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Capillary Electrophoresis Coupling with Electrochemiluminescence for Bisoprolol, Emolol, Propranolol Enantioseparation and Detection

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A simple, convenient and flexibility capillary electrophoresis coupling with electrochemiluminescence (CE-ECL) detection method was carried out for simultaneous enantioseparation of three β-blocker drugs (bisoprolol, esmolol, propranolol). In CE-ECL system, the buffers of the capillary outside and inside was not the same including the buffer composition, pH value, and concentrations. In order to simultaneously improve the detection sensitivity and enantiomer separation, the conditions of capillary inlet and outlet buffer were optimized in detail. Under the optimized conditions, the separation efficiency for enantiomers of three β-blocker drugs were improved and baseline resolution was achieved. The limit of detection (LOD) were in the range of 0.01-0.08 ng/mL for bisoprolol, esmolol, propranolol enanotiomers. The RSDs of peak areas peak, heights, and migration time were in the range of 2.1-2.5 %, 2.0-2.3 %, and 1.9-2.2 % for enanotiomers, respectively. The separation-detection techniques was applied to analysis enantiomers of bisoprolol, esmolol, propranolol in urine sample successfully, the recoveries of the six enantiomers were in the range of 94.0 % -100.7 % with RSDs less than 3.1 %.

Keywords: Enantioseparation, β-blocker drugs, capillary electrophoresis, electrochemiluminescence.

1. INTRODUCTION

It was considered to be that different enantiomers of a racemic drug usually shows some differences in their pharmacokinetics, toxicity, and pharmacological activity. One enantiomer may display helpful pharmacological activity, nevertheless, another enantiomer may provide some toxic side

effects [1-3]. Nowadays, enantioseparation of materials becomes more and more important in every field clinic chemistry [4,5], pharmaceutical industry [6-8], food and environment analysis [9-11]. Among chiral drugs, β-blocker drugs (including bisoprolol, esmolol, propranolol, et al) are considered as racemic mixtures and well-known drugs due to their stereochemical impact on pharmacodynamics and pharmacokinetics [12], but their enantiomers display some differences in the pharmacological activities and effects [13]. For example, the activity of S-propranolol is about 100 times more potent than R-propranolol [14,15]. Therefore, chiral separations of β-blocker drugs are considered to be an essential issue for further study on their pharmacological effects.

At present, analytical techniques used for chiral separations of β-blocker drugs, including high performance liquid chromatography (HPLC) [16-19], gas chromategraphy (GC) [20], capillary electrochromatography (CEC) and so on [21,22]. The other approaches are capillary electromigration techniques including capillary electrophoresis (CE) [23-26]. Liquid chromatography-Mass spectrometry/mass spectrometry (LC-MS/MS) [27], Microemulsion electrokinetic chromatography (MEKC) [28,29]. Both analytical techniques have their advantages and drawbacks. A lot of organic solvents and expensive chiral columns are required become the defect of the chromatographic methods. On the contrary, due to highly separation efficiency, powerful resolution, low solvent consumption, short separation time, and so on, CE is favored by many scholars, and have been widely used in enantioseparation analysis. But the sensitivity of CE is not very ideal, and the improving of the sensitivity of the detection techniques was also restricted because of the monitoring method.

Recently, Electrochemiluminescence (ECL) is favored more and more by people due to its simple instrumentation, high sensitivity, and wide linear range. Hence, CE coupling with ECL(CE-ECL) has been employed to detect different analytes containing tertiary amines groups [30]. At present, CE-ECL has also been employed to the analysis of chiral drugs [31,32]. However, CE-ECL was applied to enantioseparation of three β-blocker drugs (bisoprolol, esmolol, propranolol) at the same time has not been reported to, and no application to human urine sample was demonstrated.

Herein, CM-*β*-CD is used as a chiral additive to perform the enantioseparation for bisoprolol, esmolol, propranolol at the same time by CE with end-column $Ru(bpy)_3^{2+}$ ECL detection successfully. In this CE-ECL detection, the detection solution and separation buffer differed from each other, it is differed from the traditional chiral CE, in which the same detection solution and separation buffer are strictly required. In addition, adopt this end-column CE-ECL detection method, require a small amount chiral additives due to the detection buffer without any chiral additive. The influencing factors, such as CM-*β*-CD concentrations, detection potential, $Ru(bpy)_3^{2+}$ concentration, detection buffer concentration and pH, separation buffer pH and concentration, injection voltage and time, and separation voltage were investigated in detail. The proposed technique has also applied for the enantioseparation of three *β*-blocker drugs in human urine sample. It was expected to be able to supply a flexibility, simple and convenient method for enantioseparation of *β*-blocker drugs, and provide some essential information for further study on their pharmacological effects and activities.

2. EXPERIMENTAL

2.1. Samples

Human urine sample was got from a healthy male volunteer, and was conserved at -20 ˚C until separation and detection. In order to avoid the contamination on the capillary wall and the working electrode, a simple urine sample pretreatment before analysis was carried out. During the urine detection, 500 μL of urine was added to 1.5 mL of centrifuge tube, and then adding 1 mL of ethyl acetate, mixtures were put in a mechanical shaker for 6 min and subsequently centrifuged at 4000 rpm for 10 min. The upper layer was added into another tube and evaporated to dryness under a stream of dry N_2 at 30 ˚C. The solvent was evaporated, the remained dry residue was dissolved in 500 μL water, and then filtered through 0.22 μm membrane filters before assay. The urine samples spiked with standard solutions were extracted in the same way.

2.2. Apparatus

The apparatus are the same with those in our previous work [32].

