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Analysis of perphenazine and fluphenazine by capillary electrophoresis coupled with tris (2,2′-bipyridyl) ruthenium (II) electrochemiluminescence detection

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ABSTRACT

The coupling of end-column tris (2,2′-bipyridyl) ruthenium (II) electrochemiluminescence (ECL) detection with capillary electrophoresis (CE) was developed for the analysis of two antipsychotic drugs, perphenazine (PPH) and fluphenazine (FPH). The parameters related to CE separation and ECL detection, including the detection potential, the buffer pH value and concentration, the separation voltage, and $Ru(bpy)_3^{2+}$ concentration, were investigated in detail. Under optimum conditions, PPH and FPH were well separated and detected within 11 min. The linear ranges were 0.1–5 μM for PPH, and 0.1–7.5 μM for FPH, respectively. The limits of detection of PPH and FPH were 5 and 10 nM ($S/N=3$). The relative standard deviations ($n=3$) of the ECL intensity and the migration time were less than 2.5 and 0.65% in a day, and less than 3.4 and 1.7% in different three days. The proposed method was successfully applied to determine PPH and FPH in spiked urine samples with satisfactory results.

1. Introduction

Phenothiazine drugs are derivatives of phenothiazine, and they have a phenothiazine ring with different substituents attached at the 2- and 10-position. These drugs are able to control the behavior of psychiatric patients without addictions [1]. Perphenazine (PPH) and fluphenazine (FPH) belong to the phenothiazines family of drugs, and owing to their neuroleptic and antidepressive actions, they are commonly prescribed for the treatment of psychotic disorders such as schizophrenia and schizoaffective psychoses in order to decrease restlessness, aggressiveness and impulsive behavior [2,3]. Recently, PPH is found to be effective in the treatment of Parkinson's disease [4]. As shown in Fig. 1, PPH and FPH have similar chemical structure and both of them contain tertiary amines groups. To enable their clinical applications to achieve optimum therapeutic effects and minimize side effects, it is necessary to develop analytical methods with high sensitivity and free from interference. However, it is difficult to detect these trialkylamine compounds as they do not absorb very well in the UV–vis region owing to their low molar absorptivities [1]. The methods proposed for the determination of PPH, FPH and analogous analytes include electrochemiluminescence (ECL) [5], spectrophotometry [3,6], electrochemisty [4,7,8], chemiluminescence (CL) [9–11], flow injection (FI)–CL method [12], gas chromatography–mass spectrometry (GC–MS) [13], capillary electrophoresis–electrochemical detection (CE–ED) [14,15] and non-aqueous capillary electrophoresis-UV–vis spectrophotometry [16].

CE, with advantageous properties including high separation efficiency, short analysis time, low power requirements, limited consumption of chemicals, and ease of installation, operation, and maintenance, is a particularly interesting candidate for drug analysis. Because of the small physical dimensions of the separation capillaries and extremely low sample quantities injected onto the capillaries, the properties of the detector have great influence on the overall analytical performance for CE. Ideally, the detector should offer detection limits as low as possible without impacting the quality of separation.

ECL is the process whereby species generated at electrodes undergo high-energy electron-transfer reactions to form excited states that emit light [17]. It is well known as a powerful analytical technique with the advantages of high sensitivity, wide linear range, and simple instrumentation. ECL has been widely used in the areas of, for example, immunoassay, food and water testing, and biowarfare agent detection. And it has also been successfully exploited as a detector of FI analysis [18–20], high-performance liquid chromatography (HPLC) [21–23], CE [24–26], and micro total analysis (μTAS). One of the most highly studied ECL compounds is tris (2,2-bipyridyl) ruthenium (II) (namely $Ru(bpy)_3^{2+}$ due to its ability to undergo ECL reactions in aqueous solution, its excellent stability and high efficiency [27,28]. CE combining with ECL (CE–ECL) based on Ru $(bpy)_3^{2+}$ has obtained good performance for the determination of

