ELSEVIER

Journal of Chromatography B, 783 (2003) 509–515

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Determination of midecamycin by capillary zone electrophoresis with electrochemical detection

Daxing Liu[1], Wenrui Jin*

School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, China

Received 6 May 2002; received in revised form 9 September 2002; accepted 9 September 2002

Abstract

Capillary zone electrophoresis was employed for the determination of midecamycin using an end-column amperometric detection with a carbon fiber micro-disk bundle electrode at a constant potential of +1.15 V vs. saturated calomel electrode. The optimum conditions of separation and detection are 1.00×10^{-3} mol l^{-1} Na_2HPO_4–3.49×10^{-4} mol l^{-1} NaOH (pH 11.4) for the buffer solution, 20 kV for the separation voltage, 5 kV and 5 s for the injection voltage and the injection time, respectively. The limit of detection is 5.0×10^{-7} mol l^{-1} or 0.41 fmol ($S/N=3$). The linear range of the calibration curve is 1.00×10^{-6}–1.00×10^{-3} mol l^{-1}. The relative standard deviation is 1.4% for the migration time and 4.9% for the electrophoretic peak current. The method could be applied to the determination of midecamycin in human urine. In this case, a separation voltage of 14 kV was used.

Keywords: Midecamycin

1. Introduction

Midecamycin is a broad-spectrum antibiotic. Several methods have also been developed for the determination of midecamycin involving thin-layer chromatography [1], GC–MS [2], spectrophotometry [3] and liquid chromatography [4,5]. Spectrophotometry lacked sensitivity. Other methods were either relatively complex or time consuming. Capillary zone electrophoresis (CZE) has become a powerful new method for rapid separation and detection of complex mixtures including drugs [6–9]. The primary strength of CZE is its ability to provide extremely high separation efficiencies in short times. Amperometric detection provides excellent sensitivity for the small dimensions associated with CZE, while offering not only a high degree of selectivity toward electroactive species but also low cost [10]. Midecamycin is electrochemically active. There have been several studies on the voltammetric determination of midecamycin at water–oil interfaces [11–13] and on the voltammetric behavior of midecamycin in aqueous solutions [14,15]. Nevertheless, there are no reports on the determination of midecamycin by CZE.

In this work, we developed a method for the detection of midecamycin with the end-column amperometric detection at a carbon fiber micro-disk bundle electrode. The separation was performed in a

*Corresponding author. Fax: +86-531-856-5167.

E-mail address: wenrujin@jn-public.sd.cninfo.net (W. Jin).

[1]Present address: Institute of Military Medicine of Jinan Military Region, Jinan 250014, China.

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00675-X

20 μm I.D. fused-silica capillary. The detection was carried out by using potentiostatic control of the electrode potential by means of a three-electrode system. The method has been used to determine midecamycin in human urine. The main advantage of the method is simple. The samples can be directly injected. No pre-treatment of the samples is needed. The migration time is also short (approximately 6 min).

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. It 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 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. They were cut to a length of 50 cm and placed between two buffer reservoirs. High voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 20 kV. 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). The detection cell and detector were housed in a Faradaic cage in order to minimize interference from external sources of noise. 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 ground for the high potential drop mentioned above across the capillary and a SCE as the reference electrode. The arrangement of the electrochemical detection cell was illustrated in Ref. [16] in detail. The carbon fiber microdisk bundle electrodes used here were described previously [17].

2.2. Reagents and solutions

A 1.00×10^{-2} mol l^{-1} stock solution of midecamycin was prepared by dissolving an appropriate amount of midecamycin (Shandong Institute for Drug Control, China) in water containing 40% alcohol and stored at 4 °C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with corresponding buffer containing 4% 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 4~5 times between 0.2 and 1.2 V vs. SCE in the 1.00×10^{-2} mol l^{-1} Na_2HPO_4–3.49×10^{-3} mol l^{-1} NaOH buffer (containing 4% alcohol), until a steady cyclic voltammogram was obtained. The carbon fiber bundle electrode was directly inserted in the experimental solution containing midecamycin, and a cyclic voltammogram was recorded. The electrode must be cleaned in water for 2 min with the supersonic wave cleaner before each detection.

