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Determination of ranitidine in urine by capillary electrophoresis-electrochemiluminescent detection

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Abstract

The fast analysis of ranitidine is of clinical importance in understanding its efficiency and a patient's treatment history. In this paper, a novel determination method for ranitidine based on capillary electrophoresis-electrochemiluminescence detection is described. The conditions affecting separation and detection were investigated in detail. End-column detection of ranitidine in 5 mM $Ru(bpy)_3^{2+}$ solution at applied voltage of 1.20 V was performed. Favorable ECL intensity with higher column efficiency was achieved by electrokinetic injection for 10 s at 10 kV. The R.S.D. values of ECL intensity and migration time were 6.38 and 1.84% for 10^{-4} M and 6.01 and 0.60% for 10^{-5} M, respectively. A detection limit of 7×10^{-8} M (S/N = 3) was achieved. The proposed method was applied satisfactorily to the determination of ranitidine in urine in 6 min.

Keywords: Capillary electrophoresis; Electrochemiluminescence; Ranitidine

1. Introduction

Ranitidine is H_2-receptor antagonist used in the treatment of gastric and duodenal ulcers and other related disorders by inhibiting the secretion of gastric acid [1]. Due to its critical role in the pharmaceutical industry, it is important to determine ranitidine in biological fluids. Among the analytical techniques, polarography [2], high-performance liquid chromatography (HPLC) [1,3–8], thin layer chromatography (TLC) [9] and supercritical fluid chromatography [10] have been used for the determination of ranitidine. Although TLC method could simultaneously determine several samples and the standard solution, the plate coating, chamber saturation with mobile phase, and spotting were time-consuming and cost effective. The post-column fluorescence derivatization procedure for HPLC showed a better selectivity than UV detection, but post-column dilution by the fluorescent reagent solution resulted in decrease in sensitivity. Other capillary electrophoresis methods were also employed for the assay of ranitidine in biological fluids [11–13], but the poor sensitivity of UV detection limited its application. Hence, it is essentially important to develop a method with high sensitivity and selectivity for ranitidine.

Here, the coupling of capillary electrophoresis-electrochemiluminescence method was employed to detect ranitidine in urine extracts. Electrochemiluminescence (ECL) reagent $Ru(bpy)^{2+}$ was put in the cell positioned at the capillary outlet; by applying a proper potential, ranitidine can react with ruthenium species and emit photons simultaneously. By measuring the intensity of the emitted light, we can get the corresponding concentration of the drug [14–24]. Compared with the previous report the combination of ECL with CE showed several advantages: easy to operate, high selectivity to the specific analyte, fast analysis speed, low reagent consumption, etc.

In this work, a fast and sensitive technique was developed to determine ranitidine in urine using $Ru(bpy)_3^{2+}$ ECL detection after CE separation.

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2. Experimental

2.1. Apparatus

The CE-ECL detection system (Xi'an Remax Electronics Co. Ltd., Xi'an, China) consisted of a high-voltage power supply for electrophoretic separation and electrokinetic injection, potential control system, chemiluminescence detector, and data processor. Fused-silica capillary (25 μm i.d, 360 μm o.d.) with a length of 42 cm between the inlet and the detector was obtained from Yongnian Optical Fabric Factory (Hebei, China). It was flushed with 0.1 M NaOH for 20 min, double-distilled water for 2 min, running buffer for 2 min, respectively before use every day. The construction of the ECL detection cell has been presented in a previously published work [17], in which a 500 μm diameter Pt disc electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode were situated. The cell was filled with 5 mM $Ru(bpy)_3^{2+}$ containing phosphate buffer, which was replenished every 3 h. ECL detection was carried out in an end-column mode. Twenty millimolars of phosphate was used as running buffer. A voltage of 15 kV was applied at two ends of the separation capillary. The inlet end of the capillary was held at a positive potential, and the outlet end was maintained at ground. The distance between the working electrode and the outlet of the capillary was set at 150 μm with the aid of an optical microscope. The ECL intensity was collected with a photomultiplier tube positioned under the detection cell, and 800 V of the potential biased the PMT was applied. Signals were processed with a data processor controlled by a computer. All experiments were carried out at room temperature.

2.2. Reagents

Ranitidine hydrochloride was obtained from Sigma–Aldrich (St. Louis, USA). Tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification. The buffer used throughout the study was sodium phosphate system (Na_2HPO_4/NaH_2PO_4). Chemicals and reagents used (dichloromethane, sodium hydroxide) were all of analytical reagent grade. A series of ranitidine and phosphate buffers with different concentrations was prepared with double-distilled water and filtered through a 0.22 μm membrane before use.