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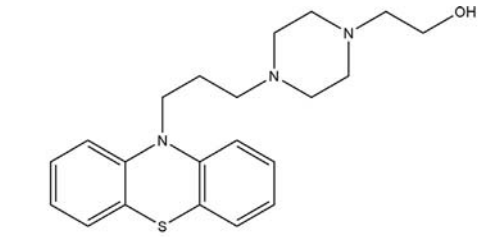
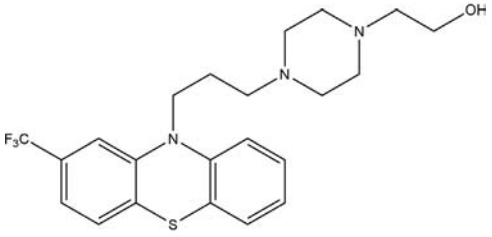


Perphenazine



Fluphenazine

Fig. 1. Chemical structures of perphenazine (PPH) and fluphenazine (FPH).

various analytes containing tertiary amines group and their derivatives [29–40]. Importantly, the CE–ECL system combined the advantages of high separation efficiency, good repeatability and short analysis time of CE and high sensitivity, non-derivation processes of ECL.

In the present work, based on the fact that the light emission of $Ru(bpy)_3^{2+}$ ECL could be enhanced by PPH and FPH containing a tertiary amines group dramatically, CE–ECL has been established for rapid separation and sensitive detection of PPH and FPH. All separation and detection conditions were systematically investigated, and the results showed that PPH and FPH were well separated within 11 min. The proposed method was successfully applied to the analysis of two analytes in human urine samples, and was expected to develop into a practical and valuable tool for the pharmaceutical monitoring of antipsychotic drugs in clinical and biofluids analysis in future.

2. Experimental

2.1. Reagents and chemicals

All the reagents were of analytical grade and were used as received without further purification. Tris (2,2′-bipyridyl) ruthenium (II) chloride hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$) was purchased from Aldrich Chemical (Milwaukee, WI, USA). PPH·HCl was purchased from Sigma (St. Louis, MO, USA). And FPH was supplied by Alfa-Ascar (Parkridge, RD, USA). Na_2HPO_4, NaH_2PO_4, H_3PO_4, NaOH were purchased from Beijing Chemicals (Beijing, China).

The stock solutions (10 mM) of $Ru(bpy)_3^{2+}$ and FPH (10 mM) were prepard by dissolving these analytes in doubly distilled water. And a 10 mM stock solution of PPH was prepared by dissolving an appropriate amount of PPH in alcohol. Dilute solutions of PPH were obtained by serial dilution of the stock solution with doubly distilled water containing 10% alcohol. All stock solutions were stored in the dark at 4 °C to avoid possible decomposition and stable for at least 1 month. Doubly distilled water was used throughout the experiment. Electrophoresis buffers in the pH range from 2.1 to 3.7 were prepared with the same concentration (20 mM) of NaH_2PO_4 and H_3PO_4 solution. Before use, all solutions were filtered through 0.22 μm cellulose acetate membrane.

2.2. Apparatus

The CE–ECL system (MPI-A, Xi'an Remex Electronic Science Tech Co., Xi'an, China) included a high-voltage power, an EC potentiostat, a chemiluminescence detector and a data collection analyzer. EC detection was performed with a CH Instrument model 800 voltammetric analyzer (Shanghai CH instruments, China).

All experiments were carried out by a conventional three electrode system, with a 500 μm diameter Pt disk as the working electrode, a Pt wire as the counter electrode, and Ag/AgCl as the reference electrode. The ECL detection cell was made from PDMS pools sticking to flat glass, and specific operation was shown in Ref. [41]. The detection cell (800 μL) was fullfilled with 5 mM $Ru(bpy)_3^{2+}$ in 100 mM phosphate buffer (PBS), which was refreshed every 3 h in order to maintain the reproducibility of the results. The working electrode was carefully positioned directly opposite the capillary outlet with the aid of an optical microscope to reach about 50 μm [42–44] between the capillary outlet and the electrode tip. All experiments were performed at room temperature. The photomultiplier tube (PMT) positioned under the detection cell, was biased at 800 V. The ECL signals were processed with a data processor controlled by a personal computer. All experiments were carried out at room temperature.

