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Short communication

Amperometric detection of perphenazine at a carbon fiber micro-disk bundle electrode by capillary zone electrophoresis

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Abstract

A simple method for determination of perphenazine by capillary zone electrophoresis with amperometric detection is described. The optimum conditions of separation and detection are 1.50×10^{-3} mol/l $Na_2B_4O_7-1.0\times10^{-3}$ mol/l NaOH (pH 9.9) for the buffer solution, 18 kV for the separation voltage, 5 kV and 5 s for the injection voltage and the injection time, and 0.80 V versus saturated calomel electrode for the detection potential, respectively. The limit of detection is 5.0×10^{-8} mol/l or 44 amol ($S/N=3$). The linear range of the calibration curve is 1.00×10^{-7} to 1.00×10^{-4} mol/l. The relative standard deviation is 1.5% for the migration time and 2.9% for the electrophoretic current at peak maximum. The method is applied to the determination of perphenazine in human urine.

Keywords: Carbon fiber micro-disk bundle electrode; Perphenazine

1. Introduction

Perphenazine, which is chemically known as 4-[3-(2-chlorophenothiazin-10-yl)propyl]-1-piperazine-ethanol (Fig. 1), is a neuroleptic drug used in the treatment of psychotic disorders such as schizophrenia and schizoaffective psychoses in order to decrease restlessness, aggressiveness and impulsive behavior [1]. The methods proposed for perphenazine determination include spectrophotometry [2–4], electrochemical method [5], high-performance liquid chromatography (HPLC) [6,7] and gas chromatography–mass spectrometry [8]. Spectrophotometry lacks the sensitivity. The interference of the substances in the biological samples is a problem for electrochemical method. HPLC is a suitable technique for biological samples, but the procedure of

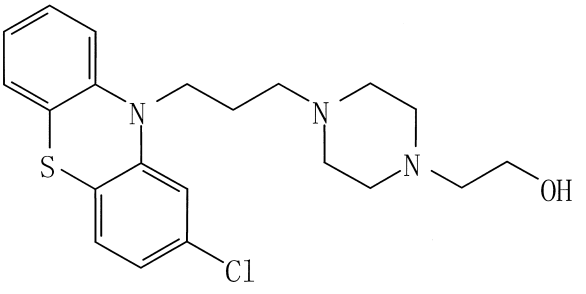

Fig. 1. Chemical structure of perphenazine.

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sample treatment and separation is elaborate and time-consuming, and requires large sample volume [7].

Capillary zone electrophoresis (CZE) appears to be one of the most suitable analytical methods for the determination and monitoring of drugs and their metabolites in biological fluids [9]. The primary strength of CZE is its ability to provide extremely high separation efficiencies in short times and need small sample volume. Wu et al. used CZE with UV detector to separate perphenazine in a drug mixture [10]. Although Maleček et al. determined perphenazine by CZE with UV detection [11], they could not separate it from other substances in urine. Moreover, the sensitivity of these methods is low. In CZE, electrochemical detection provides excellent sensitivity and high selectivity toward electroactive species [12]. There are no reports on the determination of perphenazine by CZE with electrochemical detection.

This paper demonstrates the utility of the end-column amperometric detection at a carbon fiber micro-disk bundle electrode with CZE for measurement of perphenazine. Because of its excellent selectivity and sensitivity, the method can be used to determine perphenazine in human urine.

2. Experimental

2.1. Apparatus

2.1.1. Cyclic voltammetry

A commercial polarograph (Model 83-2.5, Ningde Analytical Instruments, China) coupled with an *X*–*Y* recorder (Model 3086-11, Yokogawa Hokuskin, Japan) was used. It was used in connection with a cell, using potentiostatic control of the electrode potential by means of a three-electrode system, which consisted of a carbon fiber bundle electrode as the working electrode, a Pt wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. The reference electrode was connected to the analyte via a salt bridge filled with the same supporting electrolyte as in the cell.

