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Simultaneous determination of dysprosium and iron in urine by capillary zone electrophoresis coupled to cloud point extraction

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Abstract

Automated preconcentration strategies are needed when analyzing metals in real samples by capillary electrophoresis (CE) with UV detection. The on-line incorporation of cloud point extraction (CPE) to flow injection analysis (FIA) associated with CE for simultaneously determining dysprosium and iron at ppb levels in urine is presented and evaluated for the first time. The preconcentration step is mediated by micelles of the non-ionic surfactant polyethyleneglycol-mono-*p*-nonylphenylether (PONPE 7.5) with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol. The micellar system containing the complex was loaded into the FIA manifold at a flow rate of 8 ml min^{-1}, and the surfactant rich-phase was retained in a microcolumn packed with cotton, at pH 9.2. The surfactant-rich phase was eluted with 50 μl acetonitrile directly into the CE sample vial, allowing to reach an enrichment factor of 200-fold for a 10 ml sample urine. The type and composition of the background electrolytes (BGE) were investigated with respect to separation selectivity, reproducibility and stability. A BGE of 20 mM sodium tetraborate buffer containing 13% acetonitrile, pH 9.0 was found to be optimal for the separation of metal chelates. Detection was performed at 585 nm. An enhancement factor of 200 was obtained for the preconcentration of 10 ml of sample solution. The detection limits for the preconcentration of 10 ml of urine were 0.20 μg l^{-1} for Dy, and. 0.48 μg l^{-1} for Fe. The calibration graphs using the preconcentration system were linear with a correlation coefficient of 0.9989 (Dy) and 0.9976 (Fe) at levels near the detection limits up to at least 500 μg l^{-1}. The method was successfully applied to the determination of dysprosium and iron in urine for monitoring the elimination of dysprosium-based pharmaceuticals.

Keywords: On-line cloud point extraction; Capillary zone electrophoresis; Dysprosium; Iron; Urine; MRI contrast agents

1. Introduction

Radioimaging (RI) and magnetic resonance imaging (MRI) are important tools in the non-invasive diagnostics of diseases and tumors. Improving the contrast and the selectivity for target organs led to more sensitive and more accurate detection [1]. Physical, chemical and biological results support the use of chelated Dy complexes as NMR contrast agents; for example, the diethylenetriamine-pentaacetic acid–bis(methylamide) (DTPA–BMA) complex was used as a ligand in myocardial investigations [2] and as potential marker of cell membrane integrity [3] and 1,4,7-tris-(carboxy-methyl)-10,2'-hydroxypropyl)-1,4,7,10-tetraaza-cyclododecane (HP.DO3A) was a ligand in brain investigations [4]. Both dysprosium triethylenetriamine hexaacetate (DyTTHA) and dysprosium-bis-triphosphate (DyPPP) were used to distinguish intra- and extracellular 23 Na resonances before and after the onset of hipoxia [5]. Dy-ethoxybenzyl (EOB)–DTPA represents a potentially helpful liver-specific contrast agent in Computer Tomography [6–7].

The use of CE in pharmaceutical analysis can have benefits in terms of robustness and ruggedness, cost and time.

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The versatility of CE in the analysis of a wide array of pharmaceutically relevant analytes varying in polarity, size, and stereochemistry has been represented through the numerous accounts available in the literature [8–12].

However, CE suffers from poor concentration sensitivity when using UV detection because of the small injection volumes (typically <1% capillary length) and narrow optical pathlength. This presents a significant obstacle for routine analyses of ppb levels of a wide variety of UV-active analytes in real samples by CE [13]. Consequently, automatic preconcentration techniques in conjunction with CE represents a promising tool for analyses of pharmaceutical interest [14–16].

Surfactants are a special type of analytical reagent, which provide excellent improvements in almost every area of chemistry. In the last decades, an increasing interest is shown all over the world in developing surfactant-based methods in all fields of analytical chemistry. The analytical potential of these systems can be further increased due to the fact that non-ionic isotropic micellar solutions separate into two phases, when a certain temperature called cloud point is reached. Aqueous solutions of many non-ionic surfactant micellar systems become turbid over a narrow temperature range, when the experimental conditions have been changed. This temperature is named “cloud point temperature”. Above the cloud point, the solution separates into two phases: one, very small in volume, the surfactant-rich phase; and the other, the bulk aqueous solution containing surfactant monomers [17,18].

