaced with a vial containing the analyte solution, sample introduction was accomplished using the electrokinetic or hydrostatic method. The reproducibility of the peak-area response was 2% with automated hydrostatic injection and 5% with electrokinetic sample introduction.

#### Chemicals

All solutions, electrolytes and standards were prepared using water purified with a Milli-Q system (Millipore, Eschborn, Germany). Cerium(III) sulphate (99.99%) was obtained from Alpha Products (Karlsruhe, Germany). All other reagents were of analytical-reagent grade from Merck (Darmstadt, Germany).

#### RESULTS AND DISCUSSION

##### Choice of the counter ion for cerium(III) electrolyte

From eqn. 1 it is clear that the mobility of the

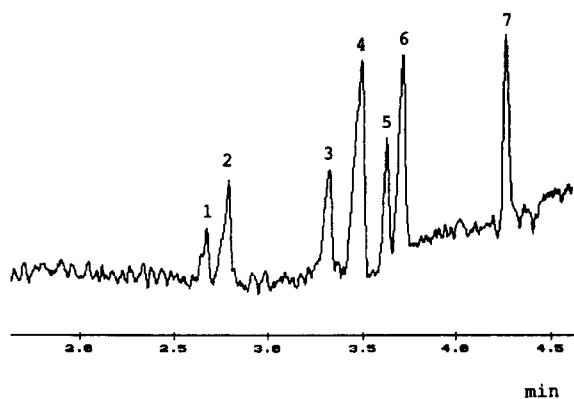


Fig. 2. Electropherogram of alkali and alkaline earth metal ions in the presence of ammonium. Carrier electrolyte, 500  $\mu\text{M}$  cerium(III) sulphate; fused-silica capillary, 55 cm  $\times$  75  $\mu\text{m}$  I.D.; high voltage, 30 kV; sample introduction was carried out by hydrostatic injection (10 cm for 30 s). Peaks: 1 = caesium; 2 = ammonium and potassium; 3 = barium; 4 = calcium and strontium; 5 = sodium; 6 = magnesium; 7 = lithium (each 50  $\mu\text{M}$ ).

counter ion,  $\mu_c$ , also has an influence on the value of  $\Delta\kappa$  and therefore on the peak shape [10]. Commercially available cerium(III) salts include the chloride, nitrate, sulphate and fluoride. Because of the small differences in the mobilities of these anions, their influence on peak shape can be ignored, whereas the solubilities of the salts play a more important role.

TABLE I

COMPARISON OF LITERATURE MOBILITIES [11] AND EFFECTIVE MOBILITIES OF CATIONS SEPARATED IN FIG. 2

The conditions for measurement are as given in Fig. 2.

Cation	$\mu_{lit}$ ( $10^{-4}$ cm <sup>2</sup> /V · s)	$\mu_{eff}$ ( $10^{-4}$ cm <sup>2</sup> /V · s)	
		Ce <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	Ce(NO <sub>3</sub> ) <sub>3</sub>
Cs <sup>+</sup>	8.03	7.32	7.76
NH <sub>4</sub> <sup>+</sup>	7.64	6.75	7.33
K <sup>+</sup>	7.64	6.75	7.33
Ba <sup>2+</sup>	6.61	5.38	6.30
Ca <sup>2+</sup>	6.18	4.88	6.05
Sr <sup>2+</sup>	6.16	4.88	6.05
Na <sup>+</sup>	5.21	4.62	5.19
Mg <sup>2+</sup>	5.51	4.44	5.44
Li <sup>+</sup>	4.02	3.66	4.00

In cerium(III) sulphate solution the equilibrium

$$2 \text{Ce}^{3+} + 3 \text{SO}_4^{2-} \rightleftharpoons \text{Ce}_2(\text{SO}_4)_3 \quad (2)$$

is shifted more to the right than that in solutions of cerium(III) nitrate and chloride because of the lower water solubility of cerium(III) sulphate. The result of this is that in cerium(III) sulphate solution the effective positive charge of Ce<sup>3+</sup> is smaller than that in nitrate or chloride solution. Therefore, in cerium(III) sulphate solution the cerium(III) ions have a lower mobility than in cerium(III) nitrate and chloride solutions.