2.1.2. Capillary zone electrophoresis

A reversible high-voltage power supply (Model GDY, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China) provided a variable voltage of 0–30 kV across the capillary with the outlet of the capillary at ground potential. Fused-silica capillaries (360 μm O.D., 20 μm I.D.) were purchased from Yongnian Optical Conductive Fiber Plant, China. Electrochemical detection at a constant potential was performed using the end-column amperometric approach with a voltammetric analyzer (Model JF-01, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China). Electrochemical detection was carried out with a three-electrode system, which consisted of a carbon fiber micro-disk bundle electrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the electrophoretic cathode, and a SCE as the reference electrode. The carbon fiber micro-disk bundle electrode was cemented onto a microscope slide, which was placed over a laboratory-made *xyz*-micromanipulator and glued in place. The electrode and the outlet end of the capillary were put in the electrochemical cell (also as the buffer reservoir of the outlet end of the separation capillary). The electrochemical cell with the three electrodes were housed in a Faradaic cage in order to minimize interference from noise of external sources. The position of the carbon fiber micro-disk bundle electrode was adjusted (under a microscope) against the outlet end of the capillary of 40 cm in length, so that the electrode and the capillary were in contact. The inlet end of the capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. The inlet end of the capillary with the syringe tip was placed in another buffer reservoir. A high voltage was applied at the electrophoretic anode, while the electrophoretic cathode (i.e., the Pt auxiliary electrode in the electrochemical cell) was held at ground potential. Separations were carried out at an applied voltage of 18 kV. The arrangement of the electrochemical cell was illustrated in Ref. [13] in detail. The carbon fiber micro-disk bundle electrodes used here were described previously [14].

2.2. Reagents and solutions

A 1.00×10^{-2} mol/l stock solution of perphenazine was prepared by dissolving an appropriate amount of perphenazine (Shandong Institute for

Drug Control, China) in alcohol and stored at 4 °C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with corresponding buffer containing 10% alcohol. All reagents were of analytical grade. All solutions were prepared with double-distilled water.

2.3. Procedure

For cyclic voltammetry the carbon fiber bundle electrode must be pre-scanned four to five times between 0 and 1 V versus SCE in 1.00×10^{-2} mol/l $Na_2B_4O_7$–2.5×10^{-3} mol/l NaOH buffer containing 10% alcohol, until a steady cyclic voltammogram was obtained. The carbon fiber bundle electrode was directly inserted in the experimental solution containing perphenazine, and a cyclic voltammogram was recorded. The electrode must be cleaned in water for 2 min with a ultrasonicator before each detection.

In CZE, before each run, the capillaries were flushed with double-distilled water, 0.1 mol/l NaOH, double-distilled water and the corresponding separation electrolyte, respectively, by means of the syringe at the inlet end of the capillary. After the electroosmotic flow reached a constant value, the electromigration injection by inserting the inlet end of the capillary into the sample solution was carried out. Then, the inlet end of the capillary was put in the running buffer. The separation voltage was applied across the capillary and the detection potential was applied at the working electrode. The electropherogram was recorded. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. All potentials were measured versus SCE.

3. Results and discussion

3.1. Optimum conditions of CZE with end-column amperometric detection

The voltammetric characteristics of perphenazine have been reported at the carbon paste electrode [5]. It was found that perphenazine could also be oxidized at the carbon fiber bundle electrode in $Na_2B_4O_7$–NaOH buffer, pH 9.5. Fig. 2 shows its typical cyclic voltammogram in this solution. An

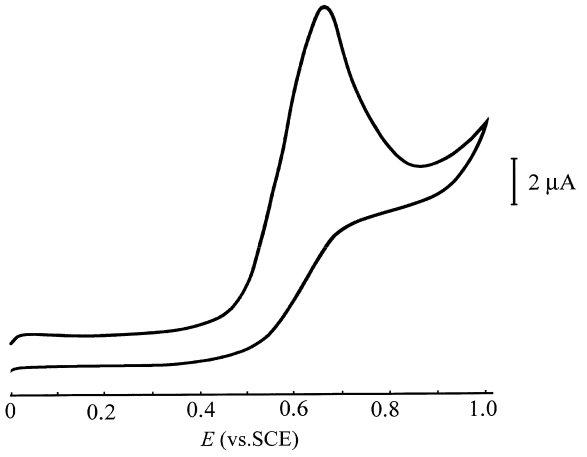

Fig. 2. Typical cyclic voltammogram of perphenazine at a carbon fiber bundle electrode in 1.00×10^{-2} mol/l $Na_2B_4O_7-2.5\times10^{-3}$ mol/l NaOH containing 10% alcohol of pH 9.5; 1.00×10^{-3} mol/l perphenazine; scan rate, 50 mV/s.

