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Research Article

Capillary electrophoresis–electrochemiluminescence detection method for the analysis of ibandronate in drug formulations and human urine

A simple, rapid and sensitive CE method coupled with electrochemiluminescence (ECL) detection for direct analysis of ibandronate (IBAN) has been developed. Using a buffer solution of 20 mM sodium phosphate (pH 9.0) and a voltage of 13.5 kV, separation of IBAN in a 30-cm length capillary was achieved in 3 min. ECL detection was performed with an indium tin oxide working electrode bias at 1.6 V (versus a Pt wire reference) in a 200-mM sodium phosphate buffer (pH 8.0) containing 3.5 mM $Ru(bpy)_3^{2+}$ (where bpy = 2,2′-bipyridyl). Derivatization of IBAN prior to CE-ECL analysis was not needed. Linear correlation ($r = 0.9992$, $n = 7$) between ECL intensity and analyte concentration was obtained in the range of 0.25–50 μM IBAN. The LOD of IBAN in water was 0.08 μM. The developed method was applied to the analysis of IBAN in a drug formulation and human urine sample. SPE using magnetic Fe_3O_4@Al_2O_3 nanoparticles as the extraction phase was employed to pretreat the urine sample before CE-ECL analysis. The linear range was 0.2–12.0 μM IBAN in human urine ($r = 0.9974$, $n = 6$). The LOD of IBAN in urine was 0.06 μM. Total analysis time including sample preparation was <1 h.

Keywords:
CE / Electrochemiluminescence detection / Ibandronate / Magnetic SPE / Urine analysis
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1 Introduction

Ibandronate sodium ([1-hydroxy-3-(methylpentylamino)propylidene]bisphosphonic acid monosodium; IBAN) (Fig. 1), a relatively new nitrogen-containing bisphosphonate drug, is a third-generation bisphosphonate for the treatment of bone disease such as malignant hypercalcemia and postmenopausal osteoporosis [1, 2]. Its once-monthly oral and quarterly intravenous (i.v.) dosage regimens have the potential to improve treatment adherence and persistence, and hence clinical outcomes, compared with other frequently administered oral bisphosphonates. Like other bisphosphonates, IBAN inhibits osteoclast-mediated bone resorption. Due to the stability of the P-C-P backbone and their polar nature, IBAN does not undergo metabolic biotransformation following oral administration and i.v. injection. It has been reported that, in a mass balance study, nearly the entire oral IBAN dose was excreted unchanged in the feces, and the majority of i.v. IBAN was excreted unchanged in the urine within 24 h [3]. For comprehensive studies of the pharmacology and pharmacodynamic effects of IBAN in biological matrices, a simple, sensitive and accurate assay is generally required.

Analysis of IBAN represents a challenge because of its high polarity and low volatility. Furthermore, IBAN possesses no chromophore or fluorophore, and its tertiary amine characteristic renders derivatization difficult. Analytical methods for IBAN reported to date are therefore limited. A highly sensitive GC-MS assay for IBAN in serum/plasma and urine was first described by Endele et al. [4]. The quantification limits could reach low ng/mL level. However, sample preparation was lengthy and tedious. Besides, in order to lower IBAN's polarity and enhance its volatility, derivatization into methyl ester prior to GC separation was required. A simple, non-derivatization detection method, evaporative light-scattering detection (ELSD), coupled with ion-pair HPLC analysis of IBAN was reported by Jiang and Xie [5]. The main drawback of this method is its relatively high LOD of IBAN (176 μg/mL). A post-column photochemical derivatization followed by sensitive fluorometric detection for HPLC determination of several bisphosphonate drugs including IBAN in human urine has been reported by Pérez-Ruiz et al. [6]. Detection was based on their oxidation to orthophosphate by the on-line peroxydisulfate-assisted

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Abbreviations: CV, cyclic voltammetry; **ECL,** electrochemiluminescence; **Fe_3O_4@Al_2O_3 NPs,** alumina-coated iron oxide nanoparticles; **IBAN,** ibandronate; **ITO,** indium tin oxide; **$Ru(bpy)_3^{2+}$,** tris(2,2′-bipyridyl)ruthenium(II)

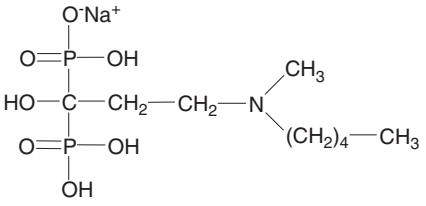

Figure 1. Molecular structure of ibandronate sodium.