Experimentally we found that cerium(III) nitrate and chloride are more suitable for the measurement of rapidly migrating ions, e.g., caesium, ammonium and potassium, whereas cerium(III) sulphate is the better choice as electrolyte for slowly migrating ions, especially lithium.

#### Effective mobilities of ammonium and alkali and alkaline earth metal ions in the cerium(III) electrolyte

From the optimization of cerium(III) concentration it turned out that the favourable range is between 300 and 600  $\mu\text{M}$ . Fig. 2 shows an electropherogram of an ammonium and alkali and alkaline earth metal ion standard, which was measured with 500  $\mu\text{M}$  cerium(III) sulphate as electrolyte. According to literature values of mobilities [11] (Table I), the retention order was as expected, with the exception of the reversal of sodium and magnesium. This effect has also been found in other systems [5,8,13] and explained by interactions of magnesium with the capillary wall [8]. As we found that with cerium(III) nitrate as electrolyte magnesium elutes before sodium, we assume that the counter ion also causes the reversed retention order. Further, in Fig. 2, ammonium and potassium and also calcium and strontium co-elute under the experimental conditions used.

The effective mobilities,  $\mu_{eff}$ , which were calculated with the equation [14]

$$\mu_{eff} = \left( \frac{1}{t_r} - \frac{1}{t_{EOF}} \right) \frac{l_w l_d}{U} \quad (3)$$

where  $t_r$  is the retention time of analyte ion (s),  $t_{EOF}$  is the retention time of the water plug of the sample (s),  $l_d$  is the length of capillary to the detector (m),  $l_w$  is the total length of capillary (m) and  $U$  is the voltage

TABLE II

DIAMETERS, EFFECTIVE MOBILITIES AND STABILITY CONSTANTS OF 18-CROWN-6 COMPLEXES FOR SEVERAL IONS

The diameters and complex stability constants were taken from the literature [15]. CE conditions as in Fig. 3.

Parameter	Cation								
	Cs <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Ba <sup>2+</sup>	Ca <sup>2+</sup>	Sr <sup>2+</sup>	Na <sup>+</sup>	Mg <sup>2+</sup>	Li <sup>+</sup>
<i>d</i> (Å)	3.40	1.72	2.66	2.76	2.10	2.36	1.96	1.50	1.40
Log <i>K<sub>s</sub></i>	0.8	0.9	2.1	4.1	1.0	2.9	1.0	—	—
<i>μ<sub>eff</sub></i> (10 <sup>-4</sup> cm <sup>2</sup> /V · s)	7.37	6.86	6.00	3.82	5.08	4.26	4.78	4.60	3.74

(V), are summarized in Table I. For barium, calcium and strontium we found the highest differences (20%) between the measured mobilities and the values cited in literature, due to the equilibrium.



Depending on the partial formation of the neutral species MSO<sub>4</sub>, the effective positive charge and the mobility of these cations will be lowered.

The effective mobilities measured with the 500 μM cerium(III) nitrate system are given in Table I. With cerium(III) chloride or nitrate as electrolyte, no decrease in the mobilities of barium, calcium and strontium was found because of the higher solubility of M(NO<sub>3</sub>)<sub>2</sub>. In this electrolyte the mobilities of these ions differed from those quoted in the literature by up to 3%.