The electrophoretic separations were performed in a 50 μm i.d. and 40 cm length fused-silica capillary (Yongnian Optical Fiber, Hebei, China) column using a running buffer of NaH_2PO_4–H_3PO_4 (10 mM, pH 2.9). The capillary was filled with 0.1 M NaOH overnight prior to use and rinsed with water to maximize free silanols on capillary surface. In the daily experiments, it was flushed with 0.1 M NaOH, double distilled water and separation buffer for 10 min, respectively. Between runs, the capillary was rinsed with doubly distilled water and running buffer for 2 min respectively to ensure the good reproducibility. ECL detection was carried out in an end-column mode. The separation voltage was fixed at 15 kV. The samples were injected electrokinetically for 10 s at 10 kV.

2.3. Sample preparation

The newly urine sample was collected from a healthy male volunteer and filtered through 0.22 μm membranes prior to analysis. And then, it was diluted with doubly distilled water by 20 times to decrease the interference of the ion strength of the sample matrix. Finally, it was spiked with 0.25–10 μM PPH, and 0.25–15 μM FPH, respectively for detection.

3. Results and discussion

3.1. Electrochemical and ECL behaviors of $Ru(bpy)_3^{2+}$/PPH and $Ru(bpy)_3^{2+}$/FPH systems

Cyclic voltammograms (CVs) and their corresponding ECL curves of $Ru(bpy)_3^{2+}$, PPH and FPH were shown in Fig. 2. There was no electrochemical response and ECL signal at a Pt electrode in 100 mM PBS (pH 7.0) in the potential range from 0 to +1.3 V (curve *a* and *a*′). With the addition of 2 mM $Ru(bpy)_3^{2+}$, the well-known reversible redox peaks appeared in the range of 1.1–1.2 V (curve *b*) and only relatively weak ECL signal could be observed (curve *b*′). In presence of 0.2 mM PPH (curve *c*) or FPH (curve *d*) led to an increase in the anodic peak current but a decrease in the cathodic one, which was ascribed to the catalysis of the analytes for the reduction of $Ru(bpy)_3^{2+}$. In addition, two oxidation peaks could be observed. The first one was the direct oxidation peak of each analyte, and the oxidation peak potentials of PPH and FPH were at about 0.7 and 0.9 V, respectively. The potential of the second oxidation peak was overlapped with that of $Ru(bpy)_3^{2+}$,

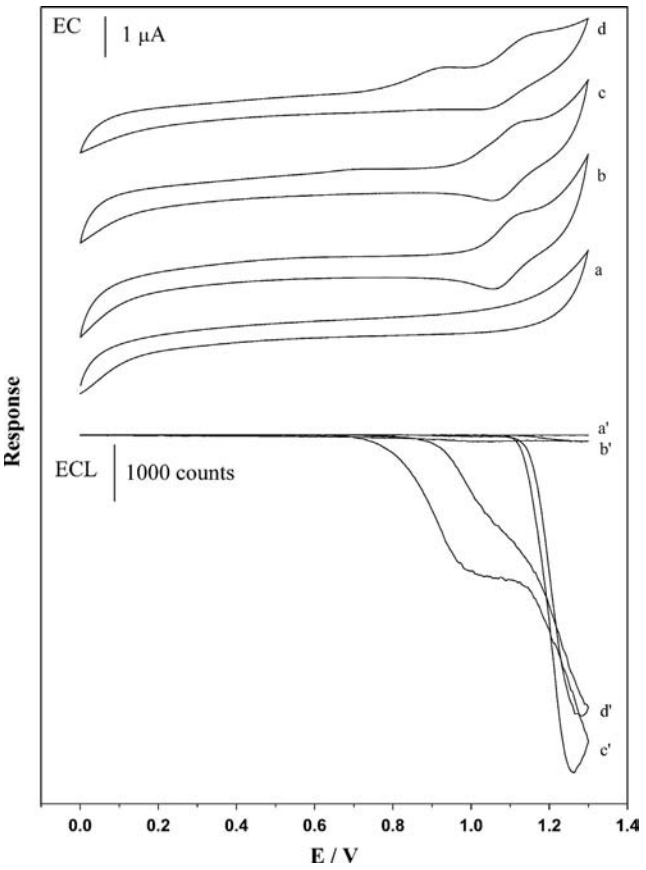


Fig. 2. Cyclic voltammograms and their corresponding ECL-potential curves. (a) 100 mM phosphate buffer at pH 7.0; (b) adding 2 mM $Ru(bpy)_3^{2+}$ to (a); (c) adding 0.2 mM PPH to (b); (d) adding 0.2 mM FPH to (b). Working electrode, 500 μm-diameter Pt electrode; scan rate, 100 mV s^{-1}; PMT, 800 V.

