Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 835 (2006) 84–89
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simultaneous electrochemiluminescence determination of sulpiride and tiapride by capillary electrophoresis with cyclodextrin additives

Jianguo Li [1], Fengjuan Zhao, Huangxian Ju *

Key Laboratory of Analytical Chemistry for Life Science (Education Ministry of China), Department of Chemistry, Nanjing University, Nanjing 210093, PR China

Received 30 November 2005; accepted 9 March 2006
Available online 29 March 2006

Abstract

Sulpiride and tiapride are often used in the treatment of depression, schizophrenia and psychopathology of senescence, gastric or duodenal ulcers and are also partly excreted by kidney. This work developed a simple and sensitive method for their simultaneous monitoring in human urine based on capillary electrophoresis coupled with electrochemiluminescence detection by end-column mode. β-Cyclodextrin (β-CD) was used as an additive to the running buffer to obtain the absolute separation of sulpiride and tiapride. Under optimized conditions the proposed method displayed a linear range from 1.0×10^{-7} to 1.0×10^{-4} M for both sulpiride and tiapride with the correlation coefficients more than 0.995 ($n = 6$). Their limits of detection were 1.0×10^{-8} M (45 amol) and 1.5×10^{-8} M (68 amol) at a signal to noise ratio of 3, respectively. The relative standard deviations for six determinations of 2.0 μM sulpiride and 3.0 μM tiapride were 1.8 and 2.5%, respectively. For practical application an extract step with ethyl acetate at pH 11 was performed to eliminate the influence of ionic strength in sample. The recoveries of sulpiride and tiapride at different levels in human urine were between 84 and 95%, which showed that the method was valuable in clinical and biochemical laboratories for monitoring sulpiride and tiapride for various purposes.

Keywords: Capillary electrophoresis; Electrochemiluminescence; Sulpiride; Tiapride; β-Cyclodextrin; Simultaneous determination

1. Introduction

Sulpiride and tiapride are substituted benzamides with analogous structures (Fig. 1) and exhibit antipsychotic, antidepressive and antiulcer properties [1]. They are antagonist of the D_2 and D_4 brain dopamine receptors with a low frequency of extrapyramidal side-effects [2] and have been used in the treatment of depression, schizophrenia and psychopathology of senescence, gastric or duodenal ulcers and irritable colon due to psychosomatic stress and various vertigo syndromes [3]. Sulpiride can be slowly and poorly absorbed from the gastrointestinal tract with peak serum levels occurring in 2–6 h [4]. It does not appear to be completely metabolized, showing that 70–90% of an intravenous dose and 15–25% of an oral dose is excreted unchanged in the urine [5,6]. Several methods, including gas chromatography [7], high performance liquid chromatography (HPLC) with ultraviolet [8], fluorescence [9–12] or mass spectrometric detection [13,14], capillary electrophoresis with electrochemiluminescence (CE–ECL) [15] and ultraviolet [16], for detection of sulpiride have been developed. In HPLC or LC analysis of sulpiride, tiapride is often used as internal standard [10,13,14,17]. They are a homogeneous class of chemicals derived originally from procainamide. Thus a sensitive and rapid method for simultaneous determination of sulpiride and tiapride is required for industrial quality control and clinical monitoring.

A column-switching high-performance liquid chromatographic method with fluorescence detection has been reported for simultaneous determination of sulpiride and tiapride within 18 min [18]. This work proposed a simple, rapid and sensitive method for their simultaneous determination using CE–ECL detection by end-column mode. Capillary electrophoresis has been considered to be a significant complementary technique to LC [19]. Its low injection volume is quite beneficial for bioanalytical applications especially in the in vivo monitoring of biofluids. Although a CE–ECL method for sulpiride has been estabilished based on a $Ru(bpy)_3^{2+}$/tertiary amine system

* Corresponding author. Tel.: +86 25 83593593; fax: +86 25 83593593.
E-mail address: hxju@nju.edu.cn (H. Ju).
[1] Permanent address: Department of Chemistry, Suzhou University, Suzhou 215006, PR China.