photolysis followed by post-column reaction with molybdate to yield phosphomolybdate. This subsequently reacted with thiamine to generate thiochrome and, finally, the fluorescence of thiochrome was measured. This detection strategy is in the nature of indirect detection mode. The LOD of IBAN was 72 nM (~23 ng/mL). A high-throughput HPLC-MS/MS method for determining IBAN in human plasma has been developed by Tarcomnicu et al. [7]. Liquid–liquid extraction and methylation derivatization were used in sample preparation. The LOQ was 0.2 ng/mL. Recently, a simple ion chromatography (IC) method for the simultaneous determination of IBAN drug and its impurities has been reported by Narendra Kumar et al. [8]. The LOD of IBAN was 38 μg/mL. ELISA, the most sensitive method for IBAN to date with a LOD at pg/mL level, also has been reported [4], but its reagents were not readily available.

CE has been a popular technique for drug analysis because of its high efficiency and versatility, very low sample and reagent consumption, low cost and minimization of environmental pollution. However, to our knowledge, there has been no CE method reported for the analysis of IBAN yet, which might be attributed to the lack of sensitive detection method for IBAN. There was a report on the use of CE-indirect UV detection for analyzing IBAN-related impurities, i.e. phosphyte and phosphate, but IBAN itself was not determined [9]. In recent years, tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$)-based electrochemiluminescence (ECL) has become an important and powerful detection method for CE due to its high sensitivity and simple instrumental setup [10]. It is known that amines, particularly tertiary amines, can be directly determined by utilizing their ECL reaction with electrogenerated $Ru(bpy)_3^{3+}$ without the need of derivatization [11, 12]. Since IBAN structurally belongs to a tertiary amine, we expect that it should be feasible to analyze IBAN with the CE-ECL method.

In this paper, we describe a simple, rapid and sensitive CE method coupled with $Ru(bpy)_3^{2+}$-ECL detection for the analysis of non-derivatized IBAN. The electrochemical and ECL behavior of IBAN and the optimal CE separation and ECL detection conditions were investigated. The applicability of this method to the analysis of IBAN in drug formulation and human urine was then examined. In order to remove the interference from urine matrix and to enrich the analyte, SPE using magnetic alumina-coated iron oxide nanoparticles (Fe_3O_4@Al_2O_3 NPs) as the extraction phase [13, 14] was employed to the pretreatment of urine sample. Report on the use of magnetic SPE for the separation of drugs from biological matrices has been scarce [13]. Application of this technique to the extraction of phosphate-bearing drugs has not been reported either. We believe this is the first report on both direct CE-ECL analysis and magnetic SPE with Fe_3O_4@Al_2O_3 NPs for IBAN.

2 Materials and methods

2.1 Apparatus

Cyclic voltammetry (CV) and ECL studies were performed with a CHI model 635 electrochemical station (Austin, TX, USA). A three-electrode cell with an indium tin oxide (ITO) working electrode (Delta Technologies, Stillwater, MN, USA), an Ag/AgCl/saturated KCl reference and a Pt wire auxiliary electrode was used for electrochemical measurements. Optical signals were captured using a Hamamatsu R928 PMT (Hamamatsu City, Japan), positioned in front of the ITO electrode and biased at −900 V.

The laboratory-assembled CE-ECL system was similar to that described previously [15]. Separation capillaries (Polymicro Technologies, Phoenix, AZ, USA) were of 50 μm id × 360 μm od × 30 cm length. Prior to use, the capillary was flushed sequentially with 0.5 M NaOH (10 min), H_2O (10 min) and electrophoretic buffer (15 min). Following each five injections, the capillary was rinsed with 0.1 M NaOH, H_2O and electrophoretic buffer for 5 min to maintain a reproducible inner surface. An ITO electrode, situated at the capillary outlet and biased at 1.6 V (versus a Pt-wire reference), was used for in situ generation of the active $Ru(bpy)_3^{3+}$. Sample injection was carried out hydrodynamically for 20 s at 10 cm height.

2.2 Chemicals

IBAN sodium (≥97%) and tris(2,2′-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3Cl_2$, 98%) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Sodium tripolyphosphate ($Na_5P_3O_{10}$) was obtained from Showa (Tokyo, Japan). Iron oxide (Fe_3O_4, particle size 20–30 nm) and sodium silicate were bought from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were of analytical-reagent grade. All solutions were filtered through a 0.45-μm pore-size membrane filter before use.