The use of micellar systems as an alternative to other techniques of separation offers several advantages including low cost, safety and high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and very high concentration factors. From an analytical point of view, the surfactant-rich phase can be used to separate and/or preconcentrate different analytes before their injection into any hydrodynamic analytical system [19–21]. That is why cloud point extraction (CPE) is particularly adequate for HPLC, flow injection analysis (FIA) and CE. Moreover, CPE uses surfactants that inhibit the absorption of non-polar analytes to glass surfaces. The coupling of CPE to CE [22] and capillary electrochromatography (CEC) [23] has already been reported.

Fang et al. [24] proposed the on-line incorporation of CPE to flow injection analysis. In previous works [17–20], we have used off-line CPE approaches in order to determine metals ions coupled to ICP–OES, HPLC, and UV–vis spectrometry. Recently, we presented and evaluated for the first time automated CPE–FI–ICP–OES methods for the determination of Gd [25] and Dy [26] in human urine. The aim of the present paper is to evaluate the feasibility of coupling an on-line CPE preconcentration to capillary zone electrophoresis for the simultaneous preconcentration of iron and dysprosium in urine.

2. Experimental

2.1. Reagents and solutions

A standard solution of 1 mg ml^{-1} Dy(III) and Fe(III) were prepared from acidic dissolution of their oxides of analytical grade purity (Aldrich, Milwaukee, WI, USA). Stock solutions were standardized by a chelatometric method [27].

A 5×10^{-2} mol l^{-1} solution of 5-Br-PADAP (Aldrich) was prepared by appropriate dissolution in ethanol (Merck, Darmstadt, Germany). Lower concentrations were prepared by serial dilution with ethanol.

As it is not possible to obtain a real aqueous solution of the surfactant polyethyleneglycol-mono-*p*-nonylphenylether (PONPE-7.5, Tokio Kasei Industries, Chuo-Ku, Tokyo, Japan) (cloud point below room temperature) it was experimentally convenient to prepare a stock surfactant solution as follows: 20 g of PONPE-7.5, 10 ml of 1 mol l^{-1} $NaCLO_4$ (Merck, Darmstadt, Germany), and 40 ml of distilled ethanol were mixed and made up to 100 ml with doubly distilled water.

The buffer solution (5×10^{-1} mol l^{-1}) was prepared by dissolving sodium tetraborate (Merck, Darmstadt, Germany) and made up to 1000 ml with ultrapure water.

Ultrapure water (resistivity: 18.3 MΩ cm) was obtained from Barnstedt EASY pure RF water system (Iowa, USA). All other reagents and solvents were of analytical grade quality. All solutions were degassed by ultrasonication (Testlab, Argentina). Running electrolytes and samples were filtered through a 0.45 μm Titan Syringe filters (Sri Inc., Eaton Town, NJ. USA).

3. Instrumental

A Beckman P/ACE MDQ instrument (Beckman Instruments, Inc., Fullerton, CA) equipped with a diode array detector and a data handling system comprising and IBM personal computer and P/ACE System MDQ Software. Detection was performed at 565 and 590 nm. The fused-silica capillaries were obtained from MicroSolv Technology Corporation and had the following dimensions: 57 cm total length, 50 cm effective length, 75 μm i.d., 375 μm o.d. The temperature of the capillary and the samples was maintained at 25 °C.

The ICP measurements were performed with a sequential ICP spectrometer [Baird (Bedford, MA, USA) ICP 2070]. The 1 m Czerny-Turner monochromator is based on a holographic grating with 1800 grooves/mm.

A Minipuls 3 peristaltic pump (Gilson, Villiers-Le-Bell, France) was used. Sample injection was achieved using a Rheodyne (Cotati, CA, USA) Model 50, four-way rotary valve. A microbore glass column (50 mm length and 3 mm i.d.) was used as the cotton holder. Tygon-type pump tubing (Ismatec, Cole-Parmer, Vernon Hills, IL, USA) was employed to propel the sample, reagent and eluent.

The pH of the electrolyte was measured by an Orion 940 pHmeter equipped with a glass-combined electrode.

All the glass instruments used were previously washed with a 10% (v/v) HNO_3 water solution and then with ultrapure water.