2.3. Reagents

Racemic bisoprolol, esmolol, propranolol and their enantiomers were analytical grade, Which were got from Sigma. Tris(2,2-bipyridyl) ruthenium (II) chloride hexahydrate, CM-*β*-CD was obtained from Yunan Cyclodextrin Factory (Guangdong, China). The stock solutions of racemic bisoprolol, esmolol, propranolol and enantiomers were prepared in alcohol solution. The stock solutions were precise diluted to prepare the working standard solutions. The buffer used in the detection cell was PBS which pH appropriate was adjusted with sodium hydroxide or orthophosphoric acid. All reagents used were dissolved with double-distilled water, and were filtered by 0.22 μm membrane filters before detection.

2.4. Experimental methods

CE separations were performed with a fused silica modified capillary (45 cm). The location of capillary-to-working electrode was positioned to be about 80 μ m by an optical microscope. After the detection reservoir was replenished every 3 h, 5 mM $Ru(bpy)_3^{2+}$ with PBS (65 mM, pH 8.0) was injected into. The detection reservoir, which in order to maintain the reproducibility of the experiment results. 4 mg/mL of CM-*β*-CD in 35 mM Tris-H_3PO_4 (pH 8.5) was used as separation buffer, the injection voltage and time were 10 kV and 12 s, the separation voltage was set as 12 kV, PMT was set at 800 V.

3. RESULTS AND DISCUSSION

3.1. The end-column CE-ECL for enantioseparation of bisoprolol, esmolol, propranolol

Owing to the difference in the effective electromigration mobilities and the inequable binding constants with the chiral selector of enantiomers, the enantioseparation could be obtained [33]. In order to obtain a stable chiral environment under each experimental condition and improve the separation efficiency, the entire separation channel is usually filled with the same buffer solution. However, the inconsistency between detection sensitivity and separation resolution limits the application of this strategy. So, to solve the disagreement, the end-column CE-ECL system with different concentration and pH of PBS and Tris-H_3PO_4 for inlet and outlet capillary buffer were carried out to the enantioseparation of bisoprolol, esmolol, propranolol simultaneously successfully.

3.2. ECL behaviors of bisoprolol, esmolol, propranolol

The ECL curves of $Ru(bpy)_3^{2+}$, bisoprolol, esmolol, propranolol were shown in Fig. 1. As Fig. 1a_0 shown, only weak ECL signal were observed when a Pt electrode in 65 mM PBS (pH 8.0) with 5 mM $Ru(bpy)_3^{2+}$ in the potential range from 0.2 to 1.25 V. However, after adding 1.5 μg/mL of bisoprolol, esmolol, propranolol, respectively, the ECL intensity increased remarkably, compared with three analytes standard solution, it can be confirmed a, b, c is the ECL intensity curves of bisoprolol, esmolol, propranolol respectively.

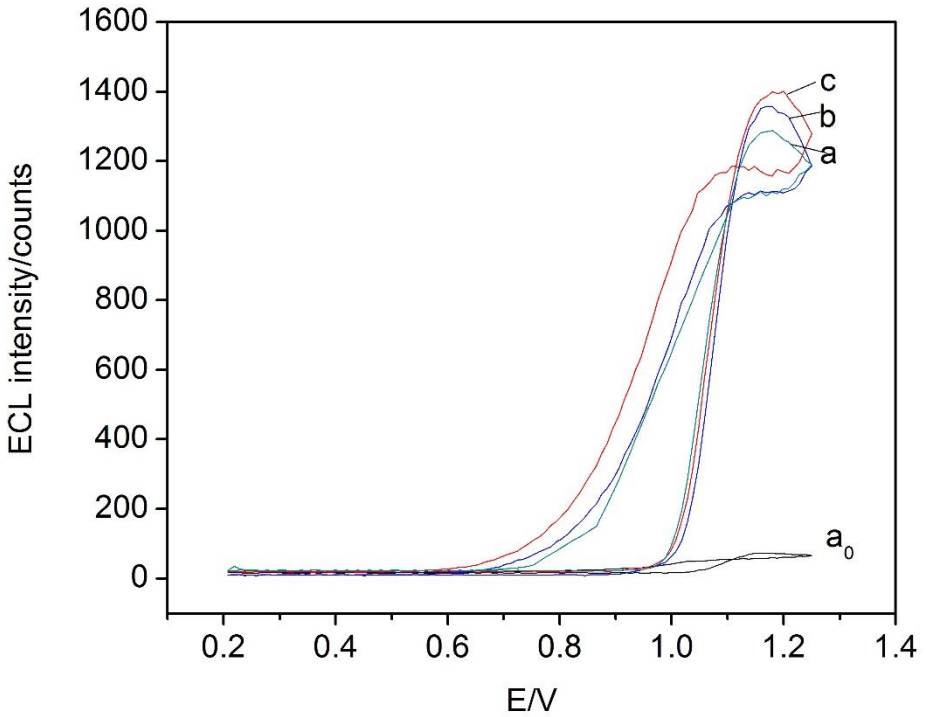

Figure 1. ECL behaviors of bisoprolol (a), esmolol (b)and propranolol（c）a_0. 65 mM PBS (pH8.0) +5 mM $Ru(bpy)_3^{2+}$; a. a_0+1.5 μg/mL bisoprolol; b. a_0+1.5 μg/mL esmolol; c. a_0+1.5 μg/mL propranolol