2.3 Urine sample preparation

The fresh human urine sample of a healthy female volunteer was acquired from a student in the laboratory. Prior to analysis, 1.0 mL urine was added with 50 μL of 0.1 M NaOH (as blank) or a standard solution of IBAN in 0.1 M NaOH (as sample) and 50 μL of 1.0 M EDTA. The solution was thoroughly mixed, followed by ultrafiltration at

14 000 × *g* for 20 min using a microcentrifuge (model 3740; Kubota, Tokyo, Japan) coupled with Millipore's Amicon® Ultra-0.5 centrifugal filter devices (nominal molecular weight limit 3 kDa). The filtrate was then subjected to magnetic SPE using Fe_3O_4@Al_2O_3 NPs as the solid phase. Preparation of the Fe_3O_4@Al_2O_3 NPs has been described previously [14]. In a 2.0-mL polypropylene vial, 1.0 mL of the above filtrate was ultrasonically mixed with 1 mg of Fe_3O_4@Al_2O_3 NPs for 5 min. The NPs that conjugated with IBAN were then aggregated by an external magnet, and the supernatant liquid was completely removed with a micropipette. The NPs were ultrasonically washed with 0.5 mL of 150 mM phosphate buffer solution (pH 9.0) for 5 min, and the supernatant liquid was discarded. This washing step was repeated twice more. Finally, IBAN on the NPs was rinsed off with 10 μL of 20 mM $Na_5P_3O_{10}$ solution (pH 9.0). The eluate was directly injected into the CE-ECL system for analysis. Due to the concern of possible contamination to the next sample, the used NPs were discarded without further cleaning.

3 Results and discussion

3.1 Electrochemical and ECL behavior of IBAN on an ITO electrode

The electrochemical and $Ru(bpy)_3^{2+}$-based ECL behavior of IBAN in aqueous solution was first studied using CV and an ITO electrode in a three-electrode cell. Figure 2A illustrates the cyclic voltammogram obtained in a 20-mM sodium phosphate buffer at pH 8.0 (curve a; dotted line). This background voltammogram is featureless in the potential range scanned (0–1.6 V). With the addition of 5 μM $Ru(bpy)_3^{2+}$, the well-known reversible $Ru^{2+/3+}$ redox waves appear at ~1.1 V (curve b; thin line). In the presence of 0.15 mM IBAN, the anodic wave for $Ru(bpy)_3^{2+}$ oxidation at ~1.1 V is significantly enhanced (curve c; thick line). The catalytic current enhancement was found to be proportional to the concentration of IBAN in the solution. The redox waves of IBAN itself were not observed in the potential range scanned. The corresponding ECL-potential curves obtained during CV scan are illustrated in Fig. 2B. There is no ECL emission in the background electrolyte (curve a in inset). In the presence of 5 μM $Ru(bpy)_3^{2+}$, weak ECL emission emerges following the oxidation of $Ru(bpy)_3^{2+}$ and reaches a maximum at ~1.4 V (curve b in inset). With the addition of 0.15 mM IBAN, significant enhancement (~100-fold) of ECL emission results (curve c), which evidences the electrocatalytic effect of IBAN on the $Ru(bpy)_3^{2+}$-based ECL. Since IBAN is a tertiary amine, its electrochemical and ECL behavior in the $Ru(bpy)_3^{2+}$ solution should be similar to that of $Ru(bpy)_3^{2+}$/tripropylamine system [16], and the mechanism of ECL reaction between $Ru(bpy)_3^{2+}$ and tertiary amine has been well-known [11]. Judging from the above results, $Ru(bpy)_3^{2+}$-based ECL can be utilized as a sensitive tool for direct detection of IBAN.