indicating PPH or FPH was able to react with $Ru(bpy)_3^{2+}$ as a co-reactant in ECL process. Meanwhile, the ECL intensity increased dramatically and reached a maximum at around 1.25 V. The ECL intensity increased 43 and 36 times by adding PPH (curve *c′*) and FPH (curve *d′*), respectively. Thus, the ECL detection method can be developed for sensitive determination of these two antipsychotic drugs. Moreover, PPH exhibited constantly larger ECL intensity than FPH under the same conditions. In general, it has been reported that electron-withdrawing groups tend to decrease the ECL intensity and electron-donating groups increase the ECL intensity [45]. Both of PPH and FPH contain electron-withdrawing groups-Cl and trifluoromethyl group that are attached to the benzyl group, respectively. But trifluoromethyl group has higher electron-withdrawing ability compared with Cl. Therefore, FPH produced a lower ECL intensity than PPH.

3.2. Hydrodynamic voltammograms (HDVs)

The ECL intensity is dependent on the rate of the light-emitting chemical reaction, and this reaction rate relies on the detection potential [28]. Therefore, the effect of the detection potential on the ECL intensity of PPH and FPH was investigated to obtain a high sensitivity. As shown in Fig. 3, PPH and FPH displayed similar profiles in the potential range of 1.0–1.3 V. When the detection potential was set at 1.00 V, the ECL responses of PPH and FPH were relatively low. After that, increasing amounts of $Ru(bpy)_3^{3+}$ was generated from $Ru(bpy)_3^{2+}$ oxidation in the system with the increase of detecting potential, so the ECL intensity increased sharply from 1.05 to 1.20 V and reached the maximum value at 1.20 V. And then it began to descend when detection potentials

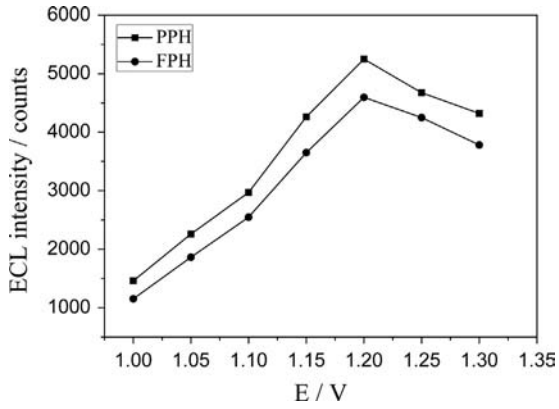


Fig. 3. Hydrodynamic voltammograms of 5 μM PPH and FPH. Conditions: separation capillary, 40 cm length (50 μm i.d.); running buffer, 20 mM NaH_2PO_4–H_3PO_4 (pH 2.9); sample injection, 10 kV × 10 s; solution in the detection reservoir, 5 mM $Ru(bpy)_3^{2+}$–100 mM PBS (pH = 7.0); separation voltage, 15 kV.

exceeded 1.20 V, probably due to the formation of Pt oxidation layer at the electrode surface. Therefore, 1.20 V was selected as the optimum detection potential.

3.3. Optimization of sample injection conditions

Electrokinetic injection was used in the experiment. And the sample injection voltage and time influence CE–ECL detection. The injection voltage was fixed at 10 kV, when the injection time was short, high resolution could be obtained but the detection sensitivity was low (data not shown). And the ECL intensity increased correspondingly with the injection time ranging from 2 to 10 s. However, too-long injection time led to low resolution due to the introduction of more analyte in the capillary and overloading occurred. As a result, an injection time of 10 s was used in subsequent experiments to compromise between the ECL intensity and the resolution.

3.4. Optimization of separation buffer

3.4.1. Effect of separation buffer type

Separation buffer has great influence on separation and detection in CE. Three kinds of buffer system including NaH_2PO_4–H_3PO_4, Tris–HCl, and citric acid–sodium citrate buffer were examined. It was found that the use of NaH_2PO_4–H_3PO_4 buffer can lead to good resolution. Additionally, the ECL stability and the *S*/*N* of PPH and FPH were best in this medium. Hence, NaH_2PO_4–H_3PO_4 was chosen as separation buffer.

