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Research Article

On-line solid phase extraction CZE for the simultaneous determination of lanthanum and gadolinium at picogram per liter levels

A non-specific on-line method is presented for the extraction and preconcentration of two rare earth elements using a microcartridge containing C_{18}-derivatized silica particles prior to their analysis by CZE. The microcartridge, named analyte concentrator, was coupled on-line to the inlet of the separation capillary (fused-silica (FS) capillary, 75 μm id × 12 cm from the inlet to the microcartidge and 37 cm from the microcartridge to the detector). The reversed-phase sorbent quantitatively retained gadolinium (Gd) and lanthanum (La) as 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complexes in the presence of non-ionic micelles of polyethylene glycol tert-octylphenyl ether, enabling sample clean-up and concentration enhancement with minimum sample handling. The rare earth elements chelates were released from the sorbent with methanol and then analyzed by CZE with diode array detection. A background electrolyte of 20 mM sodium tetraborate containing 8% ACN, pH 9.0, was found to be optimal for the separation of metal chelates. The concentration limits of detection were lowered to picogram per liter levels (20 pg/L for La and 80 pg/L for Gd). A 1000-fold improvement in concentration sensitivity for La- and Gd-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complexes with respect to CZE without preconcentration was reached.

Keywords:
CZE / Environmental analysis / Gadolinium / Lanthanum / On-line SPE
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1 Introduction

During the last three decades, the use of rare earth elements (REEs) in manufactured goods has resulted in a wide variety of electromechanical and metallurgical devices to glasses, superconductors, supermagnets, lasers, and electronic components [1, 2]. REEs have been applied in fertilizers for the agriculture with the consistent bioaccumulation of REEs in plants, soils, and water [3–5]. Indeed, they are extensively applied in medical fields; gadolinium (Gd) is widely employed as a contrast agent for magnetic resonance imaging [6–8] while lanthanum (La) is used as calcium- and aluminum-free phosphate binder for the treatment of hyperphosphatemia of chronic renal failure [9, 10]. Thus, these two chemical elements have two clinical applications both in favor of patients with severe kidney failure: Gd in the diagnostic field and La in the therapeutic one [11]. Consequently, this fact may lead to the enrichment of Gd and La in medical waste samples.

The separation and determination of REEs analytes are especially challenging due to the similarity in chemical characteristics arising from their equal charge and almost similar ionic radii. Consequently, there is an ongoing need for the development of alternative extraction/enrichment procedures for the simultaneous determination of REEs at low concentrations in complex samples, especially those of biological or environmental origin [12–15]. CE can meet many of the requirements concerning the achievement of these goals due to the high efficiency, robustness and ruggedness, low-cost, rapidness, and unique selectivity related to the technique. Nevertheless, the application of CE to metal trace level analysis has been hindered by its relatively poor concentration sensitivity; when only nanoliter sample volumes are introduced into the capillary and the sample contains a trace concentration of the analyte of interest, only a few molecules are available for detection. Therefore, the implementation of a preconcentration step prior to CE is essential in order to obtain analytical data.

Enhancement in CE sensitivity can be achieved by various preconcentration methods. Particularly, on-line

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Abbreviations: 5-Br-PADAP, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; **FS,** fused-silica; **REEs,** rare earth elements; **SPE-CE,** solid-phase extraction capillary electrophoresis; **TX-100,** polyethylene glycol tert-octylphenyl ether

solid-phase extraction capillary electrophoresis (SPE-CE) using a microextractor (named ''analyte concentrator'') has successfully been applied to the analysis of numerous compounds [16–19]. For fabrication of an analyte concentrator, different types of ligands can be immobilized directly on a portion of the inner surface of the capillary wall [20, 21], a membrane [22], beads [23–30], multi-bore capillary [31],] or monolithic roads [32–34]. Located near the inlet of the capillary, these microcartridge devices can not only be used for concentration enrichment, isolation, or extraction of analytes but also to achieve microreactions allowing the on-line analysis of reaction products [35]. This technique enables analyte(s) to be separated from either a simple matrix or a complex feedstock, a cell or tissue homogenate, biological fluid, or any other complex mixture, including pharmaceutical formulations [36]. For an overview on recent developments in analyte concentrator devices and their analytical applications, see the excellent review by Guzman *et al*. [37]. Even though on-line SPE-CE represents a promising analytical tool, a survey of the literature indicates that no methods for the simultaneous determination of metals at trace levels by SPE-CE have been developed.