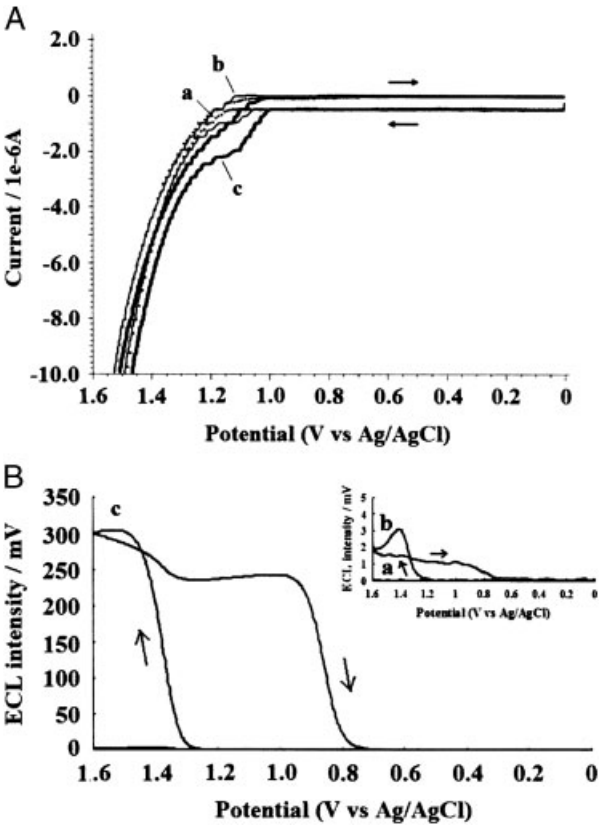

Figure 2. (A) Cyclic voltammograms of 20 mM sodium phosphate, pH 8.0 (curve a), 5 μM $Ru(bpy)_3^{2+}$ in phosphate buffer (curve b) and 0.15 mM IBAN in phosphate buffer containing 5 μM $Ru(bpy)_3^{2+}$ (curve c). (B) Corresponding ECL-potential curves of (A). Conditions: working electrode, ITO (0.25 cm^2); reference electrode, Ag/AgCl/saturated KCl; counter electrode, Pt wire; potential scan rate, 50 mV/s; PMT voltage, −900 V.

3.2 Optimization of CE-ECL procedure

3.2.1 Effect of detection potential

In the present work, a simple two-electrode cell with an ITO working electrode and a Pt wire pseudo-reference was used as the ECL detector for CE. The optimal detection potential was investigated by repeated injection of 10 μM IBAN standard into CE while varying the applied ITO electrode potential in the range 1.0–2.0 V. The ECL intensity started to increase from 1.2 V with increasing detection potential and reached a plateau at ~1.6 V (data not shown). The optimal detection potential for IBAN was therefore set at 1.6 V. This potential correlates well with the ECL result shown in curve c of Fig. 2B.

3.2.2 Effect of $Ru(bpy)_3^{2+}$ concentration in the detection cell

The variation of ECL intensities on the concentrations of $Ru(bpy)_3^{2+}$ in the range of 1–5 mM was examined next. As

expected, the intensity of ECL emission increased with increasing $Ru(bpy)_3^{2+}$ concentration in the detection cell, but the background noise also increased with higher concentration of $Ru(bpy)_3^{2+}$. The S/N ratio of ECL began to deteriorate after reaching the maximum at a $Ru(bpy)_3^{2+}$ concentration of 3.5 mM. Therefore, 3.5 mM $Ru(bpy)_3^{2+}$ was chosen as the optimum.

3.2.3 Effect of buffer pH

The effect of buffer pH on the CE of IBAN was examined in the pH range of 7.5–10. Using 20 mM sodium phosphate as the electrophoretic buffer, the optimal migration time and peak shape were found at pH 9.0. It is known that ECL intensity for the reaction of $Ru(bpy)_3^{2+}$ and tertiary amine is pH-dependent, and the optimal range is about pH 7–8 [17]. In the present study, we found that the optimum pH for ECL detection of IBAN was 8.0. Since the CE buffer (pH 9.0) in capillary continuously flowed into the detection cell during analysis, in order to offset the possible pH variation in the detector caused by CE eluate, a 200-mM sodium phosphate buffer at pH 8.0 was used in the ECL detection cell. This buffer solution was replaced with fresh one every 4 h to keep the pH value constant.

3.2.4 Effect of injection time

In the present study, sample injection was performed hydrodynamically by immersing the capillary inlet in the sample solution at 10 cm height relative to the outlet of the capillary for a fixed time period. The effect of injection time on the ECL intensity of IBAN peak was examined in the range of 5–40 s. The ECL intensity increased with increasing injection time. However, the efficiency, calculated as the number of theoretical plates, N, decreased with lengthy injection time. An injection time of 20 s was judged optimal, as a compromise between detection sensitivity and CE efficiency.