In CZE, the carbon fiber micro-disk bundle electrode was cemented onto a microscope slide, which was placed over a home-made XYZ micro-manipulator and glued in place. The position of the carbon fiber micro-disk bundle electrode was adjusted (under a microscope) against the end of the capillary, so that the electrode and the capillary were in contact. This arrangement allowed one to easily

remove and realign both the capillary and the electrode. The other 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. Before each run, the capillaries were flushed with double distilled water, 0.1 mol l^{-1} NaOH, double distilled water and the corresponding separation electrolyte, respectively, by means of a syringe. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electroosmotic current reached a constant value, the electromigration injection was carried out and the electropherogram was recorded. The separation electrolyte in the capillary was replaced after five or six runs.

All potentials were measured vs. SCE.

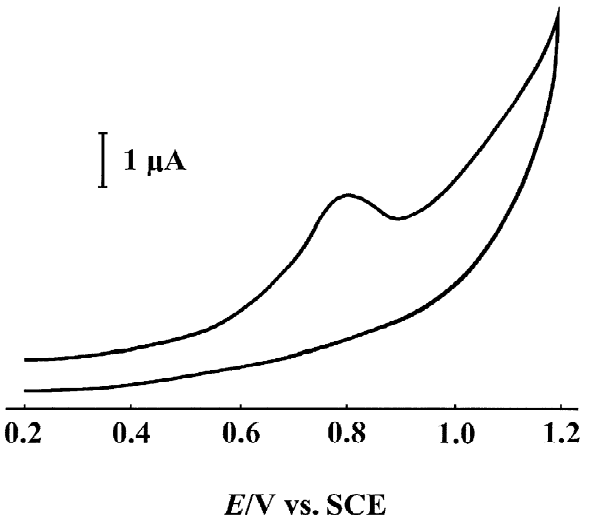

Fig. 1. Typical cyclic voltammogram of midecamycin at the carbon fiber bundle electrode in 1.00×10^{-2} mol l^{-1} Na_2HPO_4–3.49×10^{-3} mol l^{-1} NaOH (containing 4% alcohol) of pH 11.4. 1.00×10^{-3} mol l^{-1} midecamycin, $v=50$ mV s^{-1}.

3. Results and discussion

3.1. Cyclic voltammogram of midecamycin

The voltammetric characteristics of midecamycin have been reported at the mercury electrode [14] and glass carbon electrode [15]. We found that midecamycin can also be oxidized at the carbon fiber bundle electrode in Na_2HPO_4–NaOH buffer of pH 11.4. Fig. 1 shows its typical cyclic voltammogram in this solution. An oxidation peak of midecamycin at ca. 0.78 V is observed and no reduction peak appears.

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

At pH 11.4 there is an oxidation peak of midecamycin in the cyclic voltammogram shown in Fig. 1. Therefore, the electrophoretic behavior of midecamycin in five solutions with pH values around pH 11.4 was investigated. The migration time, t_m, the peak current, i_p, the width at the half-peak, $W_{1/2}$ on the electropherograms and the number of theoretical plates, N, at different pH are listed in Table 1. t_m decreases and then increases slightly with increasing pH, and there is a minimum at pH 11.4. Both i_p and N first increase and then decrease with increasing pH, and the highest i_p and N are obtained at pH 11.4. Therefore, pH=11.4 was selected. The effect of the concentration of the buffer, C_B, on t_m, i_p and N in Na_2HPO_4–NaOH is illustrated in Fig. 2. In Fig. 2, C_B only indicates the value of concentration of Na_2HPO_4, the ratio of the concentration of Na_2HPO_4 to the concentration of NaOH is 1:0.35. t_m increases with increasing C_B. i_p increases and then decreases with increasing C_B, and it has the highest value at 1.00×10^{-3} mol l^{-1}. N increases rapidly in the range of $0.50–1.00\times10^{-3}$ mol l^{-1} and then it increases much slowly with increasing C_B. In our experiments 1.00×10^{-3} mol l^{-1} Na_2HPO_4–3.49×10^{-4} mol l^{-1} NaOH was used. t_m, i_p, $W_{1/2}$ and N at different separation voltage, V_s, are listed in Table 2. With increasing V_s, i_p increases. Since V_s becomes higher, the migration velocity increases and the axial diffusion of the analyte becomes lower, which causes zone-narrowing, i.e. $W_{1/2}$ becomes smaller. In this case, the molecules of the analyte can concentrate at the working electrode. Therefore, i_p becomes larger. N is almost a constant with increasing V_s. Noise increases with increasing V_s. Considering both i_p and noise, 20 kV for V_s was chosen because of larger i_p and lower noise.