oxidation peak of perphenazine at ca. 0.66 V is observed, which means that perphenazine can be measured by electrochemical detection on the carbon fiber electrode. Therefore, the electrophoretic behavior of perphenazine in six $Na_2B_4O_7$–NaOH solutions in the pH range of 9.3–12 was investigated. The migration time, t_m, increases slowly with increasing pH. Both the current at peak maximum, i_p, and the number of theoretical plates, N, first increase and then decrease with increasing pH. The highest i_p and N are obtained at pH 9.9. Therefore, this value was selected. In this case, perphenazine in the solution was moved toward the electrophoretic cathode by electroosmotic flow. t_m increases with increasing the concentration of the buffer, C_B. Both i_p and N have the maximum when C_B is 1.50×10^{-3} mol/l. In our experiments 1.50×10^{-3} mol/l $Na_2B_4O_7-1.0\times10^{-3}$ mol/l NaOH was used. t_m decreases with increasing the separation voltage, V_s. Both i_p and N increase first and then decrease slightly with increasing V_s. The best suitable V_s is 18 kV.

Fig. 3 shows the relationship between i_p and the applied potential, E_d. When E_d is between 0.55 and 0.80 V, i_p increases rapidly with increasing E_d. When $E_d>0.80$ V, i_p is almost a constant value. When $E_d>0.80$ V is applied, the baseline of detection current gets higher, and noise gets higher. Therefore, E_d of 0.80 V is suitable for detection because of

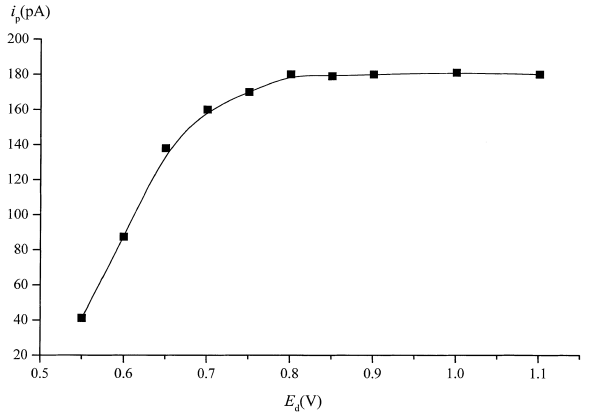

Fig. 3. Relationship between detected current at peak maximum and detection potential. 1.50×10^{-3} mol/l $Na_2B_4O_7-1.0\times10^{-3}$ mol/l NaOH. 2.00×10^{-5} mol/l perphenazine; capillary, 40 cm length, 20 mm I.D.; injection, 5 kV for 5 s; separation voltage, 18 kV; detection potential, 0.80 V.

good reproducibility, low noise of the baseline and fine shape of the electropherograms.

3.2. Reproducibility, limit of detection and linear range

The response for a series of eight injections of 2.00×10^{-5} mol/l perphenazine resulted in relative standard deviation of 1.5% for t_m and 2.9% for i_p, respectively. The limit of detection is 5.0×10^{-8} mol/l (according to the ratio of signal-to-noise of 3) or 44 amol for the injected volume calculated. A linear relationship holds between the current at peak maximum detected and the concentration of perphenazine in the range of $1.00\times10^{-7}-1.00\times10^{-4}$ mol/l. Least-squares treatment of these data yielded a slope 0.92 pA μmol^{-1} l and a correlation coefficient of 0.9999.

3.3. Determination of perphenazine in human urine

Two fresh human urine samples added perphenazine (final concentrations are 1.00×10^{-4} and 2.00×10^{-4} mol/l, respectively) were used to verify the method. After diluting 100 times, the human urine samples were injected into the capillary. Only one peak corresponding to perphenazine appears on the electropherogram of the diluted urine samples between 2 and 5 min (Fig. 4), which shows good selectivity. Usually, the internal standard method and the absolute quantitation method were used in CZE for quantitation. If some compounds can affect the concentration of the free analytes in the detection solution, the standard addition method is more suitable for quantification. The electropherograms of the diluted human urine sample containing 1.00×10^{-6} mol/l perphenazine without and with the