In the present work, the implementation of a new method for the simultaneous determination of two REEs at picogram per liter levels by on-line coupling of a solid-phase preconcentration step to CZE with diode array detection is demonstrated.

2 Materials and methods

2.1 Reagents and solutions

All chemicals were of the highest analytical grade available. Sodium tetraborate, polyethylene glycol tert-octylphenyl ether (TX-100), nitric acid, hydrochloric acid, HPLC-grade ethanol, ACN, and methanol were purchased from Merck (Darmstadt, Germany). Irregularly shaped silica-based C18 reversed-phase chromatographic media (Vydac® 218TP, polymerically bonded encapped n-octadecyl reversed-phase silica particles, 20–30 μm particle diameter, 300 Å pore size) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Jupiter® 300 C18 bulk media (spherical shaped silica-based particles, 10 μm particle diameter, 300 Å pore size) was obtained from Phenomenex (Torrance, CA, USA). Microsorb 300–10 C18 bulk packing (spherical shaped silica-based particles, 10 μm particle diameter, 300 Å pore size) was purchased from Varian (Palo Alto, CA, USA). Distilled water was purified (resistivity18.3 MΩ cm) using a Milli-Q water purification station (Millipore Intertech, Bedford, MA, USA). In total, 1 mg/mL standard solutions of Gd(III) and La(III) were prepared from acid dissolution of their oxides (Aldrich, Milwaukee, WI, USA). Stock solutions were standardized by a chelatometric method [38]. One millimolar solution of 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) (Aldrich) was prepared by reagent dissolution in ethanol. Lower concentrations of 5-Br-PADAP were obtained by serial dilution of the concentrated solution with ethanol. A 20 mM sodium tetraborate buffer solution, pH 9.0, adjusted with 0.1 M hydrochloric acid, was daily prepared. The sample solutions were prepared as follows. An aliquot of standard metal solution or spiked tap water sample was placed in a 5 mL volumetric flask and 0.10 mL of 5% TX-100 in sodium tetraborate buffer solution (v/v) was added. Next, 50 μL of a 3.75×10^{-6} M 5-Br-PADAP and 1 mL of the tetraborate buffer solution were successively added. The resultant solution was made up to volume with water. All solutions were filtered through 0.45 μm nylon membrane filters (Titan Syringe Filters, Sun Sri, Rockwood, TN, USA) and thoroughly degassed under vacuum before use. All the glass elements used to work with metal solutions and tap water samples were previously washed with a 10% v/v nitric acid/water solution and then with water.

2.2 Instrumental

All CE separations were performed in a Beckman P/ACE MDQ instrument (Beckman Coulter, Fullerton, CA, USA) equipped with a diode array detector. P/ACE System MDQ Software (Beckman) was used for data processing and calculations. Fused-silica (FS) capillaries were obtained from MicroSolv Technology Corporation (Eatontown, NJ, USA) and were 57 cm in total length (12 cm from the inlet to the analyte concentrator and 37 cm from the analyte concentrator to the detector for on-line SPE-CE, and 50 cm from the inlet to the detector for CZE without preconcentration, and 8 mm for the analyte concentrator), 75 μm id (or 150 μm id for the analyte concentrator), and 365 μm od. pH measurements were performed by an Orion 940 pH meter equipped with a glass combined electrode (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Fabrication of the analyte concentrator

Analyte concentrators were manufactured in the lab as previously described [23, 28]. The entire process was monitored under a stereomicroscope (Riechter, Vienna, Austria). A very thin piece of a polymeric porous frit structure was introduced into a PTFE tubing (365 μm id) with the help of an auxiliary capillary. A section of FS capillary (150 μm id, 365 μm od, 8 mm in length) was slid into the sleeve pushing in the frit about 2 mm. The other end of the PTFE tubing was connected to a vacuum pump to fill the 8 mm long capillary with dry reversed-phase particles (C18) by slow controlled-vacuum aspiration. The free end of the filled capillary was slid into another section of PTFE tubing containing a second frit. Both sleeves were cut at a length of 4 mm. The microcartridge was connected to two FS capillaries (75 μm id, 365 μm od) previously rinsed with 1.0 M potassium hydroxide for 5 min and water for 10 min. The total capillary length was 57 cm (12 cm from the inlet to the concentrator and 37 cm from the concentrator to the detector). The junctions were glued with a thin layer of

epoxy resin. Figure 1 shows a schematic representation of the analyte concentrator microcartridge. The MDQ CE system cartridge was carefully assembled after inserting the analyte concentrator/capillary into the cooling tubing.