2.3. Extraction of ranitidine from urine sample

Ranitidine is primarily excreted via urine from a patient's body, and 60% or more may appear in urine without modification [1]. So, it is possible to determine the quantity of ranitidine in urine as well as to learn a patient's treatment history. Primary work indicated that if urine sample was directly injected into the capillary, proteins and other biomolecules present in the urine matrix could be adsorbed to the wall of the capillary [4] and could influence the separation of the drug. Thus, a pretreatment procedure was necessary to remove the large amounts of interferential compounds [17]. The pretreatment procedure used here was a modification of the technique reported by Shah et al. [9]. In brief, 1 ml of urine (drug spiked or volunteer urine samples) was alkalinized with 50 μl of 2 M sodium hydroxide solution, and 1 ml dichloromethane was added as the extraction solvent. The procedure was repeated three times; the extract was combined and evaporated at room temperature. The dry residue was prepared in water and filtered through a 0.22 μm membrane before use.

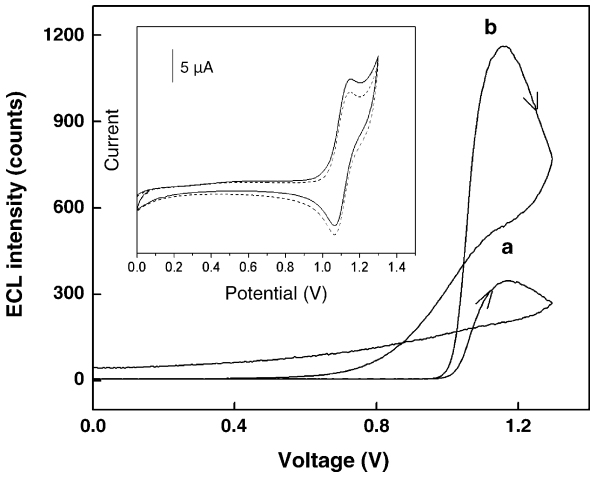

Fig. 1. Dependence of the ECL intensity on applied voltage curve of 5 mM $Ru(bpy)_3^{2+}$ without (a) and with (b) 10^{-5} M ranitidine. *Condition*: 50 mM phosphate buffer at pH 7.00 in the detection cell. Inset, cyclic voltammogram of 5 mM $Ru(bpy)_3^{2+}$ with (solid line) and without (dotted line) 10^{-4} M ranitidine in 50 mM phosphate buffer (pH 7.50) at a scan rate of 50 mV/s.

3. Results and discussion

3.1. Effect of detection voltage

Cyclic voltammetry (CV) was used to characterize the ECL behavior of ranitidine. As is shown in Fig. 1 inset, the well-known reversible CV wave of the ECL reaction of $Ru(bpy)_3^{2+}$ was observed with a peak voltage of 1.15 V (dotted line). In presence of ranitidine, rise in the anodic current and fall in the cathodal current, a marker of catalytic effect of $Ru(bpy)_3^{2+}$, were observed as the potentials were scanned to +1.15 and −1.07 V, respectively. Comparing the solid line with the dotted line, no obvious wave of oxidation was observed at voltages between 0 and 0.95 V where ranitidine was not oxidized before direct oxidation of $Ru(bpy)_3^{2+}$ at the electrode, which indicated that the peak current obtained in this system probably originated from the ECL reaction between analyte itself and ruthenium species. In Fig. 1, ECL intensity begins to increase rapidly at 1.00 V (Fig. 1a), and about 300 counts are displayed when the potential is close to 1.20 V. In a comparison, when ranitidine is added, the ECL intensity is about 1200 counts at 1.20 V (Fig. 1b). These observations indicated that ranitidine can react with the ruthenium species in the electrochemiluminescence process, and it can enhance the emitted light intensity.

