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Short communication

Separation of promethazine and thioridazine using capillary electrophoresis with end-column amperometric detection

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Abstract

Promethazine and thioridazine were separated and detected by capillary electrophoresis with end-column amperometric detection. The influence of pH value on oxidation potential, the peak current and the resolution were studied and the following conditions was selected: 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid at pH 3.0, detection potential at 1.10 V. The detection limits of these two substances were in the range of 10^{-8} mol/l. The linear range spanned two to three orders of magnitude. This method was applied to the detection of promethazine and thioridazine spiked in urine.

Keywords: Promethazine; Thioridazine

1. Introduction

Capillary electrophoresis (CE) has rapidly become a popular separation technique since its first introduction by Jorgenson and Lukacs in 1981 [1,2] because of its high efficiency, good resolution and short analysis time.

The most commonly employed detection method in CE is UV detection, because of the wide range of compounds and functional groups that absorb and its ease to use. However, due to the short light path available, the sensitivity of UV detection is low and the detection limit is high. Electrochemical detection, especially amperometric detection using a microelectrode, which was introduced first by Wallingford and Ewing [3], has been shown to be among the most sensitive detection methods for CE. It offers several advantages over other detection techniques, e.g. high sensitivity, good selectivity and economy. Furthermore, amperometric detection does not need derivation and it is not necessary to consider the pathlength problem.

Amperometric detection includes off-column, end-column and on-column detection. The most-used amperometric detection method was the off-column mode, which needed an electrically conductive joint to minimize the interference of high voltage. The joints consisted of porous materials [4,5], Nafion tubing [6,7] or cellulose acetate [8], etc. In 1991, Huang et al. [9] described another design, called an end-column amperometric detector. In this design, an electrically conductive joint was not needed and the working electrode was directly placed at the outlet of the separation capillary, which is convenient for alignment of the electrode and the capillary.

Thioridazine is an antipsychosis drug and pro-

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methazine is an antihistamine drug. The two drugs have the same phenazine ring, but, when one uses them at the same time, it is possible to cause discomfort or other undesirable reaction. It was reported [10] that 15 mg of chlorpromethazine, 50 mg of thioridazine and 75 mg of promethazine together could cause a patient to die. However, when the synthesis of thioridazine was not carefully controlled, promethazine could be present in the sample as an impurity. So, the separation and detection of the two drugs was an important task.

This article is concerned with the separation and determination of the two drugs spiked in urine using end-column amperometric detector. A newly designed and laboratory-made end-column amperometric cell was used, which was convenient for the proper alignment of the capillary and the working electrode. The influence of pH on the oxidation potential, the peak current and the resolution were studied. The treatment of a carbon fiber microdisk electrode on line was also discussed.

2. Experimental

2.1. Cyclic voltammetry

A model 832 electrochemical analyzer (CH Instruments, TN, USA) connected to a 486 computer was used for all cyclic voltammetry studies. A three-electrode system was used with a carbon fiber microdisk electrode as the working electrode, a Pt wire as the auxiliary electrode and a Ag/AgCl electrode (in saturated KCl solution) as the reference electrode.

2.2. Capillary electrophoresis

A reversible polarity high-voltage power supply (Beijing Institute of New Technique, China) provided a variable voltage of 0~30 kV. The outlet of the capillary was at ground potential with Pt wire. Fused-silica capillaries (360 μm O.D. and 25 μm I.D.) were purchased from Heibei Yongnian Optical Conductive Fiber Plant, China. Amperometric detection at a constant potential with CZE was performed using the end-column approach with a model 832 electrochemical analyzer or a voltammeter (Jinan Fourth Radio Factory, China) and a microamperometer (model 902-pA, Ningde Analytical Instrument, China). The electropherograms were recorded by a recorder (JASCO, Japan). A piece of capillary was cut to the desired length (35 cm in this experiment) and was positioned in the electrochemical cell. The detection cell and the detector were housed in a faradic cage in order to minimize the interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system with a carbon fiber microdisk electrode, a Ag/AgCl electrode and a Pt wire electrode as described above. The working electrode was placed at the outlet of the separation capillary, not inserted into the capillary.