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.03.017

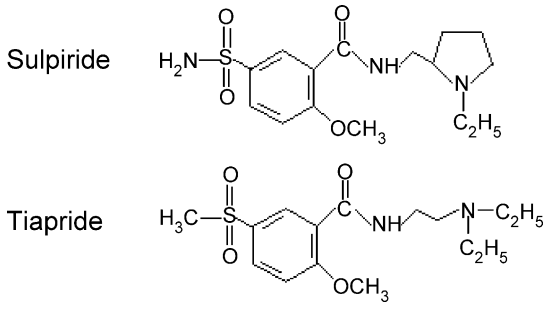

Fig. 1. Formulae of sulpiride and tiapride.

[15], its sensitivity and practicability for substituted benzamide compounds need to be further improved. Here β-cyclodextrin (β-CD) was used as an additive to the running buffer of CE separation for this purpose. With the same $Ru(bpy)_3^{2+}$/tertiary amine system the sensitivity for CE–ECL detection of sulpiride was also improved. Furthermore, a new method for CE–ECL detection of tiapride was also developed.

β-Cyclodextrin, consisting of seven glucopyranose units, is now widely used as run buffer additive for CE analyses due to the formation of inclusion complex between β-CD and analyte, which strongly influences the electroosmotic flow (EOF) of analyte [16,20,21]. In this work β-CD changed obviously the EOFs of both sulpiride and tiapride, thus the absolute separation of sulpiride and tiapride were achieved. This method could be very useful for carrying out simultaneous studies of the pharmacokinetics and pharmacodynamics of sulpiride and tiapride.

2. Materials and methods

2.1. Materials

All reagents and chemicals used were commercially available and of analytical grade. The sulpiride and tiapride were obtained from Chinese Pharmaceutical and Biological Test Institute (Beijing). Tris(2,2′-bipyridyl)ruthenium(II) chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared with water purified in a Milli-Q System (Millipore, Bedford, MA). The stock solutions of sulpiride and tiapride were stored in the refrigerator (4 °C). Standard solutions of sulpiride and tiapride were prepared by appropriate dilution with water of the stock solution. All standard solutions and phosphate buffers were weekly prepared and filtered through 0.22 μm cellulose acetate filters (Shanghai Xinya Purification Material Factory) prior to injection.

2.2. Apparatus and procedures

A programmable high-voltage power supply (0–20 kV, Remax Electronic Co., Ltd., Xi'an, China) was applied to perform the electrokinetic sample injection and electrophoretic separation. An uncoated fused-silica capillary with 50 cm length, 25 μm i.d. and 360 μm o.d. was used for separation (Yongnian Optical Fiber Factory, Hebei, China). Before use, the capillary was flushed with 0.1 M sodium hydroxide solution over night. The electrochemical measurements in CE–ECL experiments were carried out on a MPI-A multifunctional electrochemical analytical system (Xi'an Remex Electronic and Technological Co.) with a three-electrode system comprising platinum wire as counter, Ag/AgCl (3.0 M NaCl) as reference and a 500-μm platinum disk as working electrodes. The ECL emission was detected with a Model BPCL Ultraweak Chemiluminescence Analyzer (Institute of Biophysics, Beijing) in a pulse mode, which was sensitive to photons with a wavelength range of 200–800 nm. The working electrode was adjusted and fixed by three screws from three different directions to align with the capillary under the microscope. The gap between working electrode and capillary was controlled at 70 ± 5 μm [22,23]. The reference and counter electrodes were inserted into the solution above both the capillary and the working electrode. The lower layer of cell was made of a piece of optic glass through which the photons were captured by PMT, which was biased at 800 V. Four hundred and fifty microliters of 50 mM pH 6.0 phosphate buffer containing 5.0 mM $Ru(bpy)_3^{2+}$ was added to the cell for CE–ECL detection.

Electrophoresis in the capillary was driven by a high-voltage power supply (12 kV, 4 μA), which was applied at the injection end with the detection cell held at ground potential through the separation capillary guide. During the experiment, the separation voltage was applied at the injection end, with the reservoir in the ECL detection cell held at ground potential, and the detection potential was applied at the working electrode. In all experiments, sample introduction was accomplished by electrokinetic injection for 10 s at 10 kV (about 4.5 nl) [22]. Before use, the capillary was flushed with purified water and the running buffer for 15 min by means of a syringe. The running buffer (pH 5.0) contained 20 mM phosphate and 8.0 mM β-CD. After each run the electrode was treated with a cyclic voltammetric scan in a potential range of −0.5 to 0 V at 100 mV/s for 2 min [22,24], ascertaining to get better resolution and reproducibility. After a stable baseline ECL signal was reached, electromigration injection was used for sample introduction, and the electropherogram was recorded. The sample concentrations were quantified by ECL peak intensities.