We further investigated the relationship between ECL intensity and applied potential (Fig. 2). The results were correlated with the cyclic voltammetry experiments described above. At lower voltage, a very weak ECL response was obtained. Until at

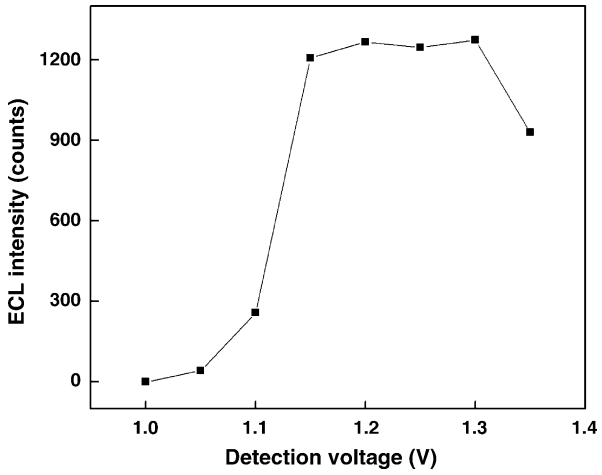

Fig. 2. Dependence of the ECL intensity on the detection potential. 10^{-4} M ranitidine aqueous solution, electrokinetic injection 8 s × 10 kV, 20 mM buffer at pH 7.00, 5 mM $Ru(bpy)_3^{2+}$ in the detection cell, separation potential 15 kV.

1.10 V, the increase in the ECL signal starts to be visible. Finally, the intensity curve reached a plateau with the most favorable detection potential at 1.20 V.

3.2. Detection conditions

$Ru(bpy)_3^{2+}$ was post-column added in our work; therefore, the ECL reaction was expected to occur when ranitidine met $Ru(bpy)_3^{2+}$ at the electrode surface. The concentration of $Ru(bpy)_3^{2+}$ may exert an effect on the detection sensitivity. Fig. 3 shows the ECL intensity changes as a function of $Ru(bpy)_3^{2+}$ concentration when ranitidine concentration was kept at a constant value of 2.5×10^{-5} M. In the range of 3–5 mM $Ru(bpy)_3^{2+}$, ECL intensity rose steadily with an increase in $Ru(bpy)_3^{2+}$ concentration. Beyond 5 mM, the ECL intensity was not enhanced any more, and background noise increased. The ECL intensity reached the maximum when the concentration of $Ru(bpy)_3^{2+}$ was 5 mM. Thus, 5 mM $Ru(bpy)_3^{2+}$ added in the cell was chosen for further experiment in light of sensitivity and stability.

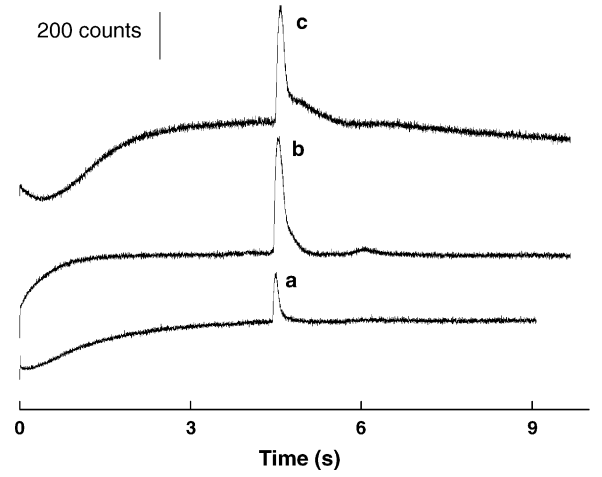

Fig. 3. Effect of $Ru(bpy)_3^{2+}$ concentration in 3 mM (a), 5 mM (b) and 10 mM (c) on ECL intensity. 2.5×10^{-5} M ranitidine aqueous solution, 20 mM running buffer at pH 7.00, 50 mM buffer in the detection cell, separation potential 15 kV.

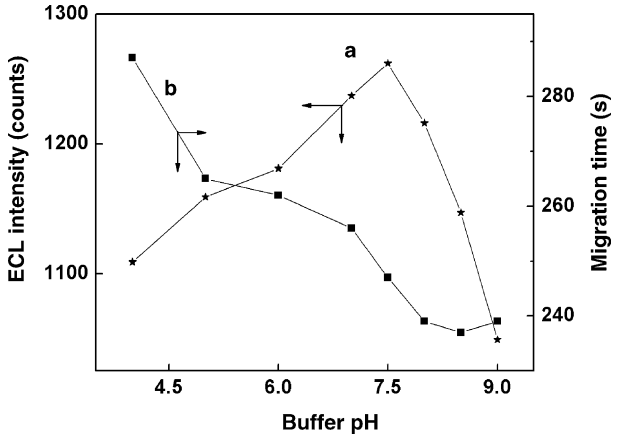

Fig. 4. Effect of the buffer pH on (a) ECL intensity and (b) migration time. 10^{-4} M ranitidine solution, separation voltage 15 kV, detection potential 1.20 V, 5 mM $Ru(bpy)_3^{2+}$, electrokinetic injection 8 s × 10 kV.