2.4 CZE and on-line SPE-CE procedures

The running BGE consisted of sodium tetraborate 20 mM, pH 9.0, containing 8% ACN, v/v, unless otherwise noted. The temperature of the system was maintained at 25°C. All solutions were pressure-introduced at the anodic side of the capillary. Runs were carried out in normal mode (cathode at the outlet side, 25 kV) and detection took place at 585 nm. New capillaries for CZE were preconditioned by rinsing with 1.0 M potassium hydroxide for 5 min, followed by a 10 min rinse with water and 10 min with running buffer. At the beginning of each day, conditioning of capillaries was performed by flushing 0.1 M potassium hydroxide, water, and running BGE for 2 min each step. Between runs, capillaries were rinsed with running BGE for 2 min. Samples were introduced at 0.5 psi for 7 s.

The time events for the extraction/preconcentration of Gd and La are presented in Table 1. Conditioning/regeneration of capillaries was performed by flushing water for 5 min, methanol for 5 min, followed by water again and running BGE, 10 min each step. Samples were introduced at the anodic side at 7.0 psi during 24 s. The REEs were retained in the analyte concentrator as REE-5-Br-PADAP complexes in micelles of TX-100 at pH 9.0 (in the 20 mM sodium tetraborate buffer solution, pH 9.0, described in Section 2.1). Removal of unretained compounds was done by flushing with separation buffer. Desorption of bound analytes was carried out introducing methanol applying 0.50 psi for 6 s. After daily use, the capillary was washed with water for 2 min followed by methanol for 2 min, and then stored at room temperature, with the inlet and outlet ends dipped into two reservoirs filled with methanol. All peak areas reported correspond to average corrected peak areas (area/migration time).

2.5 Sample collection

Tap water was allowed to run for 20 min and approximately 2000 mL of tap water was collected on a glass bottle. The water samples were filtered and processed immediately after sampling.

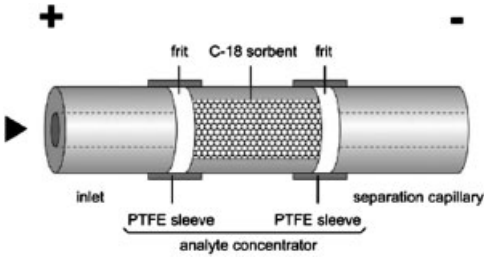

Figure 1. Schematic representation of the analyte concentrator microcartridge. Reprinted with permission from Ref. [28].

3 Results and discussion

The efficacy of the preconcentration effect was highly dependant on the operating conditions used for the analyte adsorption and elution and required a careful optimization. The effects of several experimental parameters upon the extraction, separation, and detection parameters affecting the analytical performance of the combined on-line SPE-CE methodology have been thoroughly evaluated and optimized. The optimization of the experimental conditions has been accomplished by the traditional method of one-at-a-time.

A two-stage sequence of variations was applied and during the optimization operation, all the values of the different factors except one were kept constant, and this one was the object of the survey. The first sequence involved the preconcentration conditions (cartridge conditioning/regeneration and sample injection and elution), while the second sequence implicated the electrophoretic/detection factors. The peak areas and migration times were used to evaluate the extraction efficiency and separation/quantification efficacy under different experimental conditions.

3.1 Optimization of electrophoretic conditions

The analysis of REEs was optimized in classical CZE mode by studying the optimum conditions providing the highest selectivity.

The effect of the buffer pH was investigated within the range of 6.0–10.0 at a fixed buffer concentration (10 mM), adjusted by 0.1 M sodium hydroxide and 0.1 M hydrochloric acid. It was found that when the pH was increased, resolution also increased, while time analysis decreased. The best results were obtained at pH 9.0 considering selectivity, reproducibility, and baseline and current performance.