Figure 3 illustrates a typical electropherogram of IBAN obtained with ECL detection under the optimal experimental conditions described above. Sharp and symmetric peak could be observed. The number of theoretical plates, N, calculated from $N = 5.55 \times (t_R/w_{h/2})^2$, was 1.05×10^4. Complete elution of IBAN was achieved in about 2.8 min with a capillary of 30 cm length. There is an elution band appeared at about 0.2 min in Fig. 3. Although this band showed up in most of the electropherograms obtained in this work, its cause is not clear at present. The small peak emerged at 1.3 min is probably due to impure constituent in the commercial IBAN chemical (labeled purity $\geq$97%) used for the preparation of standard solution.

3.3 Analytical performance characteristics

Calibration graph was constructed for IBAN in the concentration range 0.25–50 μM. The linear regression equation was $y = 2.14(\pm 0.17) \times 10^3 x - 0.19(\pm 0.02) \times 10^3$ ($r = 0.9992$, $n = 7$), where y is the peak area and x is the concentration (μM) of IBAN. The LOD ($S/N = 3$) and LOQ ($S/N = 10$) for IBAN in water were 0.08 and 0.25 μM, respectively, calculated from the signal height (0.348 mV) of 0.25 μM IBAN and the mean noise level (0.035 mV) at 2.8 min on the electropherograms of blank solution.

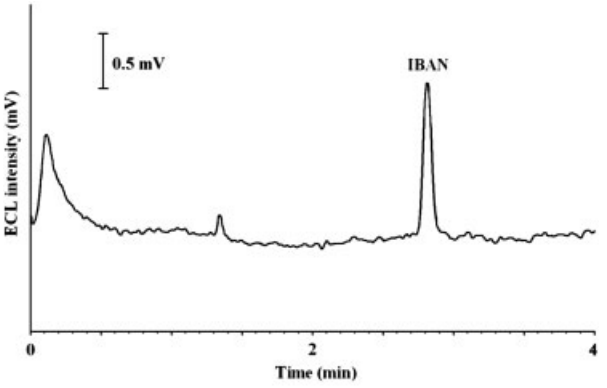

Figure 3. Electropherogram of IBAN obtained with ECL detection. Conditions: capillary size, 50 μm id × 360 μm od × 30 cm length; anodic buffer, 20 mM sodium phosphate (pH 9.0); cathodic buffer, 200 mM sodium phosphate (pH 8.0) containing 3.5 mM $Ru(bpy)_3^{2+}$; CE voltage, 13.5 kV; hydrodynamic injection, 20 s at 10 cm height; ITO potential, 1.6 V; PMT voltage, –900 V. IBAN concentration: 1 μM.

Table 1. Intra-day and inter-day precisions of migration time and peak area for IBAN at different concentrations ($n = 5$)

Concentration (μM)	RSD (%)			
	Migration time		Peak area	
	Intra-day	Inter-day	Intra-day	Inter-day
0.5	0.5	0.7	5.1	7.3
2.5	0.6	0.7	3.9	5.8
20	0.4	0.8	3.1	4.4

The intra-day precision of the method was examined by repeated injection of three standard IBAN solutions at low (0.5 μM), medium (2.5 μM) and high (20 μM) concentrations for five times on the same day. The inter-day precision was studied by analyzing the three standard samples one time each day for five consecutive days. The RSD values of the migration time and the ECL peak area are summarized in Table 1. The intra-day RSDs for migration time and peak area were $<$0.6 and 5.1%, respectively. The inter-day RSDs for migration time and peak area were $<$0.8 and 7.3%, respectively.

4 Applications

4.1 Analysis of drug formulation

The applicability of the developed CE-ECL method was first examined by analyzing an i.v. formulation of IBAN (Bonviva®)

from Roche (Switzerland). Owing to its relatively simple matrix, the only pretreatment performed was a 350-fold dilution with the CE buffer. Figure 4 shows the typical electropherogram obtained for IBAN in diluted Bonviva®. A single and sharp peak was observed. Based on triplicate analyses, the IBAN content in Bonviva® was found to be 1003 ± 75 μg/mL ($n = 3$), which agrees well with manufacturer's certified value (3 mg ibandronic acid per 3 mL solution for i.v. injection).