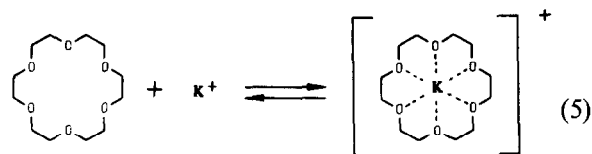
#### Separation of ammonium–potassium and calcium–strontium

The simultaneous determination of ammonium and potassium is important for food, atmospheric and water samples. For the separation of ammonium and potassium, the mobility of one of these ions has to be changed selectively. Weston *et al.* [7] slowed the migration of ammonium ions by increasing the pH of the electrolyte to 8.5. The disadvantage of this method is the formation of hydroxides with some of the analyte ions. The procedure proposed by Weston *et al.* [7] is not applicable to our electrolyte system, as cerium(III) hydroxide is formed above pH 6.

A possibility for changing the migration time is via the formation of complexes [7,8,13]. Crown

ethers having electron donor atoms such as O in their cyclic structure are suitable for forming complexes with several ions [15–18]. The complexation ability of a crown compound and the stability of the resulting complex depend on the diameter of the cavity in the crown compound relative to the diameter of the cation.

A selective macrocyclic ligand for the complexation of potassium is 18-crown-6:



In Table II the diameters of some ions and complex stability constants, *K<sub>s</sub>* [15], between these ions and 18-crown-6 (I.D. = 2.6–3.2 Å [15]) are summarized.

Fig. 3 shows the effect of 18-crown-6 as an additive to cerium(III) electrolyte on the separation of the same standard as shown in Fig. 2. In agreement with the high *K<sub>s</sub>* values, the ion mobilities of barium, strontium and potassium decreased selectively, whereas the mobility of ammonium remained constant. The apparent mobilities of the cations in this electrolyte system are summarized in Table II. Moreover, we found a strong linear correlation between the differences in retention times of potassium and ammonium and the concentration of 18-crown-6. Hence it is possible to shift the potassium peak in the electropherogram as

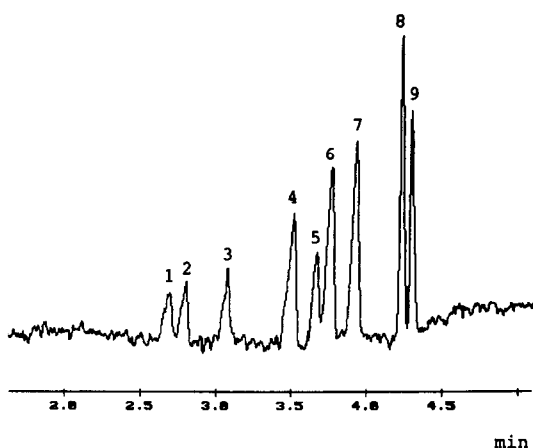


Fig. 3. Electropherogram of alkali and alkaline earth metal ions in the presence of ammonium. Carrier electrolyte,  $500 \mu\text{M}$  cerium(III) sulphate– $2.5 \text{ mM}$  18-crown-6; fused-silica capillary,  $55 \text{ cm} \times 75 \mu\text{m}$  I.D.; high voltage,  $30 \text{ kV}$ ; sample injection, hydrostatic (10 cm for 30 s). Peaks: 1 = caesium; 2 = ammonium; 3 = potassium; 4 = calcium; 5 = sodium; 6 = magnesium; 7 = strontium; 8 = barium; 9 = lithium (each  $50 \mu\text{M}$ ).

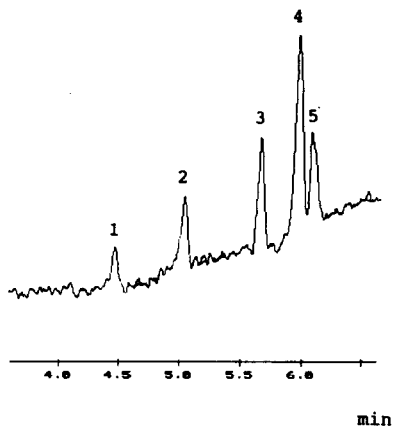


Fig. 4. Electropherogram of a rain sample. Carrier electrolyte,  $500 \mu\text{M}$  cerium(III) sulphate– $2.5 \text{ mM}$  18-crown-6; fused-silica capillary,  $60 \text{ cm} \times 75 \mu\text{m}$  I.D.; voltage,  $30 \text{ kV}$ ; electrokinetic sample injection ( $5 \text{ kV}$ , 10 s). Peaks: 1 = ammonium; 2 = potassium; 3 = calcium; 4 = sodium; 5 = magnesium.

desired. We achieved the same results in principle with dicyclohexyl-18-crown-6.