3.3. Optimization of the CM-β-CD concentrations

CD is considered as one of the most widespread applied chiral selectors, in especial, the CM-*β*-CD is usually applied to basic drugs [34]. It is reported that the apparent binding constants between analytes and selector result in enantioseparation [35]. For negatively charged CM-*β*-CD, the electrostatic forces of cationic analytes are mainly because of the apparent binding constant (36). In the proposed end-column CE-ECL detection system, three analytes and CM-*β*-CD moved in opposite directions due to three analytes with positively charged and CM-*β*-CD with negatively charged, these factors are conducive to the formation of inclusion complexes between chiral selectors and enantiomers, and lead to the enantiomers be separated. The CD concentration was considered to have a significance influence on the chiral separation [35,36], in this study, the impact of CM-*β*-CD concentrations on the enantiomer separation and ECL reaction were studied systematically. As Fig. 2 shown, when the concentration of CM-*β*-CD increased, the enantioresolution of the analyte increased too, it may be due to the increase of the number of interaction sites in the chiral selector of the analyte [37], and baseline separation of 1.5 μg/mL racemic bisoprolol, esmolol, propranolol was attained when 4 mg/mL of CM-*β*-CD concentrations was used. However, the ECL intensity decreased markedly when over 4 mg/mL of CM-*β*-CD was employed, it may be because of the higher concentrations of CM-*β*-CD, the more transient host-guest inclusion complexes, and the less free analyte was detected lead to ECL intensity decreased. In addition, when CM-*β*-CD concentrations was exceeded 4 mg/mL, the increase of enantiomer enantioresolution was slightly. Therefore, in order to get good enantioresolution and high detection sensitivity, 4 mg/mL of CM-*β*-CD was selected.

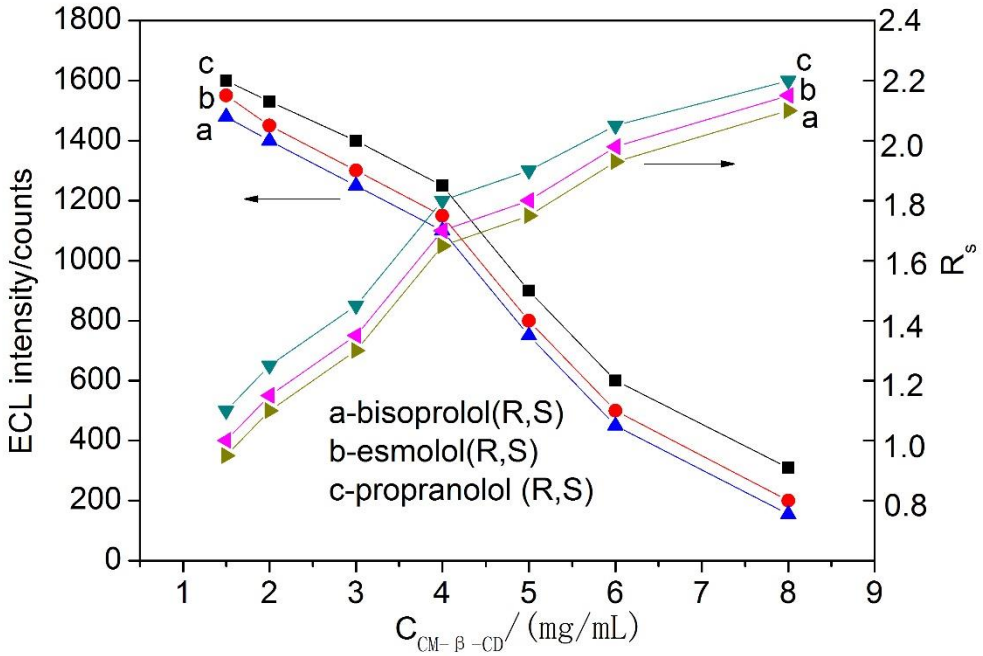

Figure 2. The effect of the CM-*β*-CD concentration on enantiomers R_s of 1.5 μg/mL bisoprolol, esmolol, propranolol. Conditions: detection potential: 1.2 V; detection buffer: 5 mM $Ru(bpy)_3^{2+}$, 65 mM PBS (pH 8.0); separation buffer: 35 mM Tris-H_3PO_4 (pH 8.5). separation voltage: 12 kV; electrokinetic injection: 10 kV×12 s.

3.4. Optimization of detection conditions

3.41 Optimization of detection potential

The ECL intensity is greatly affected by the detection potential, the ECL signal was recorded at different potentials in the range of 0.2-1.25 V. As Fig. **1** shown, lower detection potential (<1.0 V) lead to the ECL signals of bisoprolol, esmolol, propranolol extremely weak, it was due to $Ru(bpy)_3^{2+}$ was not oxidized on the surface of Pt electrode at low potential. When the detection potential rose and exceed 1.0 V, the ECL intensity of three β-blocker drugs enhance, and displayed the most value at 1.2 V, then slightly weaken. The weaken of ECL intensity could be on account of the passivated effect of the oxidation of electrode. So, 1.2 V of the detection potential at was selected in these experiments.

3.4.2 Optimization of $Ru(bpy)_3^{2+}$ concentration

In end-column CE-ECL system, the ECL signal is strong effect by the concentration of $Ru(bpy)_3^{2+}$. As the concentration of $Ru(bpy)_3^{2+}$ increases, so did the ECL intensity, this is because of the more of the electrically excited state $Ru(bpy)_3^{2+}$ is produced. when the concentration of $Ru(bpy)_3^{2+}$ was over 5 mM, the background current increased significantly, and the more noise was produced, at the same time, more consumption of the expensive reagent $Ru(bpy)_3^{2+}$ was required when its concentration is higher. Hence, in order to get better separation effect, stronger ECL signal and better signal-to-noise ratio of analytes and lower costs, 5 mM $Ru(bpy)_3^{2+}$ concentration was chosen.