Table 1
The values of t_m, i_p, $W_{1/2}$ and N in Na_2HPO_4–NaOH at different pH (5.00×10^{-5} mol l^{-1} midecamycin, capillary: 50 cm length, 20 μm I.D.; injection, 5 kV for 5 s; separation voltage, 20 kV; detection potential, 1.15 V)

Buffer	pH	t_m/s	i_p/pA	$W_{1/2}$/s	$10^{-4}N$
1.00×10^{-3} mol l^{-1} Na_2HPO_4 -1.65×10^{-4} mol l^{-1} NaOH	11.0	287	19.6	4.9	1.9
1.00×10^{-3} mol l^{-1} Na_2HPO_4 -2.40×10^{-4} mol l^{-1} NaOH	11.2	266	46.9	3.5	3.2
1.00×10^{-3} mol l^{-1} Na_2HPO_4 -3.49×10^{-4} mol l^{-1} NaOH	11.4	242	71.1	2.9	3.9
1.00×10^{-3} mol l^{-1} Na_2HPO_4 -4.90×10^{-4} mol l^{-1} NaOH	11.6	246	60.0	3.0	3.7
1.00×10^{-3} mol l^{-1} Na_2HPO_4 -6.65×10^{-4} mol l^{-1} NaOH	11.8	249	54.3	3.4	3.0

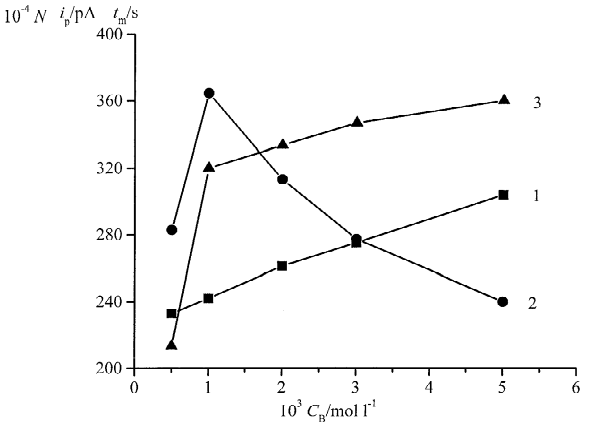

Fig. 2. Dependence of (1) the migration time, (2) the peak current detected and (3) the number of theoretical plates on the buffer concentration. Conditions as in Table 1.

Fig. 3 shows the relationship between i_p and the applied potential, E_d. When E_d is between 0.80 and 1.15 V, i_p increases rapidly with increasing E_d. When $E_d>1.15$ V, i_p is almost a constant. When $E_d>1.15$ V is applied, the base line of detection current gets higher, and noise gets higher. Therefore, E_d of 1.15 V is suitable for detection because of good reproducibility, low noise of the base line and fine shape of the electropherograms. Fig. 4 shows the typical electropherograms of 5.00×10^{-5} and 1.00×10^{-6} mol l^{-1} midecamycin at optimum conditions. The shape of the electrophoretic peak is symmetric. Small peak width and little tailing of the peak were obtained.

Table 2
The values of t_m, i_p, $W_{1/2}$ and N at different V_s (1.00×10^{-3} mol l^{-1} Na_2HPO_4–3.49×10^{-4} mol l^{-1} NaOH. Other conditions as in Table 1)

V_s/kV	t_m/s	i_p/pA	$W_{1/2}$	$10^{-4}N$
12	402	52.7	4.7	4.1
14	348	60.6	4.0	4.2
16	305	64.2	3.6	4.0
18	268	66.5	3.2	4.0
20	242	71.1	2.9	3.9
22	221	72.0	2.7	3.7

3.3. Reproducibility, limit of detection and linear range

The response for a series of six injections of 5.00×10^{-5} mol l^{-1} midecamycin resulted in relative