Table 1. Time events for the optimized SPE-CZE procedure

Step	Time (min)	Event	Value	Duration	Comment
1		Rinse-pressure	7.00 psi	3.00 min	Methanol
2		Rinse-pressure	7.00 psi	4.00 min	Water
3		Rinse-pressure	7.00 psi	6.00 min	Running BGE
4		Rinse-pressure	7.00 psi	0.40 min	Sample
5		Rinse-pressure	7.00 psi	2.00 min	Running BGE
6		Rinse-pressure	7.00 psi	0.10 min	Methanol
7		Rinse-pressure	7.00 psi	9.00 s	Running BGE
8		Autozero			
9	0.00	Separate-voltage	25 kv	30.00 min	Running BGE
10	30.00	Stop data			
11		Rinse-pressure	7.00 psi	2.00 min	Water
12	32.00	End			

Keeping all other parameters constant, the buffer concentration was varied from 5 to 75 mM. Increases in migration time as well as current were observed when the concentration of buffer increased. Resolution also increased for higher buffer concentrations, but no appreciable improvements were observed for buffer concentrations above 20 mM. However, La(III) and Gd(III) quelates were not completely separated. Therefore, ACN was used as an organic modifier to enhance the resolution. Various amounts of ACN (5–20%, v/v) were added into the 20 mM sodium tetraborate buffer, pH 9.0. The compounds were baseline separated when 8% ACN, v/v, was added. Thus, a 20 mM sodium tetraborate buffer containing 8% ACN, v/v, pH 9.0, was chosen as the running BGE as it gave a baseline separation of the analytes of interest.

3.2 Analysis of La and Gd by on-line SPE-CE

The reversed-phase microcartridge coupled to the separation capillary was tested for the extraction/preconcentration of Gd and La from standard solutions and tap water samples. The REEs were retained in the analyte concentrator as REE-5-Br-PADAP complexes in micelles of TX-100 at pH 9.0. Figure 2 shows the electropherograms of a standard mixture of La and Gd -5-Br-PADAP chelates in the presence of TX-100 micelles analyzed with and without preconcentration. A dramatic improvement in detection limit was achieved with SPE-CE compared with that obtained by CZE without preconcentration.

It is well recognized that the same type of stationary phase, *e.g.* C18 reversed-phase, obtained from diverse suppliers can provide different selectivity. The presence of partially ionized silanols or other groups carrying negative charges in the stationary phase, the type and pore morphology of the silica gel support, the carbon load, *etc.*, may sensitively affect the retention of compounds, especially those polar and ionic ones as Jandera *et al.* [39] have recently demonstrated. To compare the performance of the analyte concentrators packed with different particles, we tested two other reversed-phase chromatographic media (Jupiter® 300 C18 and Microsorb 300-10 C18) for retention of the La- and Gd-5-Br-PADAP complexes. The same electrophoretic profile was obtained by both packing materials under the same experimental conditions than those optimized for the analyte concentrators packed with Vydac® C18 chromatographic material (Fig. 3). Nevertheless, wider and shorter peaks were observed in both cases, with a loss of sensitivity in terms of LOD when compared with the results obtained with Vydac® particles. This highlights the