#### Applications

The separation of ammonium and alkali and alkaline earth metal ions by CZE could be improved

to give a routine analytical technique owing to the short analysis time ( $< 10 \text{ min}$ ) and high separation efficiency. Further, only small sample volumes and small amounts of electrolyte solution are required.

Many practical applications to different concentration ratios of analyte ions can be carried out using

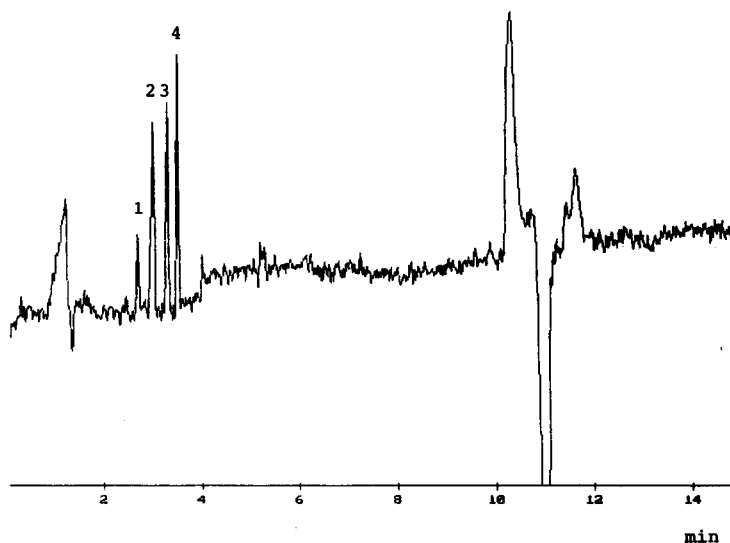


Fig. 5. Electropherogram of a cola beverage. Carrier electrolyte,  $500 \mu\text{M}$  cerium(III) sulphate– $2.5 \text{ mM}$  18-crown-6; fused-silica capillary,  $55 \text{ cm} \times 75 \mu\text{m}$  I.D.; voltage,  $30 \text{ kV}$ ; electrokinetic sample introduction ( $5 \text{ kV}$ , 10 s). Peaks: 1 = ammonium; 2 = potassium; 3 = calcium; 4 = sodium.

TABLE III

## LIMITS OF DETECTION FOR ELECTROKINETIC AND HYDROSTATIC SAMPLE INTRODUCTION

Detection limits defined as twice the noise in concentration units. Limits of detection were determined for the hydrostatic method where the sample was raised by 10 cm for 30 s and for electrokinetic injection at 20 kV for 10 s. Electrolyte systems: for  $\text{Cs}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ , 500  $\mu\text{M}$  cerium(III) chloride and 2 mM 18-crown-6; for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{Sr}^{2+}$ , 350  $\mu\text{M}$  cerium(III) chloride, 150  $\mu\text{M}$  cerium(III) sulphate, 2 mM 18-crown-6; for  $\text{Ba}^{2+}$  and  $\text{Li}^+$ , 500  $\mu\text{M}$  cerium(III) sulphate and 2 mM 18-crown-6.

Sample introduction	Limit of detection ( $\mu\text{M}$ )								
	$\text{Cs}^+$	$\text{NH}_4^+$	$\text{K}^+$	$\text{Ca}^{2+}$	$\text{Mg}^{2+}$	$\text{Na}^+$	$\text{Sr}^{2+}$	$\text{Ba}^{2+}$	$\text{Li}^+$
Electrokinetic	0.3	0.3	0.2	0.1	0.1	0.2	0.1	0.1	0.1
Hydrostatic	3.0	3.0	3.0	1.0	1.0	2.0	1.0	1.0	1.0

the cerium(III)–18-crown-6 electrolyte system described above. Figs. 4 and 5 show electropherograms of a rainwater sample and of a cola beverage. It can be seen in Fig. 5 that the complex matrix of the cola beverage does not disturb the determination of ions.