2.3. Electrode preparation

The procedure for the construction of a carbon fiber microdisk electrode was described previously [11,12]. It was constructed with 30 μm diameter carbon fiber. About 3 cm was cut and was carefully introduced into the capillary (75 μm I.D., 360 μm O.D., 2.5 cm length) until the fiber protruded about 2~3 mm from the tip. Epoxy glue was used to seal the carbon fiber at the detection end. After solidification of the epoxy glue (about 24 h), the capillary, which was enclosed with the carbon fiber electrode, was introduced into a glass capillary (0.5 mm I.D., 2 mm O.D. and 3 cm length). A copper wire was sealed with epoxy glue to the other end of the glass capillary and cured for 24 h. The microdisk electrode was carefully polished with 1.0, 0.3, 0.1 and 0.05 μm α-Al_2O_3 on a polishing cloth. Finally, the carbon fiber microdisk electrode was ultrasonicated in ethanol and double-distilled water for 1 and 3 min respectively, then was stored until required.

2.4. Capillary conditions

A new fused-silica capillary was filled with 0.1 *M* NaOH solution for 24 h before use and then flushed with doubly distilled water and the corresponding separation electrolyte for 3~5 min. To achieve good reproducibility, the capillary was refilled with H_2O, 0.1 *M* NaOH, H_2O and the running buffer for 3 min, respectively, between two injections.

2.5. Reagents and chemicals

Promethazine hydrochloride and thioridazine hydrochloride were obtained from Aldrich (USA) and were dissolved in doubly distilled water at a concentration of 10 m*M*, as a stock solution. Before use, the stock solution was diluted with doubly distilled water to desirable concentrations. All other chemicals, including buffer substances and supporting electrolytes, were of analytical reagent grade and were used as received, without further purification.

All solutions and samples were prepared from doubly distilled water. Before use, all samples and buffer solutions were filtered through 0.45 μm cellulose acetate filters (Jiangsu Qilin Medical Instrument Factory, China).

3. Results and discussion

3.1. Cyclic voltammetry of promethazine and thioridazine

Cyclic voltammograms of 10^{-4} *M* promethazine and thioridazine were recorded in a solution containing 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid at different pH values in the potential range of ~0.50–1.50 V (versus Ag/AgCl) for promethazine and ~0.20–1.30 V (versus Ag/AgCl) for thioridazine. It was proved that the pH of the buffer solution has little effect on the oxidation potential and peak current. In all buffer solutions, promethazine and thioridazine yielded an irreversible oxidation peak at 0.60–0.70 V, indicating that the two substances could be detected electrochemically. Typical cyclic voltammograms are shown in Fig. 1.

3.2. Capillary electrophoresis

3.2.1. Hydrodynamic voltammetry of promethazine and thioridazine

From the cyclic voltammetry, it was seen that the promethazine and thioridazine could be oxidized at a potential of less than 1.0 V. However, because the mobile system was different from a static system, to find the optimal detection potential, hydrodynamic voltammetry of promethazine and thioridazine was carried out and the hydrodynamic voltammograms obtained are shown in Fig. 2. The maximum current response was obtained at 1.10 V. When the potential