4. Experimental

Urine was collected and stored in plastic containers without adding preservatives. A sample mineralization was necessary since urine's natural occurring components normally do not allow Dy and Fe complexation with pyridylazo dyes. The samples of urine were digested as follows. In porcelain capsule, a 10 ml sample of urine was accurately measured into a porcelain capsule, treated with a mixture of 0.4 ml of 30% (w/w) H_2O_2 and 0.2 ml 65% (w/w) HNO_3, and then placed in sand bath. The sample was moderately heated up to cause disappearance of the amber color. Subsequently, the sample was evaporated to incipient dryness. Then, fresh portions of 65% (w/w) HNO_3 were added to the dark residue and heated to dryness. This procedure was repeated until a white ash was obtained. The residue was taken with 0.2 ml of a mixture of 0.5 mol l^{-1} HCl and 0.5 mol l^{-1} HNO_3 (3 + 1) and heated. This solution was approximately diluted up to 10 ml with ultrapure water.

Blanks were prepared with the same reagents, without the samples, undergoing an identical process of mineralization.

4.1. Preconcentration step

Before loading the column, it was conditioned for the preconcentration at the correct pH with buffer diluted solution (1×10^{-2} mol l^{-1}). The solution containing the analytes (standard solutions or samples) and a solution containing 5×10^{-5} 5-Br-PADAP, 1% (w/w) PONPE 7.5, buffered to pH 9.22 with sodium tetraborate were mixed on-line, at flow rates of 8.0 and 2.0 ml min^{-1}, respectively. The solution passed through a 100 cm mixing coil thermostatised at 30 °C, and then was loaded onto the collection column, which allowed for the surfactant-rich phase containing the chelates to be collected inside the column, while the aqueous phase passed through the column. After the loading time, further washing with buffer diluted solution served to remove the sample still present in the lines and in the column. Finally, the injection valve was switched on and the retained surfactant-rich phase was eluted with 50 μl of acetonitrile, directly into the CE sample vial. Fig. 1 shows a schematic diagram of the instrumental setup.

4.2. Simultaneous determination of dysprosium and iron by CZE

The electrolyte solution was prepared daily and filtered through a 0.45 μm Titan Syringe filters (Sri Inc.). At the beginning of the day, the capillary was conditioned with

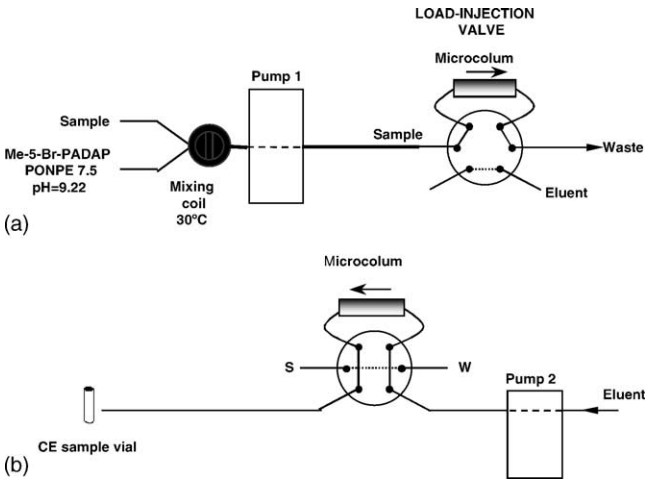

Fig. 1. Schematic diagram of the instrumental setup: (a) load position; (b) injection position.

0.1 mol l^{-1} NaOH for 5 min, followed by water for 5 min, and then with running electrolyte for 10 min before sample injection. To achieve high reproducibility of migration times and to avoid solute adsorption, the capillary was washed between analyses with ethanol for 1 min, then sodium hydroxide for 2 min, followed by water for 2 min, finally equilibrated with the running buffer for 4 min. Samples were pressure-injected at the anodic side at 0.5 psi for lengths of time 3–7 s. To avoid buffer contamination caused by adsorption of surfactant onto the outer wall of the capillary, the anodic side of the capillary was immersed in ethanol during 2 s immediately after sample injection. A constant voltage was used for all the experiments. Detection was performed at 585 nm.

Electroosmotic flow (EOF) determination was performed by using acetone as an EOF marker. The EOF marker was prepared by diluting 1 ml of acetone with the BGE and sonication for 5 min prior injection.