4.2 Analysis of human urine

In order to assess the applicability of the method for biological or clinical analysis, IBAN-spiked human urine was used as the test sample. Due to the complex matrix of human urine, direct CE-ECL analysis of IBAN in urine was difficult. Tedious and time-consuming pretreatment of biological materials was always involved prior to IBAN analysis [4, 6, 7]. In this study, we attempted to develop a simple SPE procedure for IBAN in urine sample. SPE using magnetic Fe_3O_4@Al_2O_3 NPs has been a popular technique for selective enrichment of phosphate-bearing biomolecules, e.g. phosphopeptides and phosphoproteins, from complex biological samples [18, 19]. We had also efficiently extracted a phosphate-bearing herbicide, glyphosate, from aqueous samples with SPE using Fe_3O_4@Al_2O_3 NPs as the solid phase [14]. The high specificity is due to a bidentate binding nature between the phosphate group and metal oxide surface [20]. The paramagnetic properties of these particles also allow an easy isolation of products in solution by attracting them with the aid of an external magnetic field. In addition, magnetic NPs possess large surface area which facilitates SPE. Since IBAN is a bisphosphonate compound, a specific interaction between IBAN and Fe_3O_4@Al_2O_3 NPs should also exist. A magnetic SPE procedure for IBAN in urine was therefore developed, as described in Section 2.3. This procedure was modified from our previous tactics of extracting the uniphosphonate herbicide, glyphosate, from aqueous sample with Fe_3O_4@Al_2O_3 NPs [14]. Instead of using $Na_4P_2O_7$ solution as the eluting agent, IBAN attached on the magnetic NPs was rinsed off with $Na_5P_3O_{10}$ solution. This modification was necessary because $P_3O_{10}^{5-}$ revealed a higher eluting strength than $P_2O_7^{3-}$ for IBAN. The optimal concentration of $Na_5P_3O_{10}$ was found to be 20 mM. Figure 5(A) shows the typical electropherogram of a urine sample spiked with 0.5 μM IBAN and subjected to ultrafiltration only. Despite the selectivity of $Ru(bpy)_3^{2+}$-ECL detection, large extraneous peaks appear in the background, indicating that it is not possible to remove the interfering components in urine matrix by a single ultrafiltration pretreatment. Accurate determination of IBAN signal size would be difficult because the analyte peak situated very close to the huge extraneous peaks. Furthermore, the stability of CE capillary as well as the sensitivity of ITO electrode in the detection cell deteriorated rapidly with consecutive injections of urine sample, probably caused by fouling of the capillary wall and electrode surface. The electropherogram of the same urine sample subjected to ultrafiltration followed by SPE with Fe_3O_4@Al_2O_3 NPs is illustrated in Fig. 5B. In comparison with Fig. 5A, those large matrix peaks totally disappear and just a single IBAN peak shows up. Besides, the peak size of IBAN in Fig. 5B is significantly larger than that in Fig. 5A, which evidences the efficiency and specificity of SPE with Fe_3O_4@Al_2O_3 NPs for IBAN. The slight spreading of peak shape and longer migration time of IBAN observed in Fig. 5B, compared with Figs 3 and 4, may be due to conductivity and/or pH differences between injected analyte zone and CE background electrolyte [21].

Calibration graph was constructed using IBAN-spiked human urine in the concentration range of 0.2–12.0 μM. The linear regression equation was $y = 11.43(\pm 0.67) \times 10^3 x - 0.99(\pm 0.12) \times 10^3$ ($r = 0.9974$, $n = 6$, where y is the peak area and x is the IBAN concentration in μM). The LOD and LOQ for IBAN in urine were 0.06 and 0.2 μM (19 and 64 ng/mL), respectively. As a comparison, Table 2 lists the reported LOD and/or LOQ values of IBAN in various sample matrices by different analytical methods. The LOD/LOQ values obtained with the developed CE-ECL method

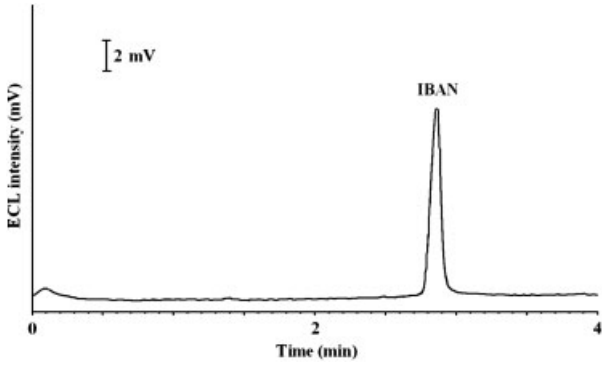

Figure 4. Electropherogram of an intravenous formulation of IBAN (350-fold diluted Bonviva®). Conditions as in Fig. 3.