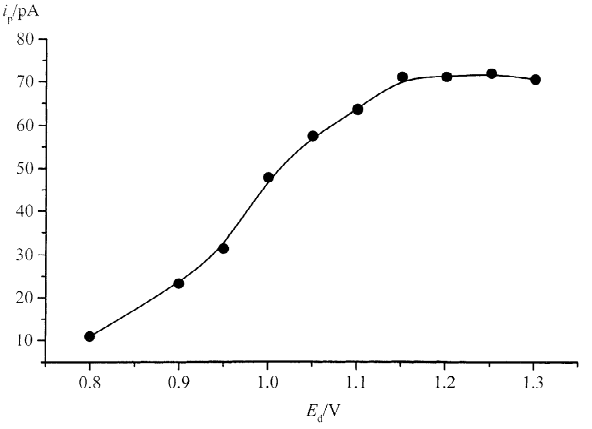

Fig. 3. Relationship between detected peak current and detection potential. 1.00×10^{-3} mol l^{-1} Na_2HPO_4–3.49×10^{-4} mol l^{-1} NaOH. Other conditions as in Table 1.

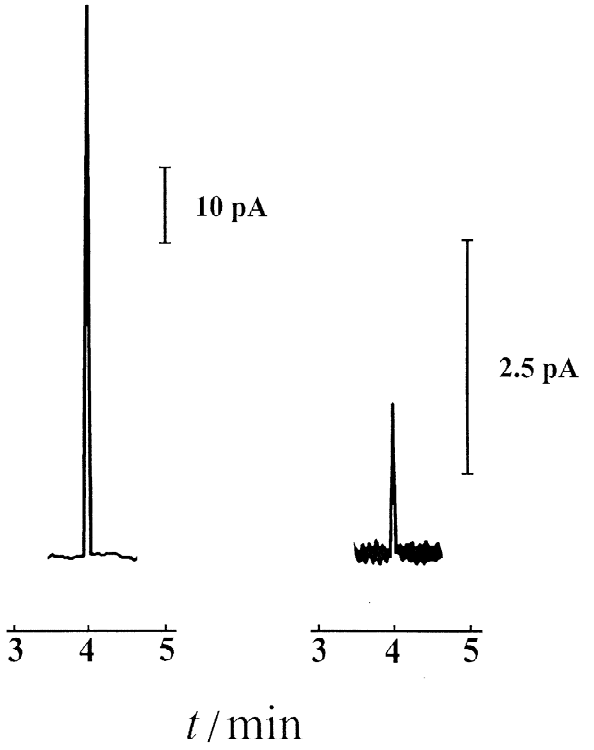

Fig. 4. Typical electropherograms of midecamycin. Concentration of midecamycin: (1) 5.00×10^{-5} mol l^{-1}; (2) 1.00×10^{-6} mol l^{-1}. Conditions as in Fig. 3.

standard deviation of 1.4% for t_m and 4.9% for i_p, respectively. The limit of detection is 5.0×10^{-7} mol l^{-1} (according to ratio of signal-to-noise of 3), which was estimated from the electropherograms obtained for 1.00×10^{-6} mol l^{-1} midecamycin (see Fig. 4. curve 2), or 0.41 fmol for the injected volume calculated. A linear relationship holds between the peak current detected and concentration in the range of 1.00×10^{-6}–1.00×10^{-3} mol l^{-1}. Least-squares treatment of these data yielded a slope 1.49 pA μmol^{-1} l and a correlation coefficient of 0.9998.

3.4. Determination of midecamycin in human urine

Fig. 5 shows the electropherograms of midecamycin (curve 1), urine sample without midecamycin (curve 2) and with 1.00×10^{-5} mol l^{-1} midecamycin (curve 3) under the conditions chosen above. Peak A and B in the electropherograms of the urine sample were not identified. From Fig. 5, it can