5. Results and discussion

For an adequate selection of the dysprosium-based contrast agent, it has to be considered that the kinetics of dissociation in vivo are more important than thermodynamics stability due to the fact that lanthanide chelates should be excreted as rapidly as possible (elimination half times on the order of hours) consistent with their use in imaging [1,28,29]. It has been demonstrated that some endogeneous metals such as zinc, copper, nickel and iron could compete with Dy for the complexing agent [28]. Consequently, Fe was chosen as a "tester" for the electrophoretic studies considering the following factors: electrophoretic behavior, complexing characteristics, and, specially, iron whole body content (Fe: 3–7 g, Zn: 2.5 g, Cu: 50–120 mg, Ni: 0.5 mg).

5.1. Development of the preconcentration step

Recently, we evaluated the on-line CPE preconcentration of dysprosium in urine with a simple FI system, which was easily hyphenated to ICP–OES with pneumatic nebulization [26]. In the present paper, our research aim was to expand the potentiality of this automated preconcentration system by studying the feasibility to couple an on-line CPE system for Dy to capillary electrophoresis, able to give baseline separation of the lanthanide from other urine's components with good resolution, reproducibility and accuracy compatible with pharmaceutical analysis.

The preconcentration system 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 to form 5-Br-PADAP complexes shown by these elements.

The effect of several experimental parameters upon the extraction parameters and sensitivity have been thoroughly evaluated and optimized.

The retention conditions of the metal complexes were optimized and dysprosium and iron signals were monitored by CE while changing the pH of the sample solution that passes through the micro-column. The optimal pH values were in the 8.5–10.0 range, in accordance with the optimal complex formation pH range. Considering these results, the selected pH was 9.20.

The minimum reagent to metal ion molar ratios for each analyte necessary to reach the optimum response was 10 for Dy and 8 for Fe. Above such excess, no variation on the analytical response was observed.

5.1.1. Surfactant concentration

The effect of PONPE 7.5 concentration upon sensitivity and extraction parameters was studied within the surfactant concentration range 0.1–0.8 % (w/w). Quantitative Dy and Fe extractions were observed for an amphiphile concentration higher than 0.20% (w/w). In order to achieve a good preconcentration factor, 0.25 % (w/w) was chosen as optimal.

5.1.2. Collection column

A home-made column packed with suitable filtering material was employed to carry out phase separation. Commercial cotton proved to be highly efficient at retaining the surfactant rich-phase. In accordance with previous work [25], the internal diameter of the microcolumn was set at 3 mm. The column length was varied within the range of 20–100 mm, allowing the optimal retention for column lengths >30 mm. A column length of 30 mm was chosen.

5.1.3. Loading and elution

The flow rate sample through the microcolumn is a very important parameter, since this is one of the steps that controls the time of analysis. We could verify that with flow-rates up to 8 ml min^{-1} there is no effect on the analyte recovery, while at higher loading flow-rates the recovery decreases.

In order to elute the surfactant-rich phase retained on the column, acetonitrile was used as the eluent. The analyte was completely eluted from the cotton with 50 μl acetonitrile.

5.2. Development of the separation conditions

In order to propose a specific and accurate way of analyzing CPE-preconcentrated urine containing Dy by using capillary zone electrophoresis, it is essential to find the best experimental conditions in which the analytes can be separated from each other. The optimization was performed using a normal urine sample spiked with a known amount of Dy. Urine's iron content was previously determined by ICP–OES. The following parameters were consecutively optimized: sample conditioning, pH, BGE composition and concentration, sample and capillary temperatures, and other electrophoretic parameters such as separation voltage, injection mode and length, etc.

5.2.1. Effect of pH

The buffer pH plays an important role for improving selectivity in CE especially for closely related compounds, because it affects both the overall charges of the solute and the electroosmotic flow (EOF). The effect of the buffer pH was investigated within the range of 6.0–10.0 at a fixed buffer concentration, adjusted by 0.1 mol l^{-1} NaOH and 0.1 mol l^{-1} HCl. It was found that when the pH was increased, resolution also increased, while time analysis decreased. At pH 9.00 baseline separation was achieved.

5.2.2. Effect of buffer composition and concentration

Buffer concentration has also a significant effect on the separation performance through its influence on the EOF and the current produced in the capillary. Different BGEs have been tested, but the one producing the best results considering selectivity, reproducibility, baseline and current performance, was sodium tetraborate containing acetonitrile, pH 9.00. Keeping other parameters constant (pH: 9.00, 25 kV, 25 °C) the buffer concentration was varied from 5–75 mM. Increases in migration times as well 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, Dy and Fe were not completely separated.