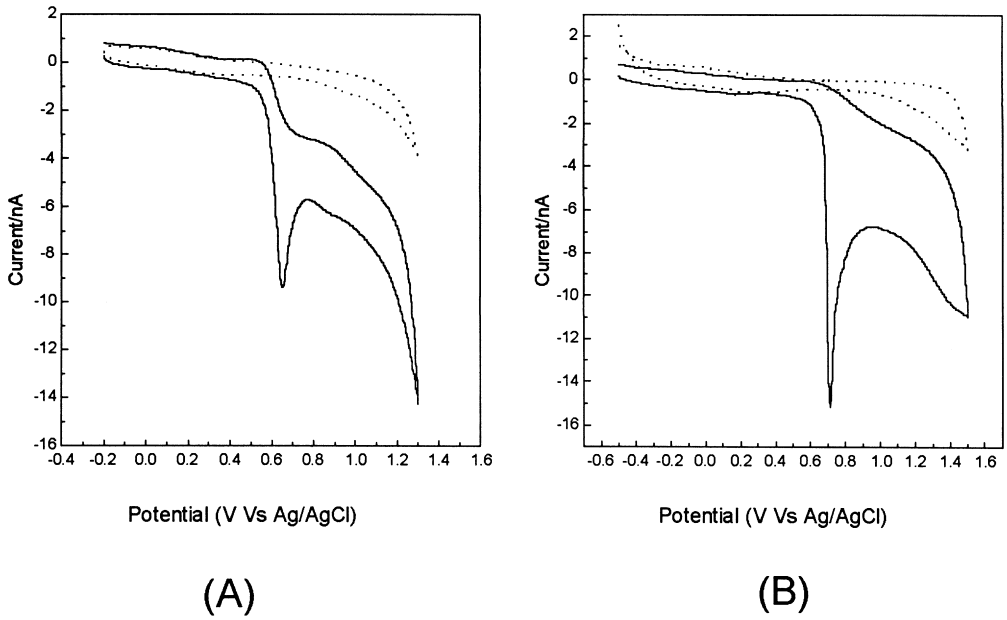

Fig. 1. Cyclic voltammograms of (A) promethazine and (B) thioridazine. Conditions: 10^{-4} *M* promethazine and thioridazine. Buffer: 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid at pH 3.0, v=0.05 V/s.

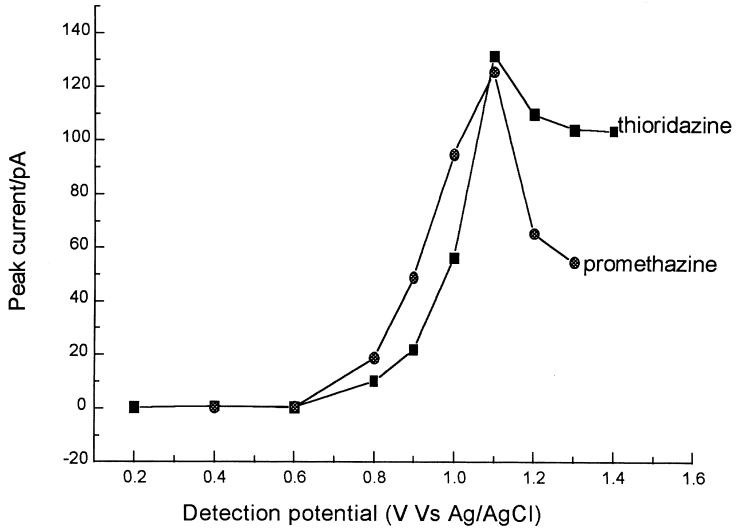

Fig. 2. Hydrodynamic voltammograms of promethazine and thioridazine Conditions: 3×10^{-6} *M* promethazine or thioridazine. Buffer: 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid, pH 3.0. Capillary: 25 μm I.D., 35 cm. Injection: by electromigration, 10 kV, 3 s. Separation voltage: 10 kV.

was less positive than 1.10 V, the response of the two substances increased with increasing detection potential, but when the detection potential was more positive than 1.10 V, the response became smaller with increasing potential. The optimum detection potential in hydrodynamic voltammetry shifted towards positive potential compared to that in cyclic voltammetry. This shift was probably caused by resistance effects with the flow cell. From the hydrodynamic voltammetry experiments, a detection potential of 1.10 V was selected.

3.2.2. Electrochemical treatment of the electrode on-line

Promethazine and thioridazine had certain absorptions on the carbon fiber electrode and the response became smaller when samples were injected several times without treatment. Ultrasonic cleaning was an effective treatment method when the electrode was not mounted to the detection cell. However, when the electrode and the capillary had been mounted to the cell, it was no longer suitable. In order to obtain reproducible results, an on-line treatment method had to be found. Several electrochemical treatment methods were tried and the following conditions were selected: anodic polarization at 2.0 V for 120 s, cathodic polarization at −2.0 V for 10 s, then, stabilization at 0.0 V for 120 s. The procedure was repeated again. The conditions were suitable for thioridazine, but the peak current also decreased slightly for promethazine. The results are shown in Fig. 3.