3.4.3 Optimization of the detection buffer pH and concentration

In end-column CE-ECL system, the detection buffer has great influence on the intensity of ECL. The effect of the PBS pH on ECL intensity of analytes and the ECL background signal intensity with pH in the range of 6.0 ~10.0 were investigated. As shown in Fig. 3, at low pH, the ECL signal of bisoprolol, esmolol, propranolol enantiomers were poor, and increased with the increased of pH. At pH 8.0, the ECL signal of analytes reached the maximum and then decreased. The ECL background signal also increased with the pH in the range of 6.0~8.0 and then decreased more than 8.0. The reason may be, at low pH, the reaction between bisoprolol, esmolol, propranolol enantiomers and $Ru(bpy)_3^{2+}$ was delayed, while high pH led to stronger ECL intensity was due to the reaction was quick. The ECL background intensity decreased over 8.0 mainly on account of the fact that the higher pH solution caused the instability of $Ru(bpy)_3^{3+}$ [38]. So, pH 8.0 was selected as the optimal pH for the further experiments.

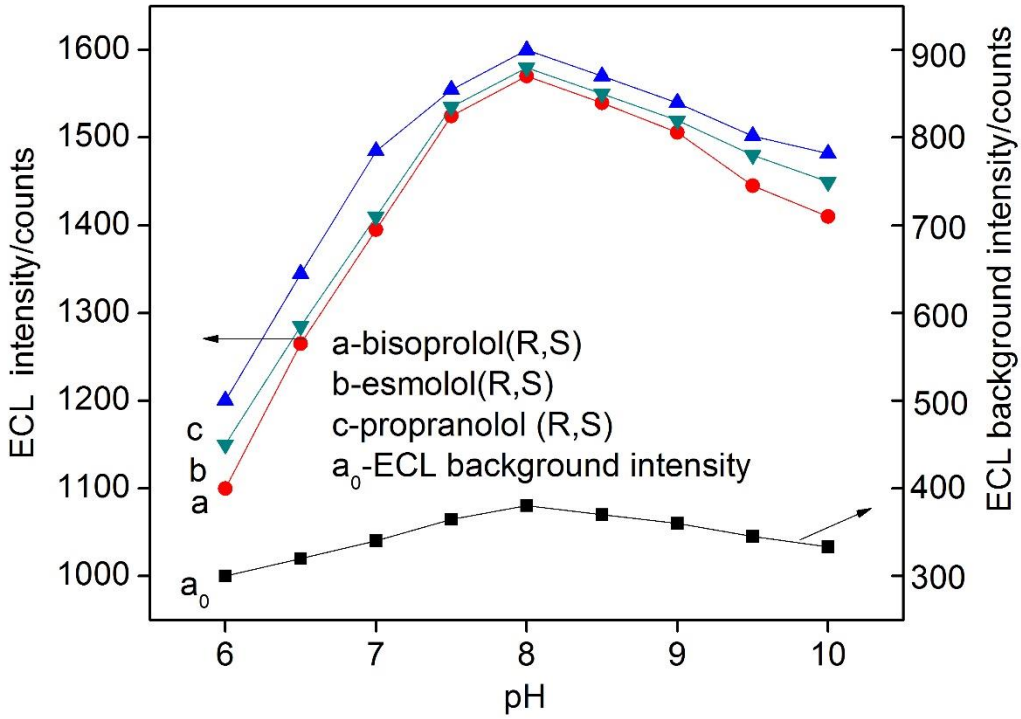

Figure 3. The effect of detection buffer pH values on ECL intensity of 1.5 μg/mL bisoprolol, esmolol, propranolol and the ECL background signal intensity. Except for detection buffer pH, other conditions as Fig. 2.

The effect of the detection buffer concentration on ECL intensity of bisoprolol, esmolol, propranolol enantiomers was tested in the range of 30~80 mM too. The result shown, when the concentrationat of the detection buffer is low, the ECL intensity of bisoprolol, esmolol, propranolol enantiomers increased with the increase of PBS concentration, and the highest ECL intensity was gotten at 65 mM, when the PBS concentration exceeded 65 mM, the ECL signal of analytes enantiomers decreased. What's more, it produce higher baseline noise in experiment when the concentration of PBS exceeded 65 mM. So, a 65 mM PBS was selected in end-column CE-ECL system for the further experiments.

3.5. Optimization of separation conditions

3.5.1 Optimization of the separation buffer solutions

The effect of Tris-H_3PO_4, HAc-NaOH, HAc-NaOH, Glycine-HCl separation buffer solutions on R_s and apparent migration ratio (Δμ) of bisoprolol, esmolol, propranolol enantiomers was investigated. In end-column CE-ECL system, the Tris-H_3PO_4 can obtain the best R_s and Δμ, it was due to the Tris with positively charged can play a role with CM-*β*-CD, and in favour of improving the chiral recognition ability and enantiomers separation. Comprehensive consideration, Tris-H_3PO_4 were chosen as separation buffer in end-column CE-ECL system.

3.5.2 Effect of the separation buffer pH and concentration

To our knowledge, in end-column CE-ECL system, the separation buffer pH is an important parameter affecting CE separation due to its influence on the electromigration and the charge of bisoprolol, esmolol, propranolol enantiomers. So, the separation buffer pH is a key factor to be optimized during the separation. In this experiment, the separation buffer pH was tested in the range from 6.5 to 11.0. When 7.0<pH≦8.5, there are larger difference of migration rate between bisoprolol, esmolol, propranolol enantiomers in capillary. But the EOF increases rapidly with the rise of pH value, which led to the migration time of enantiomers in capillary was shortened, and was unfavorable for the separation of the enantiomers. It can be seen from Fig. **4** (a_0, b_0), the best R_s of the enantiomers was obtained when the pH value was 8.5. So, the best separation buffer pH was chosen as 8.5.