It has been reported that organic modifiers are very important to improve separation in many systems [12]. Acetonitrile was used as an organic modifier to enhance the resolution. Various amounts of acetonitrile (5, 10 and 15% (v/v)) were added into the 20 mM sodium sodium tetraborate buffer, pH 9.00. The compounds were baseline separated when 13% (v/v) of acetonitrile was added. So, a 20 mM sodium sodium tetraborate buffer containing 13% acetonitrile, pH 9.00 was chosen as the BGE as it gave a full separation of the analytes of interest in under 9 min. Separa-

tion of the analytes under optimal conditions is shown in Fig. 2.

5.2.3. Injection

The injection mode giving the best response concerning reproducibility and linear range was the hydrodynamic mode, while the electrokinetic mode gave a slightly greater sensitivity. Injection parameters were optimized by varying the lengths of sample (3–7 s) and pressure injection until optimum conditions were reached. The best results were obtained for the following experimental parameters: hydrodynamic injection mode 0.5 psi, 5 s.

Due to the high viscosity of the sample, buffer contamination caused by adsorption of surfactant onto the outer wall of the capillary was observed with consequent loss of separation efficiency and reproducibility. To avoid such effects, the anodic side of the capillary was immersed in ethanol during 2 s immediately after sample injection.

5.3. Separation performance evaluation of the combined methodology

Fig. 2 shows the sample solution electropherograms obtained using the optimized experimental conditions. The retention times of Dy and Fe were found to be 8.79 and 8.89 min, respectively. Acetone was used as an EOF marker. These migration times did not vary to any considerable degree during and in between analyses (%R.S.D. <1% for the retention time of each peak). Resolution of the Dy from Fe was 4.73. The analytes under study were baseline separated in <10 min, giving separation efficiencies of 88,267 average experimental electrophoretic plates (*N*) (Fig. 3).

An extraction percentage higher than 99.9% was achieved when the procedure was carried out under the optimal experimental conditions. Consequently, the enrichment factor achieved for this system was 200-fold.

The limits of detection of the analytes for the preconcentration of 10 ml of urine, based on a signal-to-noise ratio of 3 were 0.20 $\mu g\,l^{-1}$ for Dy and 0.48 $\mu g\,l^{-1}$ for Fe. This represents an improvement in detection limits for lanthanides up to 1200-fold by CE over previously published methods [30]. An enrichment factor of 200-fold was obtained for the preconcentration of a 10 ml sample urine. The calibration graphs using the preconcentration system were linear with a correlation coefficient of 0.9989 (Dy) and 0.9976 (Fe) at levels near the detection limits up to at least 500 $\mu g\,l^{-1}$.

Once the conditions for extraction, separation and quantification were established, the CPE–CE method was applied for the determination of dysprosium and iron in human urine (Fig. 2). In order to develop recovery studies the following

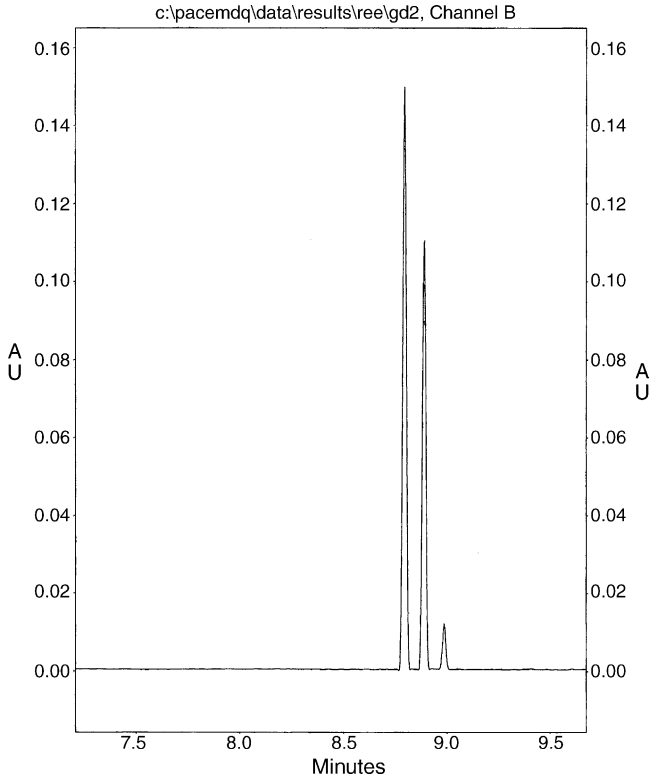

Fig. 2. Electropherogram of an iron–dysprosium spiked aqueous solution. Conditions: 20 mM sodium sodium tetraborate buffer containing 13% acetonitrile, pH 9.00, capillary: 57 cm total length, 50 cm effective length, 75 μm i.d., 375 μm o.d., hydrodynamic injection mode 0.5 psi, 5 s, 25 kV constant voltage, detection by direct UV–vis absorbance at 585 nm.