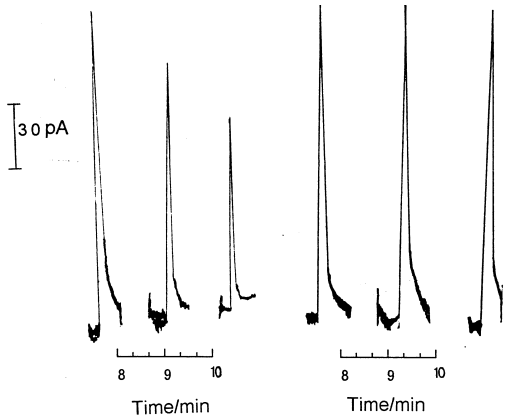

Fig. 3. Influence of electrode treatment on the response of thioridazine (A) without treatment and (B) after treatment. Conditions: 3×10^{-6} *M* thioridazine; detection potential, 1.10 V (versus Ag/AgCl); buffer: 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid, pH 3.0; Capillary, 25 μm I.D., 35 cm long; injection by electromigration at 10 kV for 3s; separation voltage, 10 kV.

3.2.3. Effect of pH on resolution

The pH value of the electrolyte solution in CE is a significant influence on the separation. In our experiments, the pH of the buffer solution was changed from 3.0 to 7.0 in 0.5 pH units. It was proved that, on increasing the pH value, the resolution became poorer. This was probably because promethazine and thioridazine were both cations in the pH range studied; their electrophoretic mobilities were almost stable, but the electroosmotic flow increased with increasing pH, and the apparent electrophoretic mobilities became bigger, which led to the poor resolution. Based on the experimental results, a pH value of 3.0 was selected. Typical separation electropherograms are shown in Fig. 4, where the response of the promethazine was larger than that of thioridazine at the same concentration, but, from the hydrodynamic voltammograms shown in Fig. 2, it can be seen that the peak currents of thioridazine and promethazine were almost the same; the reason was probably that the promethazine absorbed at the carbon fiber electrode, it was detected at electrode first, the electrode was deactivated, so the response of thioridazine became smaller. This explanation can be proved by the following experiments: the response of thioridazine and promethazine was almost the same when they were at the same concentration if injecting them respectively (see Fig. 5).

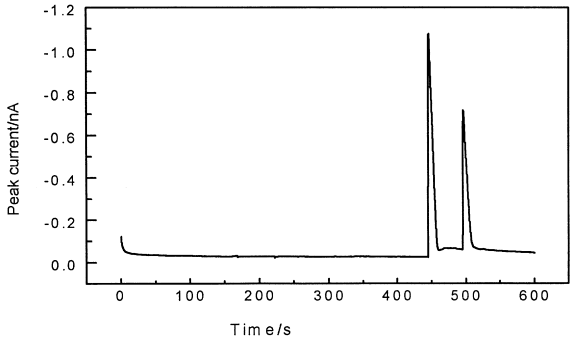

Fig. 4. Electropherograms of (A) promethazine and (B) thioridazine. Conditions: 10^{-5} *M* promethazine or thioridazine; detection potential (versus Ag/AgCl), 1.10 V; buffer, 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid, pH 3.0; Capillary, 25 μm I.D., 35 cm long; injection, electromigration at 10 kV for 3 s; separation voltage, 10 kV.

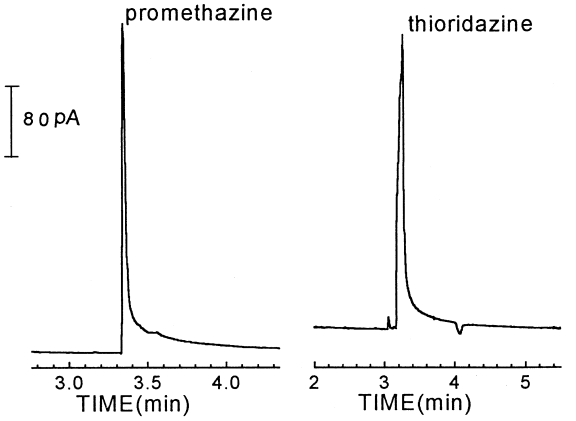

Fig. 5. Electropherograms of 10^{-5} *M* promethazine and thioridazine, injected respectively. Buffer: 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid, pH 3.0. Capillary: 25 μm I.D., 35 cm long. Injection: electromigration at 10 kV for 3 s. Separation voltage: 10 kV.