2.3. Sample preparation

The blank urine samples of healthy people collected from student volunteers in the laboratory were used as matrix to spike sulpiride and tiapride. To eliminate the influence of ionic strength in sample and obtain clear electrophoretic profile a modified Rurak's extraction procedure [25] was done before electrophoresis. Urine samples (200 μl) or the spiked samples were pipetted into clean 2.0 ml centrifugation tubes and were alkalinized by adding 100 μl of saturated sodium carbonate solution. One milliliter of ethyl acetate was then added to each sample and the tubes were capped. The samples were vortically mixed using a medium motion on a shaker for 30 min, and then centrifugated at 2000 rpm for 10 min. The top organic layer was separated and transferred into a clean set of centrifugation tubes. This procedure was repeated and the obtained organic layers were mixed, which was evaporated to dryness under a

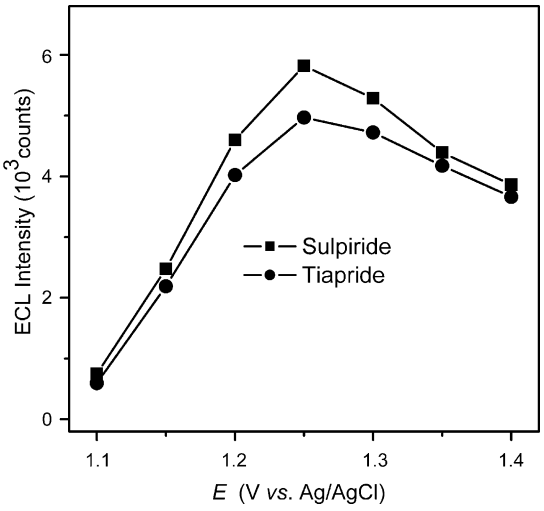

Fig. 2. Effects of applied potential on ECL intensity of 4.0 μM sulpiride and tiapride. Injection, 10 kV for 10 s; running buffer, 20 mM PBS + 8.0 mM β-CD at pH 5.0; separation voltage, 12 kV; ECL cell, 5.0 mM $Ru(bpy)_3^{2+}$ + 50 mM PBS at pH 6.0; PMT voltage, −800 V.

gentle stream of nitrogen at 35 °C in a water bath. The residue was reconstituted with 200 μl of water and vortically mixed for 60 s. Finally the sample solution of about 4.5 nl was injected into the electrophoresis system by electrokinetic injection. The extraction efficiency was estimated by measuring the peak intensity of non-extracted standard solution compared with that of corresponding spiked sample after extracting at each analyte concentration.

3. Results and discussion

3.1. Effect of applied potential on ECL

As shown in Fig. 2, the applied potential for ECL detection was investigated towards sulpiride and tiapride by changing from +1.10 to +1.40 V. When the applied potential was less than +1.10 V, light emission was not observed since $Ru(bpy)_3^{2+}$ was not oxidized on the electrode. With the increasing applied potential from +1.1 V the ECL intensities for both sulpiride and tiapride increased and reached the maximum values at +1.25 V, which was selected as the optimum potential for ECL detection of sulpiride and tiapride. It was noted that with a cyclic voltammetric treatment of the electrode the ECL detector could keep excellent stability after working for two months without polishing the electrode, and the sensitivity did not change significantly.

3.2. Effects of pHs of ECL detection solution and CE running buffer

The pH of running buffer is one of the most important parameters for improving selectivity in CE, especially for closely related compounds. At the same pH condition of the running buffer and detection solution, the ECL intensity for both sulpiride and tiapride reached the maximum value at pH 6.0 (Fig. 3A), therefore, pH 6.0 was selected for ECL detection. However, the optimal pH for the resolution occurred at the running buffer of pH 5.0 (Fig. 3B). The maximum resolution was 1.85. When pH was more than 6.0, the resolution of sulpiride/tiapride was lower than 1.4. This was because the pH of running buffer influenced not only the electroosmosis of the complexes but also the interaction between the hydroxyl groups on the rim of β-CD and substituents near the asymmetric center of the analyte. In order to obtain a good separation result, we selected pH 5.0 as the pH value of running buffer for following experiments.