3.3. Buffer pH

The pH of detection buffer has a significant effect on the ECL response. The previous work indicated that $Ru(bpy)_3^{2+}$ has a good ECL efficiency in a weak basic solution because of the deprotonation of the amine species to form a reducing free radical intermediate and the reaction between radical intermediate and ruthenium species [14–19]. Also, the pH of separation buffer influences electroosmotic flow (EOF) and the analyte ionization and thus, the separation efficiency. Moreover, if the pH value of separation buffer is different from that of detection buffer, a pH gradient will be noticed along the capillary; so, to eliminate the effect of the difference between detection buffer and separation buffer, two buffers with same pH were used. Fig. 4 gives the effect of buffer pH on the detection sensitivity and migration time. Similar to the amine ECL reaction, the highest ECL intensity of ranitidine was at pH 7.50. With increasing pH of the buffer, the eletroosmotic flow inside capillary increased remarkably, and the migration time of ranitidine reduced gradually. To maintain good sensitivity and a suitable analysis time, both the

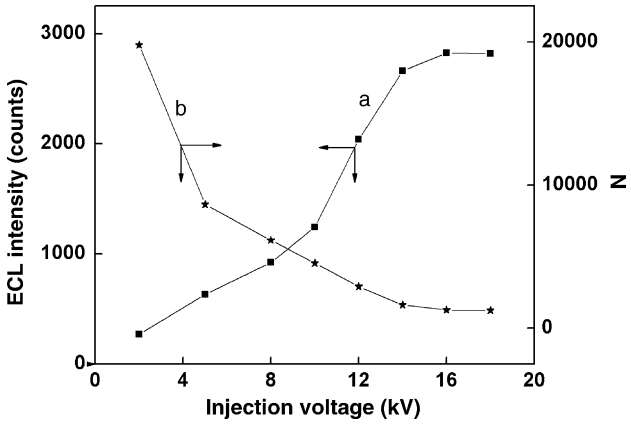

Fig. 5. Dependence of the ECL intensity (a) and separation efficiency (b) on the injection voltage. Injection time 8 s, buffer at pH 7.50, 10^{-4} M ranitidine solution, separation voltage 15 kV, detection potential 1.20 V, 5 mM $Ru(bpy)_3^{2+}$.

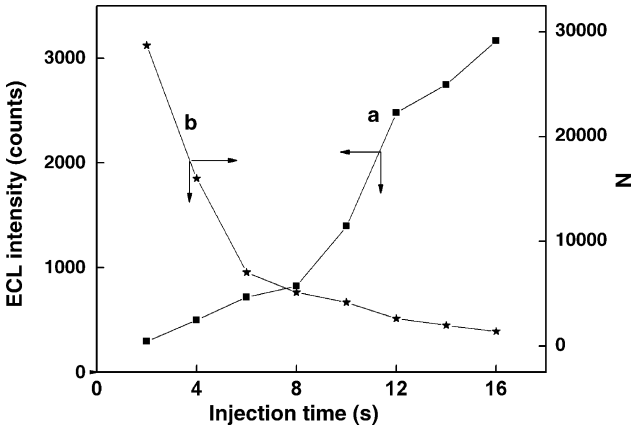

Fig. 6. Dependence of the ECL intensity (a) and separation efficiency (b) on the injection time. Injection voltage 10 kV, buffer at pH 7.50, 10^{-4} M ranitidine solution, separation voltage 15 kV, detection potential 1.20 V, 5 mM $Ru(bpy)_3^{2+}$.