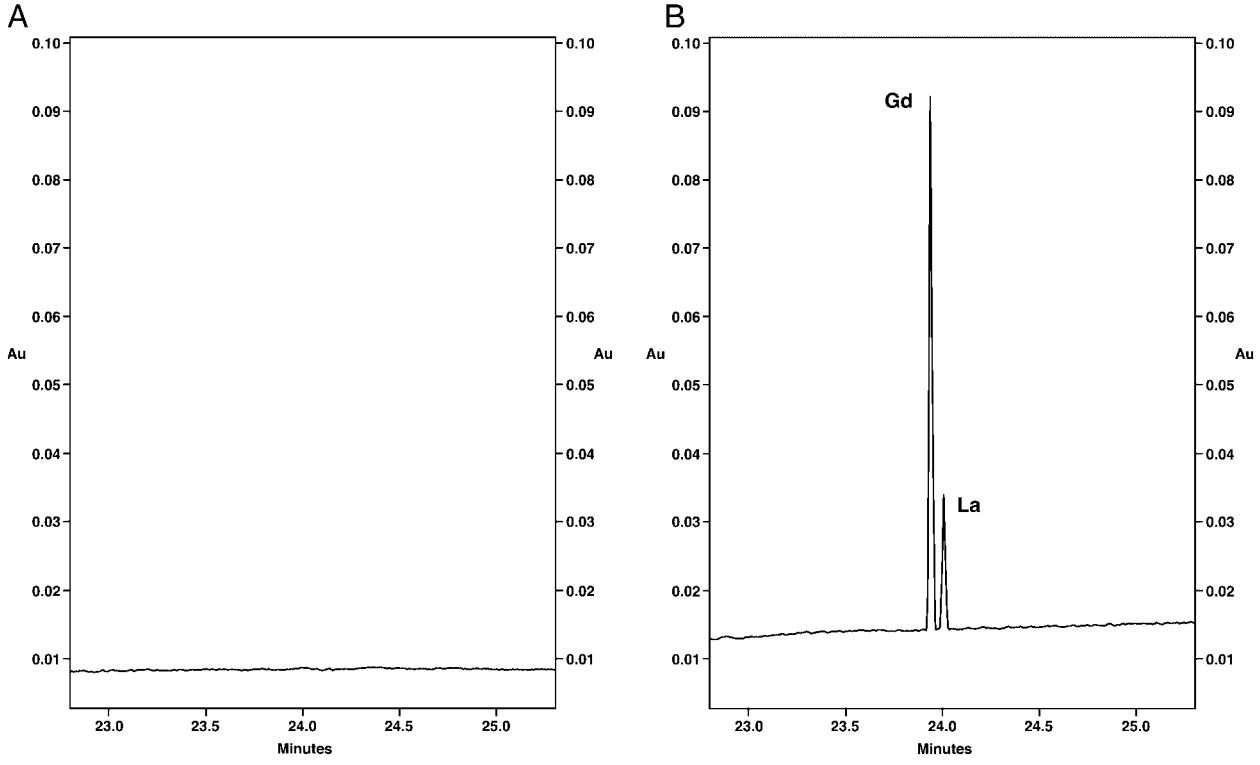

Figure 2. Analysis of La- and Gd-5-Br-PADAP chelates: sample, La(III) = 100 pg/L, Gd = 200 pg/L, 5-Br-PADAP = 4×10^{-8} M, TX-100 = 0.1%, v/v; running BGE: 20 mM sodium tetraborate with 8%, v/v ACN, pH = 9.0; 25°C, detection at 585 nm, 25 kV. (A) CZE: sample introduction, 0.50 psi for 7 s; capillary, 75 μm id × 57 cm length (50 cm to detector). (B) On-line SPE-CE: sample introduction, 7.0 psi for 24 s; elution with methanol, 0.50 psi for 6 s, followed by injection of running BGE, 0.50 psi, 9 s; analyte concentrator, 150 μm id × 8 mm length, packed with Vydac® 218TP particles; capillary, 75 μm id × 57 cm length (12 cm from the inlet to the analyte concentrator and 37 cm from the analyte concentrator to the detector).