The influence of the Tris-H_3PO_4 concentrations in the range 5~50 mM on bisoprolol, esmolol, propranolol enantioseparation was investigated. As shown in Fig. **4** (a_1, b_1), when the Tris-H_3PO_4 concentration varied from 5 mM to 35 mM, the R_s of analytes were both increased significantly. However, when the buffer concentration varied from 35 mM to 50 mM, the R_s decreased. The efficiency of enantioseparation decreased was probably resulted from increasing Joule heating that was caused by the high concentration of the Tris-H_3PO_4. Therefore, 35 mM of Tris-H_3PO_4 concentration was selected.

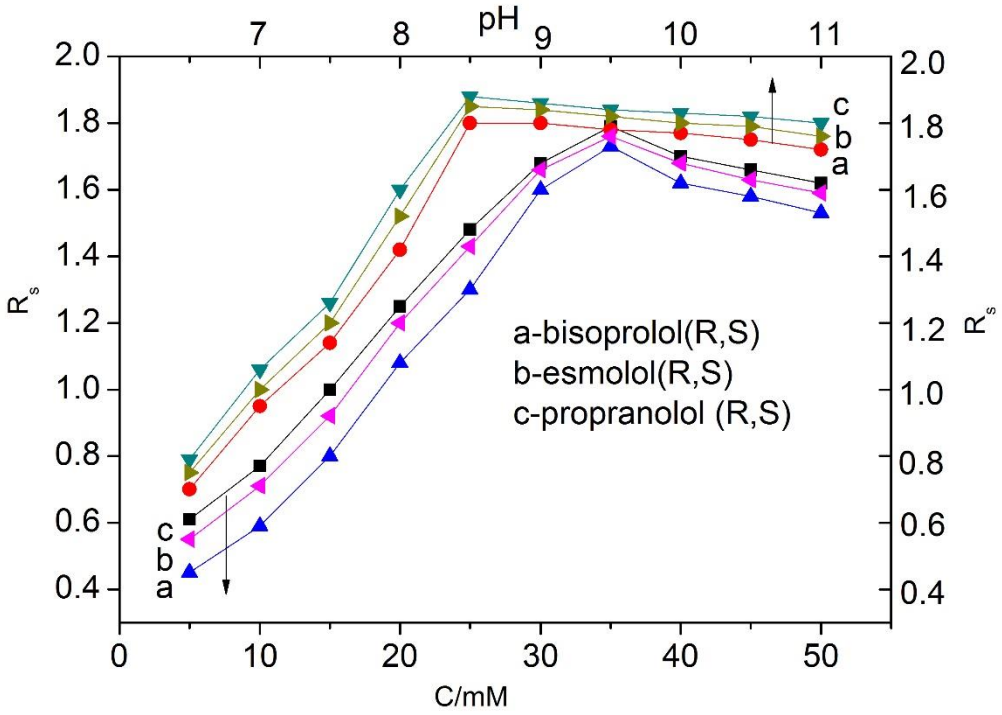

Figure 4. The effect of the separation buffer pH and concentration on enantiomers R_s of 1.5 μg/mL bisoprolol, esmolol, propranolol. Except for the separation buffer pH and concentration, other conditions as Fig. 2.

3.5.3 Effect of separation voltage

The separation voltage was also optimized, when the separation voltage was increased from 8.0

kV to 18.0 kV, the changes of R_s and ECL intensity of bisoprolol, esmolol, propranolol enantiomers were shown in Fig. 5. As can be seen from the result, the higher separation voltage, the stronger ECL signal and shorter analysis time were obtained, it was because of high separation voltage was produced a large number of analytes, which resulted in the reaction rate of $Ru(bpy)_3^{2+}$ and analytes increases, and led to strong ECL signal, good peak shape and short analysis time. But too high voltage will lead to the Joule heat and affect the enantiomer separation of the analytes. In end-column CE-ECL system, the R_s of bisoprolol, esmolol, propranolol enantiomers increased with the separation voltage increased from 8.0 kV to 12 kV. When the separation voltage was higher than 12 kV, the background noise generated greater, and led to the R_s decreased rapidly. Therefore, comprehensive consideration the separation efficiency and peak shape, 12 kV was chosen as the optimized separation voltage in subsequent experiments.

3.6. Optimization of injection conditions

In order to choose the best injection voltage, The trend of ECL signal was investigated when the injection voltage was increased from 2 to 18 kV, the changes of ECL signal and theoretical tray number were shown in Fig. 5.