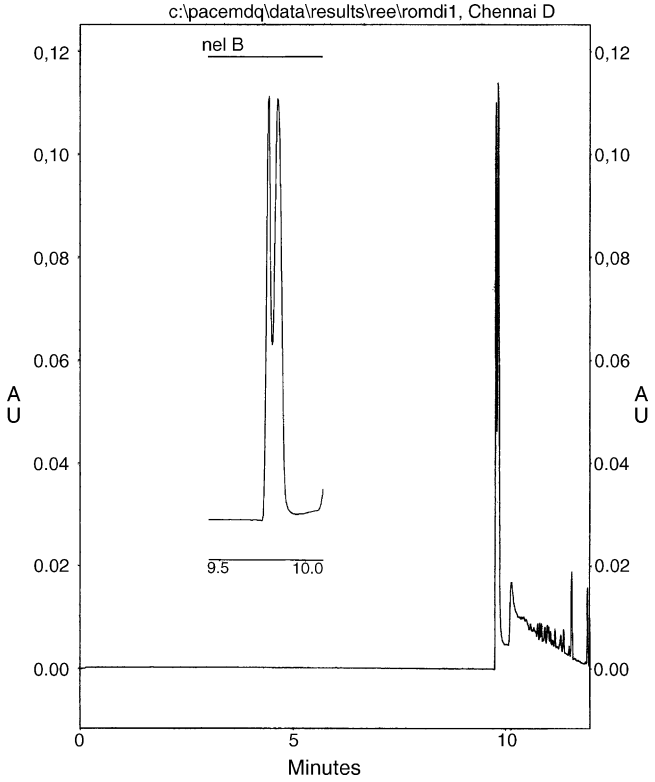

Fig. 3. Electropherogram of normal human urine spiked with dysprosium. Conditions: 20 mM sodium sodium tetraborate buffer containing 13% acetonitrile, pH 9.00, capillary: 57 cm total length, 50 cm effective length, 75 μm i.d., 375 μm o.d., hydrodynamic injection mode 0.5 psi, 5 s, 25 kV constant voltage, detection by direct UV–vis absorbance at 585 nm.

Table 1
Recovery experiments

	Base value ($\mu g\,l^{-1}$)	Quantity added ($\mu g\,l^{-1}$)	Quantity found[a] ($\mu g\,l^{-1}$)	Recovery (%)[b]
Aliquot I				
Dy	–	2.00	1.98	99.00
Fe	5.80	0.00	5.79	–
Aliquot II				
Dy	0.00	0.00	0.00	0.00
Fe	5.80	10.00	15.81	100.10
Aliquot III				
Dy	0.00	12.00	12.25	102.08
Fe	5.80	20.00	25.70	99.50
Aliquot IV				
Dy	0.00	22.00	21.98	99.90
Fe	5.80	40.00	45.88	100.20

[a] Mean value ($n = 6$).
[b] [(Found-base)/added] × 100.

experiment was carried out: 100 ml of urine samples were collected and divided into 10 portions of 10 ml each. The proposed method was applied to four portions, and the average quantity and iron was taken as a base value. The content of dysprosium was under the quantification limit of this method. The, increasing quantities of dysprosium and iron were added to the other aliquots. The results are given in Table 1. The recovery obtained (99.00–102.08%) was highly satisfactory.

6. Conclusions

The results for this work demonstrated for the first time the feasibility of coupling an on-line cloud point preconcentration step to capillary zone electrophoresis. Substantial improvements were attained with the proposed method compared to published reports. The recommended procedure presented herein was found to be easily applicable to the analysis of human urine, providing a sensitive method for the determination of dysprosium and other metal ions with minimal sample handling and high reproducibility.

The basic research involved in improving the pharmaceutical quality control of these lanthanide chelates-based RMI contrast agents should contribute to our understanding in other areas such as the relationships among structure, thermodynamic stability, kinetic liability, water solubility and relaxivity.

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