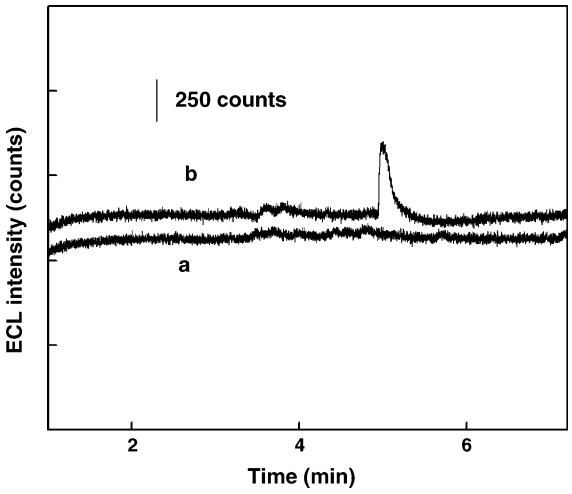

Fig. 7. Electrophoregrams of urine sample of blank (a) and containing ranitidine (b). Electrokinetic injection 10 s at 10 kV, buffer at pH 7.50, separation voltage 15 kV, detection potential 1.20 V, 5 mM $Ru(bpy)_3^{2+}$.

detection buffer and the separation buffer with pH 7.50 were used.

3.4. Effect of injection time and voltage

Fig. 5 shows the effect of injection voltage on ECL intensity and theoretical plate number (N) as injection time set at 8 s, and Fig. 6 shows that ECL intensity and N are influenced by the injection time ranging from 2 to 18 s. Theoretical plate efficiencies of ranitidine are calculated using the equation: $N = 5.54(t_R/W_h)^2$, where t_R is the retention time and W_h is the width at half the maximum peak height. As illustrated in Figs. 5 and 6, long injection time and high voltage led to strong ECL signal and low separation efficiency due to the introduction of more analyte in the detection cell. However, when shorter injection time and lower injection voltage were used, it was difficult to obtain favorable ECL intensity though high column efficiency could be achieved. So, electrokinetic injection for 10 s at 10 kV was used as a compromise.

3.5. Analytical performance of the present CE-ECL for ranitidine

Under the optimized conditions, namely, detection voltage of 1.20 V, buffer at pH 7.50, electrokinetic injection for 10 s at 10 kV, and 5 mM $Ru(bpy)_3^{2+}$ in the detection cell, the reproducibility of the method was tested by consecutive injection of ranitidine standard solution of 10^{-4} and 10^{-5} M ($n = 7$). The R.S.D. values of ECL intensity and migration time were 6.38 and 1.84% for 10^{-4} M and 6.01 and 0.60% for 10^{-5} M, respectively. A series of standard solutions with different concentration was tested to determine the linearity. The calibration curve obtained exhibited satisfactory linear behavior over the concentration ranging from 2×10^{-6} M to 1×10^{-4} M with a detection limit of 7×10^{-8} M (S/N = 3). Compared with other optical detection methods, the detection limit of ranitidine was lower than that reported by Wu et al. and Pérez-Ruiz et al. in system of CE-UV [11–13]. The present method showed the advantages of selectivity, sensitivity, reproducibility, linearity and small sample consumption. The results of the above analysis are presented in Table 1.

4. Applications

The proposed CE-ECL method was employed for the determination of ranitidine in human urine samples. Three healthy volunteers were treated simultaneously with an oral administration of 150 mg ranitidine hydrochloride capsule. The urine samples were collected at 5 h after oral administration of the drug. The urine collected before dosing was employed as a blank. All urine samples were extracted as shown in Section 2 and examined with CE-ECL system (injection volume 18 nl). Electropherograms of ranitidine extracted from real urine sample are shown in Fig. 7; no interference existed in the extracts (from 1.05 to 3.51 mg); ranitidine was detected, which corresponded to 4.11 mg/l in urine on an average. This phenomenon could explain that many factors, such as the capability of absorbing the drug, the metabolizing cycle of the drug and drinking volumes of water, were not entirely same for different subjects.

Table 1
The linearity, detection limit and reproducibility of the determination of ranitidine by the proposed method

Regression equation ($n = 6$)	Linear range (M)	Correlation coefficient	Detection limit (M)	R.S.D. (%, $n = 7$)	
				For ECL intensity	For migration time
$y = a+bx$, $a = 294.57 \pm 18.37$, $b = 10.514 \pm 0.394$	2×10^{-6} to 1×10^{-4}	0.997	7×10^{-8}	6.01–6.38	0.60–1.84

Conditions: detection voltage 1.20 V, buffer at pH 7.50, electrokinetic injection for 10 s at 10 kV, 5 mM $Ru(bpy)_3^{2+}$ in the detection cell.

5. Conclusion

A new method has been developed to determine ranitidine in urine. Under the optimized conditions, the approach of CE-ECL with $Ru(bpy)_3^{2+}$ showed good performance in terms of selectivity, sensitivity, repeatability, short analysis time and linearity. The validated method could be a good tool for the assay of ranitidine in urine.

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