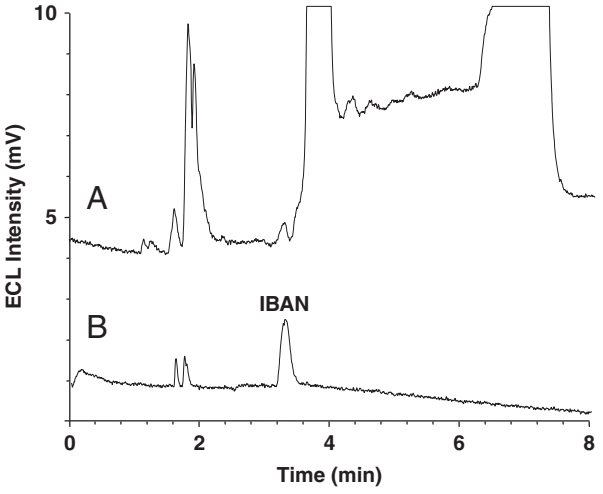

Figure 5. Electropherograms of human urine sample containing 0.5 μM IBAN. (A) Sample pretreatment with ultrafiltration only. (B) Sample pretreatment with ultrafiltration followed by SPE using magnetic Fe_3O_4@Al_2O_3 NPs as the solid phase. Other conditions as in Fig. 3.

Table 2. Comparison of reported LOD/LOQ values of IBAN by various analytical methods

Method	Sample	LOD/LOQ (ng/mL)	Reference
GC-MS[a)]	Plasma	–/1	4
	Urine	–/2	
ELISA	Serum	–/0.05	4
HPLC-MS/MS[a)]	Plasma	–/0.2	7
HPLC-ELSD	Water	176 000/–	5
IC-conductometry	Drug	38 000/113 000	8
HPLC-fluorometry[a)]	Water	23/–	6
CE-ECL	Urine	19/64	This work

a) Derivatization required.

are more than three orders of magnitude lower than those of HPLC-ELSD [5] and IC conductometry [8] methods, and are similar to that of the technically complicated HPLC-photochemical derivatization-fluorometric detection method [6]. Although GC-MS [4] and HPLC-MS/MS [7] methods could provide lower ng/mL level LOQ for IBAN in biological fluids, both analyte derivatization and high-cost instrumentation were required. ELISA is the most sensitive and selective method for IBAN reported to date. However, its specific reagent is not readily available.

The intra-day and inter-day precisions of the method were evaluated using urine samples spiked with 0.5 and 5.0 μM IBAN. The intra-day RSDs ($n = 5$) for 0.5 and 5 μM IBAN were 7.7 and 4.6%, respectively. The inter-day RSDs ($n = 5$) for 0.5 and 5 μM IBAN were 9.4 and 6.1%, respectively. Mean recoveries, calculated as the ratio of IBAN concentration found to that originally spiked, for 0.5 and 5 μM IBAN were 102 and 97% ($n = 5$), respectively. Total analysis time for each urine sample including pretreatment, SPE and CE-ECL was <1 h.

5 Concluding remarks

A simple, rapid and sensitive CE-ECL method for the analysis of IBAN has been developed. Compared with most chromatographic methods for IBAN reported to date, derivatization of the analyte prior to CE-ECL analysis is not necessary. High-cost instrumentation is also not required. The feasibility of efficient and accurate determination of IBAN in drug formulation by the new method was demonstrated. In couple with SPE pretreatment using magnetic Fe_3O_4@Al_2O_3 NPs as solid phase, the proposed method was successfully applied to the determination of IBAN at sub-μM level in human urine. The novel magnetic SPE procedure developed for IBAN in urine sample was simpler and faster than other reported pretreatment methods. Combining the selectivity of $Ru(bpy)_3^{2+}$-ECL detection and the specificity of Fe_3O_4@Al_2O_3 NPs extraction, this method seems particularly suitable for the analysis of phosphate-containing amines in biological and environmental materials. To our knowledge, this is the first report on the development of both CE-ECL method and magnetic SPE pretreatment for trace IBAN in biological sample. Research on the development of CE-ECL method for other structurally similar bisphosphonate drugs is in progress.

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The authors have declared no conflict of interest.

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