*Limits of detection and quantitative analysis*

The peak symmetry and peak height of ions with different mobilities depend on the choice of the electrolyte counter ion. For the determination of the limits of detection for the fast ions ( $\text{Cs}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ), the best results were obtained with cerium(III)

chloride.  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Sr}^{2+}$  were measured with an electrolyte solution consisting of cerium(III) chloride–cerium(III) sulphate (70:30). For  $\text{Li}^+$  and  $\text{Ba}^{2+}$ , cerium(III) sulphate was used.

The limits of detection achieved with these different cerium(III)–18-crown-6 electrolyte systems are given in Table III. These detection limits were determined for hydrostatic injection, where the sample was raised by 10 cm for 30 s, and for electrokinetic injection at 20 kV for 10 s. The measured limits of detection are in an intermediate position between those given by indirect UV detection and indirect laser-induced fluorescence detection.

Quantitative determinations using hydrostatic injection can be carried out on the basis of a linear relationship between peak area and sample concentration over several orders of magnitude. Fig. 6 shows the calibration graph for lithium as a characteristic example for all other cations. The correlation coefficient is  $r = 0.9998$ .

With electrokinetic injection, samples of lower concentration can be analysed because an accumulation of the analyte ions in the capillary during the sample introduction takes place. However, if the ionic strength of the sample varies significantly from that of the standard solution systematic errors are possible, because of the amount of analyte injected depends on the total ionic strength of the sample. Hence the hydrostatic injection method will be preferred for quantitative determinations in CZE, except when the analyte concentration is too low for successful detection.

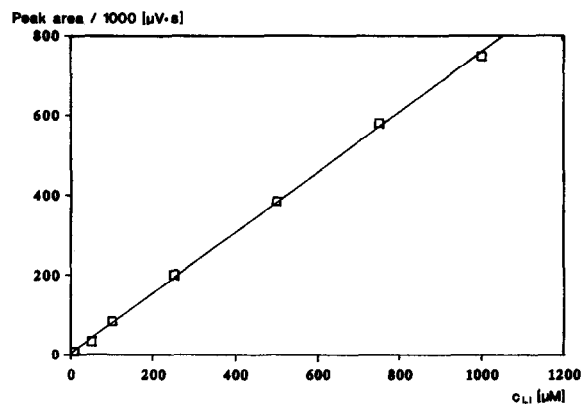


Fig. 6. Calibration graph for lithium determinations. Carrier electrolyte, 500  $\mu\text{M}$  cerium(III) sulphate; fused-silica capillary, 60 cm  $\times$  75  $\mu\text{m}$  I.D.; voltage, 30 kV; hydrostatic sample introduction (10 cm, 30 s).

## CONCLUSIONS

Cerium(III) satisfies the condition that must be fulfilled by an electrolyte co-ion in CZE as far as resolution and detection are concerned for the determination of alkali and alkaline earth metal ions. The limits of detection are in the 0.1–0.3  $\mu\text{M}$  range with a commercially available fluorescence detector for normal HPLC. The separation of potassium and ammonium and of strontium and calcium can be achieved by adding 18-crown-6 to the electrolyte solution. The migration behaviour of the analyte ions can be influenced by changing the crown ether concentration, so that this system can be optimized for the analysis of various types of samples. Further, it is possible to optimize the peak symmetry, either of the highly mobile ions ( $\text{K}^+$ ,  $\text{NH}_4^+$ ) or of the less mobile ions ( $\text{Be}^{2+}$ ,  $\text{Li}^+$ ), by the choice of the counter ion.

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