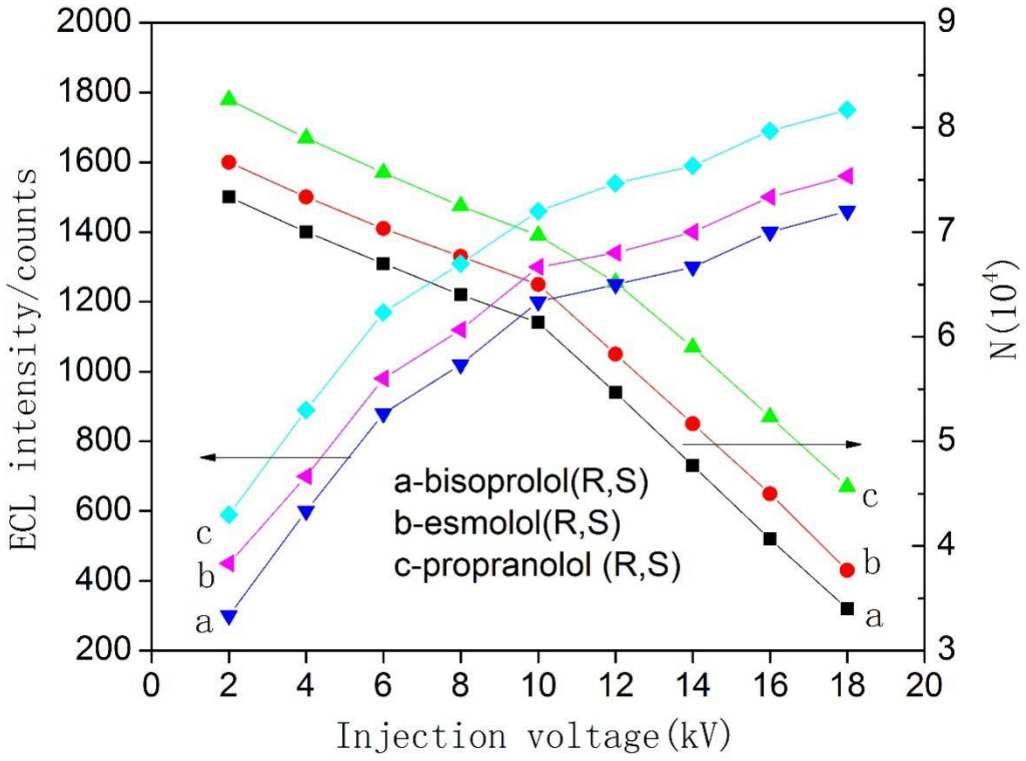

Figure 5. The effect of the injection voltage on enantiomers R_s of 1.5 μg/mL bisoprolol, esmolol, propranolol. Except for the injection voltage, other conditions as Fig. 2.

The theoretical plates number was obtained by the equation: $N = 5.54(t_R/W_h)^2$, where t_R is the migration time and W_h is the width at half the maximum peak height. As Fig. **5** shown, lower injection voltage lead to weaker ECL intensity of bisoprolol, esmolol, propranolol enantiomers. Nevertheless, the ECL signal was strong and the N was decreased with injection voltage was high, it was due to the introduction of more analyte in the detection cell. When injection voltage was 10 kV, the ECL signal was stronger and N was higher too, so 10 kV was chosen as the best injection voltage. Setting the injection voltage at 10 kV, the effects of the injection time varied from 2 to 16 s on ECL intensity and theoretical plate number of bisoprolol, esmolol, propranolol enantiomers was also studied. The ECL signal enhancement with the injection time, while the N decreased. When injection time was at 12 s, better ECL intensity and N was acquired. As a result, injection time for 12 s was chosen in the experiment.

3.7. Linearity, detection limits and reproducibility

After optimization, the experimental conditions are obtained as follows: 1.2 V was used as detection potential; 5mM $Ru(bpy)_3^{2+}$ and 65 mM PBS (pH 8.0) were used as ECL solution containing; 4 mg/mL of CM-*β*-CD in 35 mM Tris-H_3PO_4 (pH 8.5) as separation buffer; the separation voltage was 12 kV; the injection voltage and time were 10 kV and 12 s.

Table 1. Regression equation, linear range and limits detection for enantiomers (n=6)

Analyte	Regression equation	r	Linear range (ng/mL)	LOD (ng/mL)	R_s	RSD (%, n=6)		
						A	h	t
R- bisoprolol	I=285.62ρ+242.16	0.9991	0.5~15000	0.05	1.80	2.3	2.1	2.1
S- bisoprolol	I=280.15ρ+239.93	0.9989	0.5~15000	0.05	1.79	2.4	2.0	2.0
R- esmolol	I=276.48ρ+216.76	0.9988	0.9~10000	0.08	1.81	2.2	2.2	2.2
S- esmolol	I=261.05ρ+207.50	0.9989	0.9~10000	0.08	1.82	2.5	2.3	2.2
R-propranolol	I=259.13ρ+205.43	0.9992	0.1~15000	0.01	1.78	2.2	2.0	2.0
S-propranolol	I=258.45ρ+200.78	0.9990	0.1~15000	0.01	1.75	2.1	2.1	1.9

I: ECL intensity, ρ: analyte concentration, r: correlation coefficient, R_s: resolution, RSD: relative standard deviation, A: peak area, h: peak height, t: migration times

Table 2. The results of this method are compared with other methods

Method	Analytes	Linear range(ng/mL)	Detection limit(ng/mL)	Ref
The present method	R, S- bisoprolol	0.5~15000	0.05	--
	R, S- esmolol	0.9~10000	0.08	--
	R, S- propranolol	75~5000	0.01	--
HPLC	R, S- bisoprolol	75~5000	25 and 26	17
CEC	R, S - propranolol	1.0~6000	0.3	22
	R, S - esmolol	0.80~6000	0.2	
CE-FASI	R, S -β-blocker	--	0.15–0.80	24
CE-UV/FASI	R, S- esmolol	0.25~25	50	26
MEKC	R, S- propranolol	1.0~500	0.02	29

Both bisoprolol, esmolol, propranolol enantiomers achieved baseline separation by end-column CE-ECL system (Fig. **6.**a), the repeatability were gotten by 6 time continuous injections of 0.5 μg/mL bisoprolol, esmolol, propranolol standard solutions, the experimental results were shown in Table **1**. The enantiomer detection results of this method and other methods are listed in Table **2**. Compared with HPLC [17], CEC [22], CE-UV/FASI [24-26], and MEEKC [29], the detection limit of the present method was the lowest, meanwhile, the linear range is also the widest. It indicated the linearity and sensitive of the proposed separation detection technology was better in enantioseparation.