3.4.2. Effect of the separation buffer pH

The pH of searation buffer affects the electroosmotic flow (EOF) in the capillary and the extent of ionization of each analyte, which determines the migration time, the resolution as well as the sensitivity of the analytes. The results indicated that when pH was higher than 3.5, PPH and FPH could not be separated and only one overlapped peak was obtained in electropherogram. So the effect of pH value of running buffer on ECL intensity and resolution in 20 mM NaH_2PO_4–H_3PO_4 in pH range of 2.3–3.5 (0.2 increments unit) was investigated in detail (Fig. 4). The ECL intensity and resolution increased as pH changed from 2.3 to 2.9, and then decreased when the pH value exceeded 2.9. Therefore, the optimum pH value for separation buffer was set at 2.9 for further experiments.

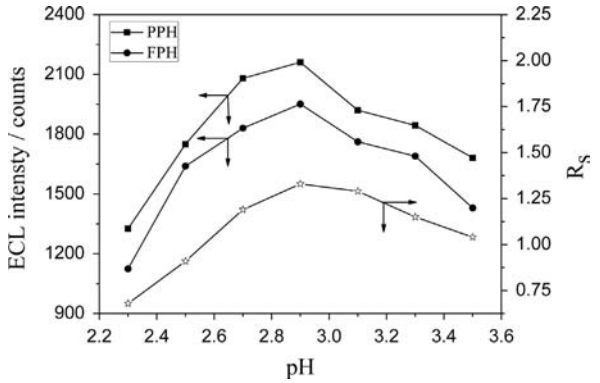


Fig. 4. Effect of running buffer pH on ECL intensity and resolution (R_s) of 2.5 μM PPH and FPH. Conditions: detection potential, 1.20 V. Other conditions as in Fig. 3.

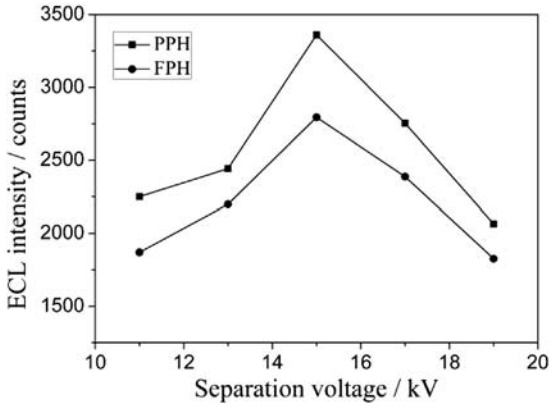


Fig. 5. Effect of separation voltage on ECL intensity. Conditions: detection potential, 1.20 V; running buffer, 10 mM NaH_2PO_4-H_3PO_4 (pH 2.9). Other conditions as in Fig. 3.

3.4.3. *Effect of the separation buffer concentration*

At pH 2.9, the effect of separation buffer concentration was also investigated. The results showed that the highest ECL signal was obtained when the buffer concentration was 10 mM. And the resolution was not improved with the concentration of buffer increasing from 5 to 20 mM. When the concentration was above 20 mM, the separation was worse, the migration time of analytes prolonged gradually and the baseline became unstable. This was ascribed to the increased Joule heating caused by the increased ionic strength. In order to obtain ideal separation and shorter migrating time, 10 mM buffer concentration was selected as the optimum condition.

3.4.4. *Effect of the separation voltage*

The ECL intensity of PPH and FPH was systematically investigated in the separation voltage over the range of 11–19 kV (Fig. 5). For both of the analytes, ECL intensity reached a maximum value at 15 kV and then decreased at a higher value. Since the increase of separation voltage would cause the current to increase, the migration time of analytes would be shortened. However, higher separation voltages resulted in much higher noise as the Joule heating inside the capillary increased from 0.7 to 4.7 μA in the separation voltage range. So 15 kV was chosen to carry out the experiment.