3.3. Effect of concentration of β-CD additive on resolution

The separate selectivity depends on the relative concentration of β-CD [20]. Fig. 4 shows the effect of β-CD concentration on the CE–ECL electrophoregram of sulpiride/tiapride. With the increasing concentration of additive, the retention time increased and the separation resolution was improved. The electrophoregram showed the best resolution at the β-CD concentrations more than 8.0 mM. Hence, 8.0 mM β-CD was used for simultaneous CE–ECL detection of sulpiride and tiapride so that the good separation and short analysis time could be gained.

3.4. Effect of separation voltage on CE–ECL

The increase of separation voltage would cause the current to increase, and the analysis time would be shortened. However, the too high voltage led to the Joule's heat and affected the separation of the tested analyte. The effect of separation volt-

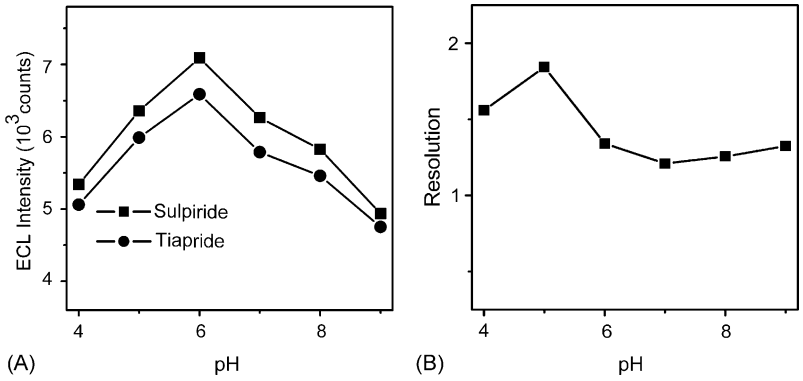

Fig. 3. Effects of pH on ECL intensity (A) and resolution (B) of 5.0 μM sulpiride and tiapride. Detection potential, +1.25 V; other conditions as in Fig. 2.

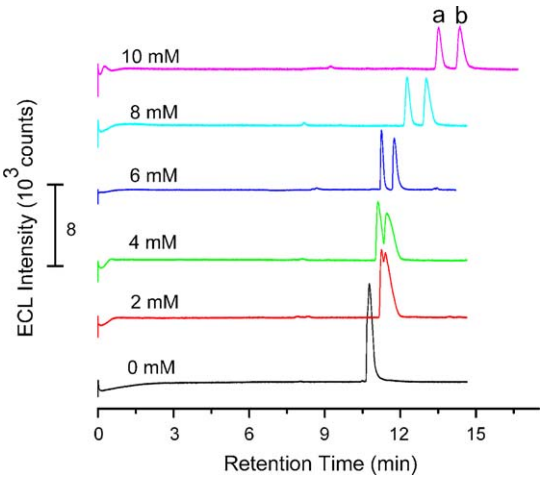

Fig. 4. Effect of β-CD concentration in running buffer on separation of 4.0 μM sulpiride (a) and 6.0 μM tiapride (b) under other optimal conditions.

age was optimized in the range of 8–18 kV. Fig. 5 shows the electrophoregrams of sulpiride and tiapride obtained at various CE voltages. With the increasing separation voltage, the EOF increased, thus more analyte in the effluent arrived in the diffusion layer of working electrode within a given time, higher ECL signal and better peak shape could be obtained. This observation was in agreement with the results reported [24,26]. Although part of the electrophoretic current might still leak into the ground through the working electrode, it only shifted the redox potential of the $Ru(bpy)_3^{2+}/Ru(bpy)_3^{3+}$ couple, but did not affect the ECL reaction [25]. To obtain a good separation and short analysis time, the separation voltage of 12 kV was used as the optimal condition.