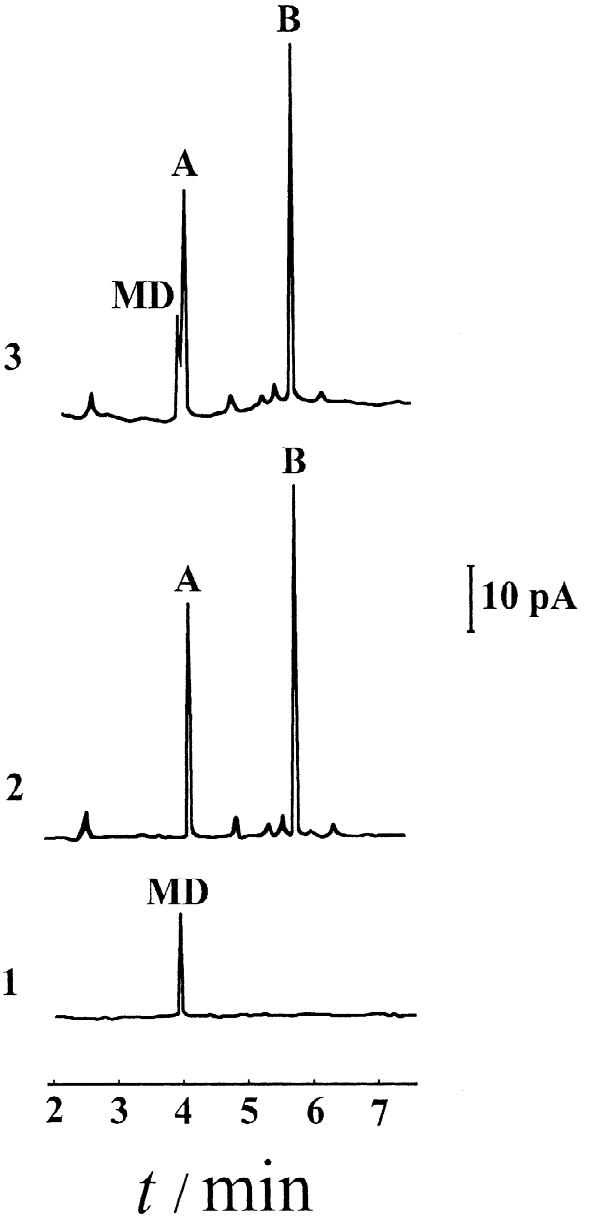

Fig. 5. Electropherograms of (1) the standard midecamycin, MD, (2) the urine sample and (3) the urine sample containing 1.00×10^{-5} mol l^{-1} midecamycin. Separation voltage, 20 kV. Conditions as in Fig. 3.

be found that midecamycin can not be separated completely from the peak A on the electropherogram. In order to separate midecamycin from the peak A in urine sample, a lower separation voltage is

used. When a separation voltage of 14 kV is used, midecamycin can be well separated (see Fig. 6). The calculated resolution is 1.5.

Usually, the internal standard method and the absolute quantitation method are used in CZE for quantification. If some compounds present in the sample solution can affect the concentration of the free analytes, the standard addition method is more suitable for quantification. Two human urine samples containing 1.00×10^{-3} and 5.00×10^{-3} mol l^{-1} midecamycin, respectively, were used to verify the possibility of the standard addition method. After diluting 100 times, the human urine sample was injected into the capillary. The electropherograms of the diluted human urine sample containing 1.00×10^{-3} mol l^{-1} midecamycin without and with the standard solution of midecamycin are shown in Fig. 7. The results obtained for the urine sample by using the standard addition method are listed in Table 3. The concentrations of midecamycin in two human urine samples obtained by the standard addition method are 1.04×10^{-3} and 5.25×10^{-3} mol l^{-1}, respectively, which agree with the values in the human urine samples. The recovery is between 97 and 106%.

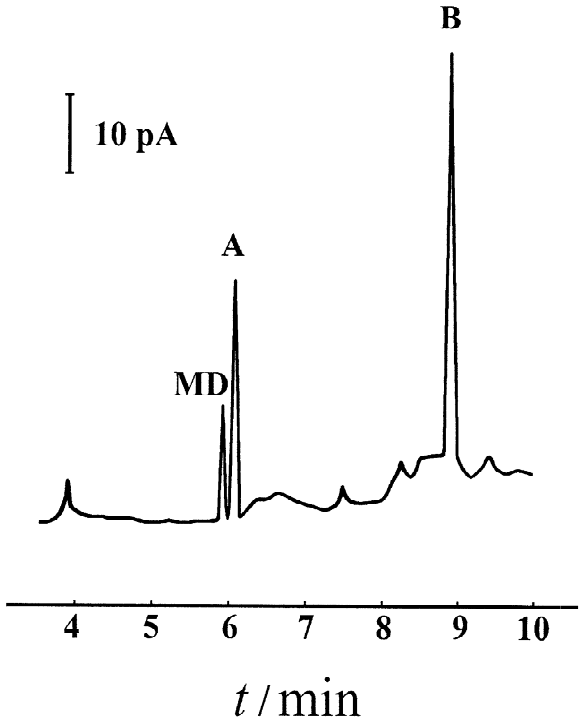

Fig. 6. Electropherograms of urine containing 1.00×10^{-5} mol l^{-1} midecamycin with the separation voltage at 14 kV. Other conditions as in Fig. 3.