3.5. *Optimization of detection conditions in the ECL cell*

3.5.1. *Effect of pH value of the detection buffer*

It was well known that the ECL reaction of $Ru(bpy)_3^{2+}$ with tertiary amine depends on the buffer pH to a great extent [46], so the influence of buffer pH value in the detection cell on ECL intensity was evaluated with pH value range from 6.0 to 9.0 (0.5 as an interval). As shown in Fig. 6, the ECL emissions of PPH and FPH increased rapidly with increasing pH to a maximum at pH 7.0. The ECL intensity was faint due to the protonation of tertiary amine group under acidic conditions. It was noticed that the ECL intensity was decreased gradually when the pH value was higher than 7.0. The reason might be the competition of the reaction of $Ru(bpy)_3^{3+}$ with OH^- ions in higher pH value [46]. When the pH value exceeded 9.0, the noise of baseline was very large. Thereby the optimized pH value was selected at 7.0 in this study.

3.5.2. *Effect of $Ru(bpy)_3^{2+}$ concentration*

$Ru(bpy)_3^{2+}$ was used as the ECL reagent in the system and its concentration had a great influence on the ECL signal. The ECL intensity increased markedly with increasing $Ru(bpy)_3^{2+}$ concentration in the range from 1.0 to 10.0 mM due to the generation of more amount of electrogenerated excited species $Ru(bpy)_3^{2+*}$. Unfortunately, the background current increased significantly when its concentration exceeded 5 mM. In this work, 5 mM $Ru(bpy)_3^{2+}$ in 100 mM phosphate buffer (pH 7.0) was adopted to produce optimal *S*/*N* value.

3.6. *Method performances*

The optimized CE–ECL detection conditions were as follows: detection potential at 1.2 V (vs. Ag/AgCl), 10 mM NaH_2PO_4–H_3PO_4 (pH=2.9) as running buffer, electrokinetic injection 10 s at 10 kV, separation voltage at 15 kV, 5 mM $Ru(bpy)_3^{2+}$ and 100 mM PBS at pH 7.0 in the detection cell. Under the optimal conditions, the CE–ECL method was successfully applied for the separation and detection of antipsychotic drugs PPH and FPH. The analytical results were summarised in Table 1. The linear range was 0.1–5 μM for PPH with the detection limit of 5 nM (*S*/*N*=3). For FPH, the linear range was 0.1–7.5 μM with the detection limit of 10 nM. The repeatability of this method was obtained from three continuous injections of the standard solution of PPH and FPH at 2.5 μM, the intraday RSD values of the migration time and ECL intensity was less than 0.65 and 2.5%, respectively. The inter-day variation (n=3) was less than 1.7 and 3.4% for the migration time and ECL intensity, respectively.

A comparison of the results obtained by CE–ECL with other methods is clearly shown in Table 2. The detection limit was lower than those by spectrophotometry [3,6], CL [9–11], and CE–ED [14,15], and was comparable to that achieved in ED [4,7,8] and GC–MS [13], but was higher than ECL [5] and FI–CL [12].

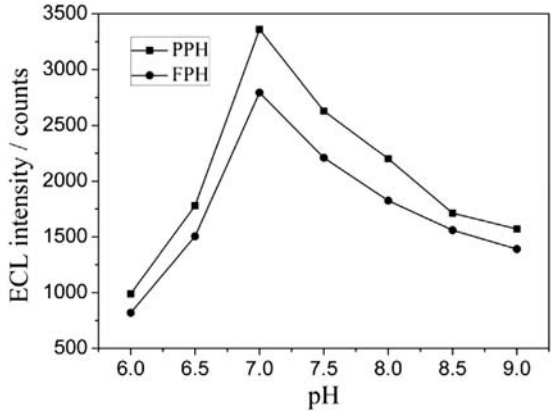


Fig. 6. Effect of the pH value in the detection reservoir on ECL intensity of 2.5 μM PPH and FPH. Conditions as in Fig. 5.

Table 1
Linear ranges, detection limits and repeatability of PPH and FPH detection.

Analyte	Linear range (μM)	Regression equation $Y=BX+A$	Correlation coefficient	Detection limit (nM)	Repeatability (RSD, %)	
					Migration time	ECL intensity
PPH	0.1–5	$Y=1158X+156$	0.9987	5	0.48[a], 1.6[b]	2.5[a], 3.4[b]
FPH	0.1–7.5	$Y=1070X+43$	0.9996	10	0.65[a], 1.7[b]	1.6[a], 3.0[b]

Y represents the ECL intensity; X represents the concentration of PPH or FPH.
[a] The intra-day RSD ($n=3$).
[b] The inter-day RSD ($n=3$).