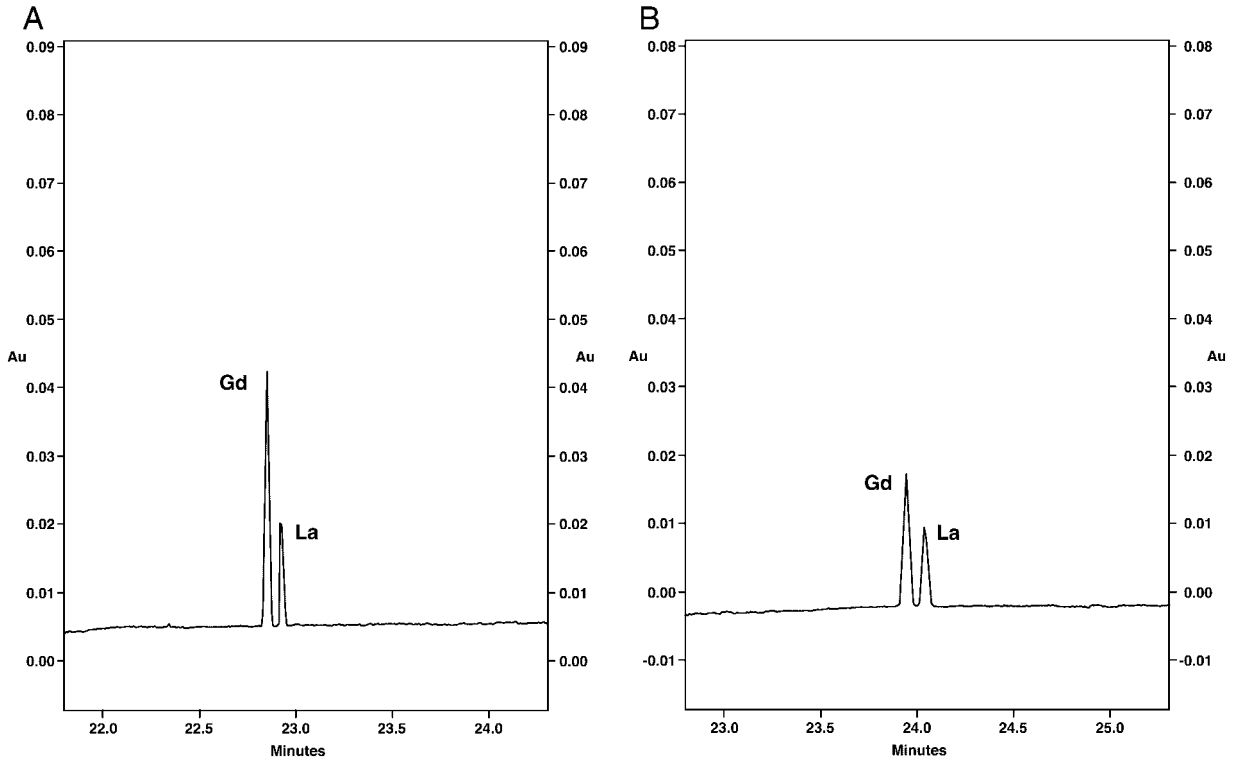

Figure 3. Analysis of La- and Gd-5-Br-PADAP chelates by on-line SPE-CE. Analyte concentrators were packed with (A) Jupiter® 300 C18 particles and (B) Microsorb 300-10 C18. Other conditions are the same as in Fig. 2.

importance of considering the chromatographic properties of the stationary phase and the chemical characteristics of the analytes for the fabrication of the analyte concentrator and evaluating the chromatographic behavior of different packing materials to get successful results. Further work has to be done to find the optimal conditions to improve, if possible, the detection sensitivity when using those chromatographic materials.

3.3 Separation performance: Evaluation of the methodology

The method showed linear response in the ranges of 0.06–8.00 ng/L (La) and 0.22–9.00 ng/L (Gd). The intra-day repeatability ($n = 6$) RSD values for migration time, peak area, and normalized peak area were lower than 0.72, 3.96, and 3.66%, respectively, while inter-day repeatability ($n = 5$ days) RSD values were lower than 2.74, 9.41, and 9.83%, respectively. The concentration limits of detection were lowered to picogram per liter levels (20 pg/L for La and 80 pg/L for Gd). A 1000-fold improvement in concentration sensitivity for La- and Gd-5-Br-PADAP complexes with respect to CZE without preconcentration was reached. The analyte concentrator could be reused at least ten times without loss of resolution or loss of the retention capacity with the capillary conditioning described in Section 3.2. Precision between analyte concentrators was 8% (RSD, $n = 3$).

Table 2. Recovery study (95% confidence level, $n = 6$)

Aliquots	La added (pg/L)	La found (pg/L)	Recovery (%)[a)]
1–6	0.00	NF[b)]	–
7	80.00	82.00	102.50
8	120.00	116.00	96.67
	Gd added (pg/L)	Gd found (pg/L)	Recovery (%)[b)]
1–6	0.00	NF[b)]	–
9	250.00	260.00	104.00
10	350.00	355.00	101.42

a) $\%R = 100 \times \frac{(C_r - C_b)}{C_a}$.
b) NF, not found.

REEs are widely used in agriculture, medicine, and in many industrial applications [1–11]. As a result, large amounts of REEs may be discharged to receiving water bodies. Indeed, the potential toxicity of free REEs has already been recognized [12, 40, 41]. Consequently, their determination in water, especially tap water, is a subject of great public concern. The method was applied to the analysis of spiked water samples in order to validate its accuracy. Tap water was collected and divided into portions of 1000 μL each. The proposed method was applied to six portions and the average quantities of La and Gd obtained were taken as base values. Then, increasing quantities of the two analytes were added to the aliquots of sample, and