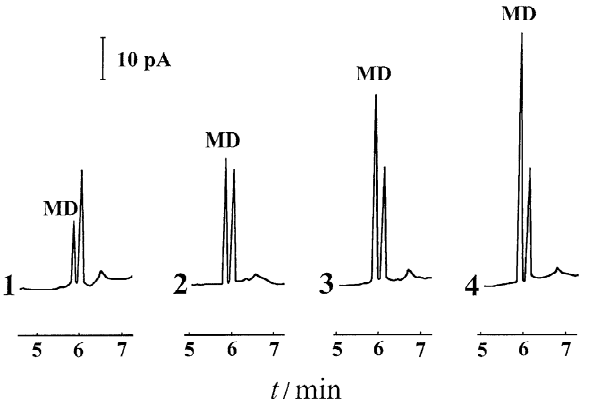

Fig. 7. Electropherograms of midecamycin in the human urine sample. The concentration of midecamycin (mol/l): 1, sample; 2, (1)+1.00×10^{-5}; 3, (1)+2.00×10^{-5}; 4, (1)+3.00×10^{-5}. V_s=14 kV, other conditions as in Fig. 3.

Table 3
Results of determination of midecamycin in synthetic human urine samples

Sample	Concentration (10^{-3} mol l^{-1})	Average concentration (10^{-3} mol l^{-1})	RSD (%)
A	1.05, 1.03, 1.04	1.04	0.96
B	5.25, 5.45, 5.05	5.25	3.8

Acknowledgements

This project was supported by the National Natural Science Foundation of China and the State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

References

[1] H. Vanderhaeghe, L. Kerremans, J. Chromatogr. 193 (1980) 119.

[2] K. Takasuki, S. Suzuki, N. Sato, I. Shaito, J. Assoc. Off. Anal. Chem. 70 (1987) 708.
[3] K. Li, Q. Deng, J. Shao, S. Yan, Zhongshan Daxue Xuebao 29 (1990) 124.
[4] H. He, R. Yu, Q. Yang, Z. Yu, X. Wang, Chin. Acta Pharm. Sinica 26 (1991) 156.
[5] Y. Yuan, J. Zhou, F. Zhao, Chin. J. Pharm. Anal. 13 (1993) 116.
[6] S. Furlanetto, S. Orlandini, G. Massolini, M.T. Faucci, E. La Porta, S. Pinzauti, Analyst 126 (2001) 1700.
[7] J.A. Prieto, U. Akesolo, R.M. Jimenez, R.M. Alonso, J. Chromatogr. A 916 (2001) 279.
[8] C. Garcia-Ruiz, A.L. Crego, J.L. Lavandera, M.L. Marina, Electrophoresis 22 (2001) 2775.
[9] S. Hillaert, W. Van den Bossche, J. Pharm. Biomed. Anal. 21 (1999) 65.
[10] P.D. Voegel, R.P. Baldwin, Electrophoresis 18 (1997) 2267.
[11] R. Fan, Y. Chang, J. Electroanal. Chem. 256 (1988) 207.
[12] R. Fan, S. Li, W. Huang, Chinese J. Anal. Chem. 18 (1990) 912.
[13] Z. Yu, Y. Zhao, D. Guo, E. Wang, Chinese J. Appl. Chem. 8 (3) (1991) 33.
[14] R. Fan, F. Ji, J. Qian, Q. Wu, C. Bian, Y. Yang, Acta Pharmacol. Sin. 30 (1995) 34.
[15] Y. Yang, S. Li, Chin. J. Anal. Chem. 28 (2000) 974.
[16] W. Jin, Q. Weng, J. Wu, Anal. Chim. Acta 342 (1997) 67.
[17] W. Jin, D. Yu, Q. Dong, X. Ye, Electrophoresis 21 (2000) 925.