3.8. Analysis of urine sample

To verify the proposed end-column CE-ECL method can be adopted to detected three *β*-blocker drugs enantiomers in complex human urine sample, the strategy was used to the analysis of bisoprolol, esmolol, propranolol enantiomers in healthy persons urine sample (prepared as 2.3). Under optimum conditions, the electropherograms of the urine sample (Fig. **6**.b) and the urine sample added with 0.25 μg/mL bisoprolol, esmolol, propranolol standard solutions (Fig. **6**.c) were obtained under the optimum conditions. Compared to the typical electropherograms of bisoprolol, esmolol, propranolol standard solutions (Fig. **6**.a), except two unknown peaks was found, no peaks of bisoprolol, esmolol, propranolol enantiomers appeared to urine sample, when added with 0.25 μg/mL of bisoprolol, esmolol, propranolol, the characteristic peaks of bisoprolol, esmolol, propranolol enantiomers electrophoresis peaks appeared (Fig. **6**.c).

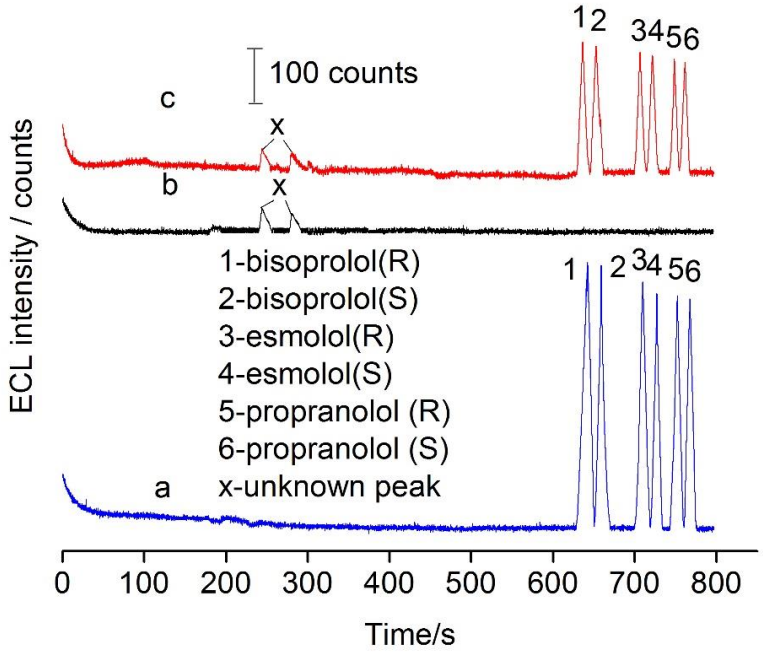

Figure 6. The electropherograms of the enantiomers separation for bisoprolol, esmolol, propranolol. a. 0.5 μg/mL bisoprolol, esmolol, propranolol standard solutions; b. the blank urine; c. b spiked with 0.25 μg/mL bisoprolol, esmolol, propranolol standard solutions; 4mg/mL CM-*β*-CD, other conditions as Fig. 2.

Table 3. Recoveries and precisions for analytes in human urine samples (n=5)

Sample	Added(μg/mL)	Found(μg/mL)	Recovery (%)	RSD (%, n=5)
R- bisoprolol	0.100	0.096	96.2	2.5
	0.500	0.480	96.0	2.4
	1.000	1.007	100.7	2.1
S- bisoprolol	0.100	0.098	98.0	2.7
	0.500	0.488	97.5	2.6
	1.000	1.001	100.1	2.2
R- esmolol	0.100	0.094	94.0	3.1
	0.500	0.475	95.0	2.8
	1.000	0.975	97.5	2.6
S- esmolol	0.100	0.095	95.0	2.7
	0.500	0.482	96.4	2.5
	1.000	0.993	99.3	2.4
R-propranolol	0.100	0.094	94.0	2.6
	0.500	0.490	98.1	2.5
	1.000	0.992	99.2	2.2
S-propranolol	0.100	0.097	97.0	2.5
	0.500	0.486	97.2	2.4
	1.000	1.004	100.4	2.3

The results shown that the resolution of the enantiomers have been maintained in the urine sample matrix as the standards in buffer solution (Fig. **6**.a to c). Therefore, the end-column CE-ECL with CM-*β*-CD method was proved to avoid the interferents of urine matrix, and offer an efficient strategy for quantitative determination of chiral drugs in human urine. To study the reproducibility, a urine sample were continuously tested by this method for five times. The recoveries of enantiomers were in the range of 94.0%~100.7%, the RSDs were less than 3.1%, and the other results are showed in Table **3**.

4. CONCLUSIONS

The enantiomers of three β-blocker drugs (bisoprolol, esmolol, propranolol) were successfully separated by end-column CE-ECL with CM-*β*-CD as chiral additive. It indicated that this method can improve the efficiency of separation, and can provide a reliable and rapid method for simultaneous separation of three racemic drugs in human urine under optimized experimental conditions.

CONFLICTS OF INTEREST
There are no conflicts to declare.