Table 2
Detection of PPH and FPH by different methods.

Method	Analyte	Linear range (μM)	LOD (nM)	References
ECL	PPH	0.001–3	0.31	[5]
Spectrophotometry	PPH	0.12–61.9	121.3	[6]
ED	PPH	0.006–0.5; 0.5–5	–	[4]
CE–ED	PPH	0.1–100	50	[14]
FI–CL	PPH	0.003–3.22	1	[12]
GC–MS	PPH	0.005–0.16	5	[13]
CL	PPH	0.124–24.8	49.5	[9]
CL	FPH	0.16–11.4	91.4	[11]
CL	FPH	1.14–45.7	34.3	[10]
ED	FPH	0.5–50	5	[7]
ED	FPH	0.05–15	10	[8]
Spectrophotometry	FPH	18.3–73.1	9150	[3]
CE–ED	PPH	1.86–186	149	[15]
	FPH	1.71–171	171	
CE–ECL	PPH	0.1–5	5	This work
	FPH	0.1–7.5	10	

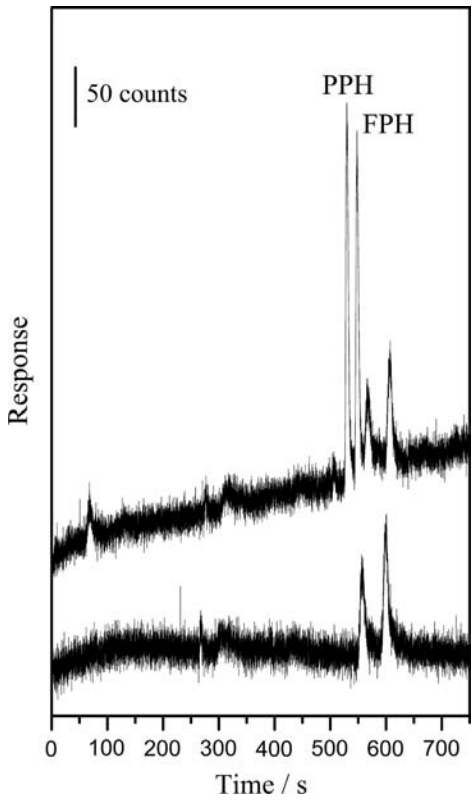


Fig. 7. Electropherograms of 20-fold diluted blank urine sample (a), 20-fold diluted urine spiked with 0.5 μM PPH and FPH. Conditions: separation voltage, 15 kV; other conditions as in Fig. 5.

3.7. Sample analysis

The applicability was also examined by analyzing the antipsychotic drugs in human urine. The typical electropherograms of blank urine sample from a healthy male person and also spiked with 0.5 μM of PPH and FPH are illustrated in Fig. 7. It can be observed that PPH and FPH could be well separated and the unknown compounds from the urine matrix did not interfere with the peaks of the analytes. Calibration graphs were constructed using PPH and FPH-spiked human urine in the concentration range of 0.25–10 μM, and 0.25–15 μM, respectively. The linear regression equation were $Y=596.2X+30.5$ ($R=0.9906$) for PPH and $Y=519.7X+35.3$ ($R=0.9997$) for FPH, where Y was the ECL intensity and X was the concentration of PPH or FPH in μM). And the detection limits of PPH and FPH in urine were 0.10 and 0.15 μM, respectively. The inter-day RSDs of ECL intensity and migration time were less than 4.4 and 1.8%. Compared the calibration curves and results of spiked urine sample with that of standard solution, results including slope, line range and detection limits were different, which might be due to the matrix effects of urine sample. However, matrix did not interfere with the detection of the analytes, which meant the proposed method showed good selectivity in actual sample detection.

4. Conclusion

This work demonstrated a new analytical procedure based on CE–ECL for determination of two antipsychotic drugs PPH and FPH. The two analytes could be well separated within 11 min with high sensitivity, wide linear range, and good reproducibility. In addition, it is an efficient approach for the routine study of PPH and FPH in urine. The established procedure showed its high separation efficiency and sensitivity for PPH and FPH analysis with good selectivity.

Acknowledgements

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