3.2.4. Reproducibility, detection limit, linear range and stability of the electrode

The response for a series of eight injections of 1×10^{-5} *M* promethazine resulted in a relative standard deviation of 0.6% for t_m (migration time) and 8.5% for i_p (peak current of promethazine and thioridazine), respectively, and for thioridazine, the relative standard deviation was 0.3% for t_m and 5.2% for i_p, respectively. With a signal-to-noise ratio of three, the detection limits for promethazine and thioridazine were 3×10^{-8} *M* and 1×10^{-8} *M*, respectively. Regression analysis for promethazine over the concentration range 1×10^{-7}–3×10^{-5} *M* resulted in a correlation coefficient of 0.9991 ($n=6$), and the slope of the curves obtained was 27.9 pA. For thioridazine, the linear concentration range was 1×10^{-7}–1×10^{-4} *M*, the linear correlation coefficient was 0.9986 ($n=7$), and the slope of the curve was 13.46 pA. The calibration curves were carried out in samples containing promethazine and thioridazine, respectively. The theoretical plate numbers were 26 300 and 33 100 for promethazine and thioridazine, respectively.

The carbon fiber microdisk electrode used in the experiments was stable and its current response did not change much over a period of three months if the electrode surface was refreshed. We used the same electrode for all of the experiments. Furthermore, the differences between electrodes were very small.

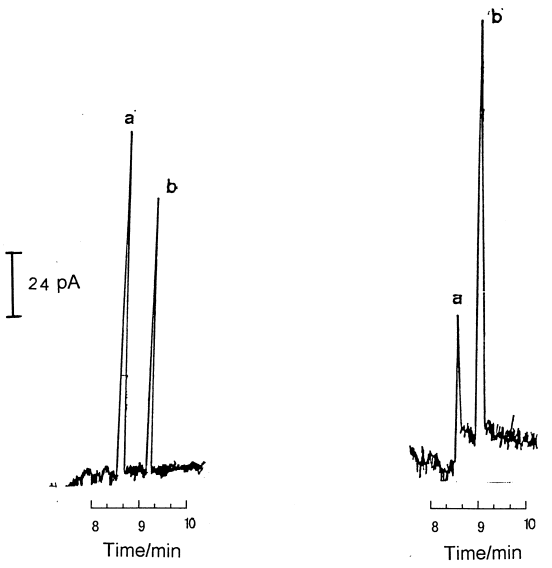

Fig. 6. Electropherograms of two substances in urine. (A) Promethazine (3×10^{-6} *M*) (a) and 5×10^{-6} *M* thioridazine (b), and (B) 1×10^{-6} *M* promethazine (a) and 1×10^{-5} *M* thioridazine (b). Buffer: 0.03 *M* Na_2HPO_4 and 0.015 *M* citric acid, pH 3.0. Capillary: 25 μm I.D., 35 cm long. Injection, by electromigration at 10 kV for 3 s. Separation voltage: 10 kV.

3.2.5. Urine sample

The purpose of this work was to determine promethazine and thioridazine in urine samples. To examine the feasibility of this method, the following experiment was done:

Normal human urine was diluted ten times with doubly distilled water, as the blank solution. Promethazine (3×10^{-6} *M*) and thioridazine (1×10^{-6} *M*) or 1×10^{-6} *M* promethazine and 1×10^{-5} *M* thioridazine were added to the urine. Under optimum separation conditions, the two substances were separated and detected. Other components in urine did not interfere with the detection. The method was simple, sensitive and rapid. It could be used for clinical detection. The electropherograms are shown in Fig. 6A and B.

4. Conclusion

Promethazine and thioridazine could be baseline-separated using capillary electrophoresis with end-column amperometric detection using a carbon fiber microdisk electrode. The method had good sensitivity, selectivity, and the analysis time was short (less than 10 min). Electrochemical treatment of the electrode on line was also found. This method could be used for the analysis of urine from patients.

Acknowledgements

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