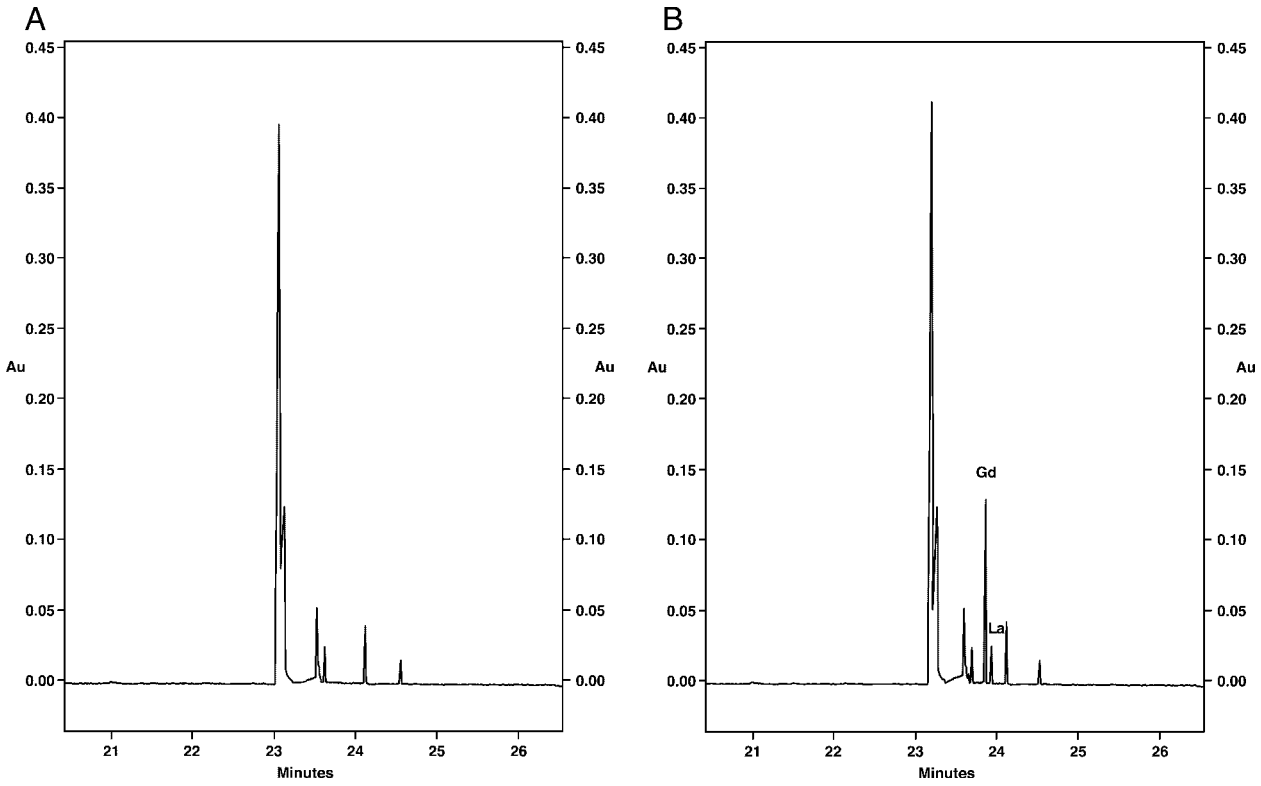

Figure 4. Electropherogram of a tap water sample from San Luis Province, Argentina, analyzed by on-line SPE-CE. (A) Non-spiked tap water sample and (B) spiked tap water sample. Concentrations added: La(III) = 150 pg/L, Gd = 350 pg/L.

La and Gd were determined by means of the described procedure. The results are given in Table 2. Figure 4 shows the electropherograms of a tap water sample from San Luis, Argentina (with and without La and Gd spiking) using the optimized experimental conditions obtained for the on-line solid phase extraction-capillary zone electrophoresis methodology. The preconcentration method proposed in this paper allows the elimination of great part of the saline content in the sample, principally sodium and potassium, due to the limited tendency of these elements to form 5-Br-PADAP complexes. Although the proposed preconcentration methodology is non-specific taking into account the complexation and retention processes, the combined on-line SPE-CE approach offers a highly selective and sensitive approach for the simultaneous determination of REEs.

4 Concluding remarks

The sample preconcentration strategy employed for the present approach involves no additional modification of the commercially available standard CE instrument, and it can be easily accomplished by carefully controlling the operation conditions. It allows large-volume injection of the sample avoiding diffusion phenomena and band broadening effects by maintaining the resolution and separation efficiency that strongly characterizes the CZE. A large sensitivity enhancement factor was obtained in the study allowing the simultaneous determination of La and Gd at picogram per liter levels.

The possibility of performing on-line SPE/preconcentration for the determination of trace elements by CE opens up an attractive alternative in the area of automated separation methods, particularly in view of the excellent extraction efficiencies, preconcentration factors, and selectivity associated with the methodology.

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The authors have declared no conflict of interest.

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