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References

1. P. Paoli, L. Chelazzi and L. Conti, *Acta. Cryst. C.,* 75 (2019) 87.
2. M.E. Tiritan, M. Pinto and C. Fernandes, Molecules., 25 (2020) 1713.
3. C. Höcht, F. M. Bertera, Y. S. Plantamura, L. Parola, J. S Del Mauro and A. H. Polizio, *Expert. Opin. Drug. Met.,* 15 (2019) 1.
4. F. Chen, Q. Bai, Q. Wang Q, S. Chen, X. Ma,C. Cai, D. Wang, A. Waqas and P. Gong, *Curr. Pharm. Biotechno.,* 21 (2020) 1.
5. J. Lee, S. Kim, J. Y. Sim, D. Lee, H. H. Kim, J. S. Hwang, Lee G. Dong, Z-Y. Park and I. J. Kim, *BBA-Biomembranes,* 1861 (2019) 34.
6. A. Petri, V. Colonna and O. Piccolo, *J. Org. Chem.,* 15 (2019) 60.
7. J. Zhang, Q. Liu, W. Wu, B. He, X. Wang, H. H. Sung, M. Chen, B. S. Li, S. H. Liu, W. Y. Lam Jacky and B. Z. Tang, *ACS Nano,* 13 (2019) 3618.
8. J. Zou, X-Q. Chen, G-Q Zhao, X-Y. Jiang, F-P. Jiao and J-G. Yu, *Talanta,* 195 (2019) 628.
9. Y. Liu, L. Wu, P. Kohli, R. Kumar, H. Stryhanyuk, I. Nijenhuis, R. Lal and H-H. Richnow, *Environ. Sci. Technol.,* 53 (2019) 8715.
10. X. Zhang, Y. Zhang, H. Zhang, Y. Quan, Y. Li, Y. Cheng and S. Ye, *Org. Lett,* 21 (2019) 439.
11. W-Y. Zhao, W-Y. Zhou, J-J. Chen, G-D. Yao, B. Lin, X-B. Wang, X-X. Huang and S-J. Song, *Phytochemistry,* 159 (2019) 39.
12. R-H. He, Y-J. He, Y-J. Tang, H-H. Zhou, H. L. McLeod and J. Liu, *Pharmacogenomics,* 17 (2016) 74.
13. J. Xu, H. Sun, Y. Zhang and A. C. Aldera, *Sci. Total. Environ.,* 650 (2019) 1122.
14. Y. Yi, D. Zhang, Y. Ma, X. Wu and G. Zhu, *Anal. Chem.,* 91 (2019) 2908.
15. V. K. Vashistha and A. Kumar, *Chirality,* 32 (2020) 722.
16. S-B. Lee, T-V. Pham, X-L. Mai, T-A-T. Le, T-N-V. Nguyen, J-S. Kang, W. Mar and K. H. Kim. *J. Anal. Sci & Technol.,* 33 (2020) 59.
17. M. Pocrnić, M. Ansorge, M. Dovhunová, E. Tesařová and N. Galić, *Arh. Hig. Rada. Toksiko.,* 71 (2020) 56.
18. O. M. Younes, F. A. Ali and Z. A. Assaf, *Research. J. Pharm. Tech.,* 13 (2020) 1679.
19. T. Yan, F. Li, S. Qi, J. Tian, R. Tian, J. Hou, Q. Luo, Z. Dong, J. Xu and J. Liu, *Chem. Commun.,* 56 (2020) 149.
20. S. Batra and R. Bhushan. *Biom. Chromatogr.,* 33 (2019) 1.
21. A. P. Kumar and J. H. Park, *J. Chromatogr. A.,* 1218 (2011) 5369.
22. M. Lu, L. Zhang, B. Qiu, Q. Feng, S. Xia and G. Chen, *J. Chromatogr. A.,* 1193 (2008) 156.
23. J. Guo, J. Wang, H. Lin, Y. Feng, H. Shen, R. Huang, L. Liu and Z. Zhao, *J. Sep. Sci.,* 42 (2019) 1077.
24. J. Wu, X. Xiao, Z. Li and J. Li, *Anal. Bioanal. Chem*., 411 (2019) 2121.
25. S. Hu, M. Zhang, F. Li and M. C. Breadmore, *J. Chromatogr. A.,* 1596 (2019) 233.
26. K.B. Borges, M. T. Pupo and P. S. Bonato, *Electrophoresis,* 30 (2009) 3910.
27. M. C. O. Souza, M. P. Marques, G. Duarte and V. L. Lanchote, *J. Pharmaceut. Biomed.,* 164 (2019) 268.
28. Y. Ma, H. Zhang, H. Chen and X. Chen, *Electrophoresis,* 35 (2014) 3345.
29. Y. Ma, H. Zhang, Z. Rahman Z, W. Wang, X. Li, H. Chen and X. Chen, *Electrophoresis,* 35 (2014) 2772.
30. Y. Hu, *J. Chromatogr. B.,* 143 (2015) 98.
31. Z. Xu, G. Jin, H. Shao, S. Fan, X. Li, S. Shi and F. Yan, *J. Chromatogr. Sci.,* 8 (2020) 1.
32. Y. Hu and X. Wei, *Curr. Anal. Chem.,* 14 (2018) 504.
33. R. Vespalec and P. Bocek, *Chem. Rev.,* 100 (2000) 3715.
34. W. P. Guo, Z. B. Rong and Y. H. Li, *Electrophoresis,* 34 (2013) 2962.
35. M. P. Vaccher, E. Lipka and J. P. Bonte, *Electrophoresis,* 26 (2005) 2974.

36. M. P. Vaccher, E. Lipka and J. P. Bonte, *Electrophoresis,* 25 (2004) 1111.
37. S. Trkulja and K. A. Kovar, *J. Sep. Sci.,* 27 (2004) 557.
38. S. E. Hooper and M. R. Anderson, *Electroanalysis,* 19 (2007) 652.