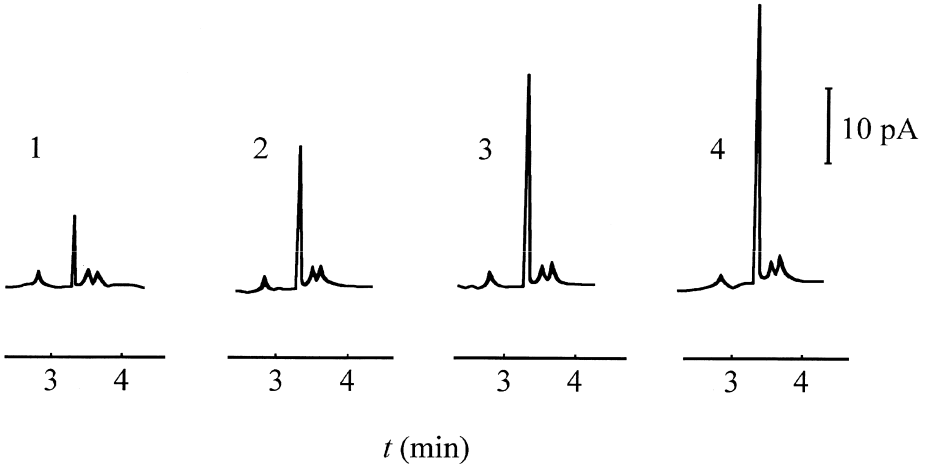

Fig. 4. Electropherograms of the diluted human urine sample containing 1.00×10^{-6} mol/l perphenazine. Added concentration of perphenazine (mol/l): 1, 0; 2, 1.00×10^{-6}; 3, 2.00×10^{-6}; 4, 3.00×10^{-6}. Conditions as in Fig. 3.

Table 1
Results of determination of perphenazine in the diluted synthetic human urine samples

Sample	Concentration (10^{-6} mol/l)	Average concentration (10^{-6} mol/l)	RSD (%)
A	0.960, 1.04, 0.980	0.993	4.2
B	2.05, 1.90, 2.05	2.00	4.3

standard solution of perphenazine are shown in Fig. 4. The results obtained for the urine samples by using the standard addition method are listed in Table 1. The recovery of the method is between 95 and 107%. The concentrations of perphenazine in two human urine samples estimated by the standard addition method are 0.993×10^{-4} and 2.00×10^{-4} mol/l, respectively, which agree with the values in the human urine samples.

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References

[1] C. Dollery (Ed.), Therapeutic Drugs, Vol. 2, Churchill Livingstone, Edinburgh, London, Melbourne, New York, Tokyo and Madrid, 1991, p. 36.
[2] E. Regulska, M. Tarasiewicz, H. Puzanowska-Tarasiewicz, J. Pharm. Biomed. Anal. 27 (2002) 335.
[3] J.M. García, A.I. Jimenez, F. Jimenez, J.J. Arias, Anal. Lett. 25 (1992) 1511.
[4] J.M. García, A.I. Jimenez, F. Jimenez, J.J. Arias, Anal. Chim. Acta 252 (1991) 107.
[5] T.Z. Peng, Z.P. Yang, R.S. Lu, Chin. Anal. Pharm. Sinica 25 (1990) 277.
[6] J.P. Foglia, D. Sorisio, M.A. Kirshner, B.H. Mulsant, J.M. Perel, J. Chromatogr. B 668 (1995) 291.
[7] N.E. Larsen, L.B. Hansen, P. Knudsen, J. Chromatogr. 341 (1985) 244.
[8] R. Ventura, M. Casasampere, R. Bergés, J. Femández-Morán, J. Segura, J. Chromatogr. B 769 (2002) 79.
[9] K.D. Altria, in: Analysis of Pharmaceuticals by Capillary Electrophoresis, F. Vieweg & Sohn, Braunschweig/Wiesbaden, 1998.
[10] H.F. Wu, F.Y. Guan, Y. Luo, Chin. Anal. Pharm. Sinica 32 (1997) 377.
[11] J. Maleček, E. Hadašova, J. Havel, J. Cap. Elec. Microchiptech. 6 (1999) 151.
[12] P.D. Voegel, R.P. Baldwin, Electrophoresis 18 (1997) 2267.
[13] W. Jin, Q. Weng, J. Wu, Anal. Chim. Acta 342 (1997) 67.
[14] W. Jin, D. Yu, Q. Dong, X. Ye, Electrophoresis 21 (2000) 925.