3.5. Detection limit, linearity and reproducibility

Under optimal conditions the typical electrophoregrams of the two analytes at different concentrations were shown in Fig. 6. The retention times of two compounds were independent of their

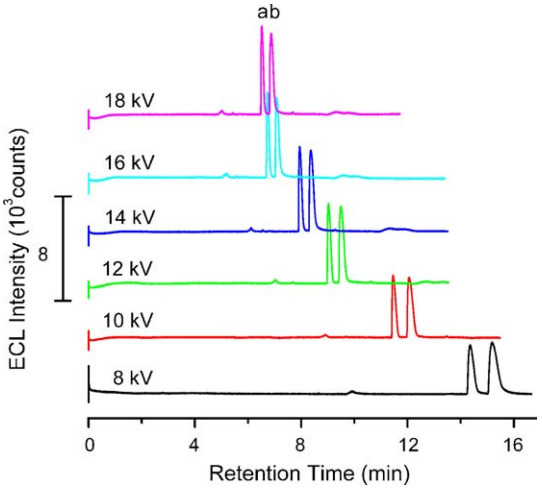

Fig. 5. CE–ECL electropherograms for separation of 4.0 μM sulpiride (a) and 6.0 μM tiapride (b) at different separation voltages under other optimal conditions.

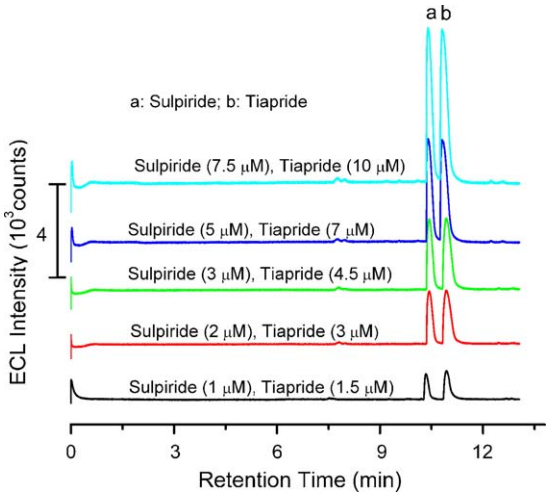

Fig. 6. CE–ECL Electropherograms for separation of sulpiride (a) and tiapride (b) at different concentrations under optimal conditions.

concentrations and within 12.0 min. The separation efficiencies of were 52125 and 40236 plates/m, respectively. In the concentration range of 0.1–100 μM, the ECL intensity was proportional to the concentration with the correlation coefficients of 0.9989 for sulpiride and 0.9985 for tiapride with the regression equations of ECL intensity = $(958.6 \pm 182.2) + (2631.5 \pm 60.5)\,c$ μM and $(705.3 \pm 207.2) + (2293.5 \pm 55.7)\,c$ μM, respectively. The limit of detection at a signal to noise ratio of 3 was determined to be 1.0×10^{-8} M (45 amol) and 1.5×10^{-8} M (68 amol), respectively. The repeatability of this method was examined by five consecutive injections of standard mixture of 2.0 μM sulpiride and 3.0 μM tiapride. As shown in Fig. 7, the relative standard derivations (R.S.D.) of the ECL intensity and migration times were 1.8 and 0.67% for sulpiride and 2.5 and 0.62% for tiapride, respectively.

3.6. Application

Because electrokinetic injection mode was employed in this work, the ionic strength of sample matrix would influence the injection of samples. On the other hand, some organic compounds in urine might influence the ECL reaction. Therefore, an extraction procedure was performed to remove the ions and some

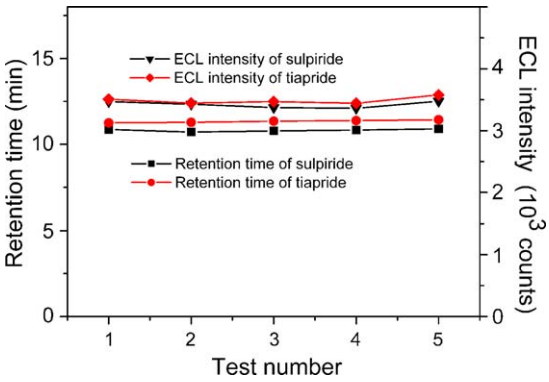

Fig. 7. Reproducibility of ECL intensity and retention time for sulpiride and tiapride under optimal conditions.

Table 1
Recoveries of sulpiride and tiapride in urine sample.

Sulpiride ($n=5$)			Tiapride ($n=5$)		
Concentration (μM)	Recoveries (%)	R.S.D. (%)	Concentration (μM)	Recoveries (%)	R.S.D. (%)
0.6	91	3.5	0.9	95	4.1
2.0	89	5.7	3.0	93	5.4
5.0	84	4.2	7.5	87	6.3

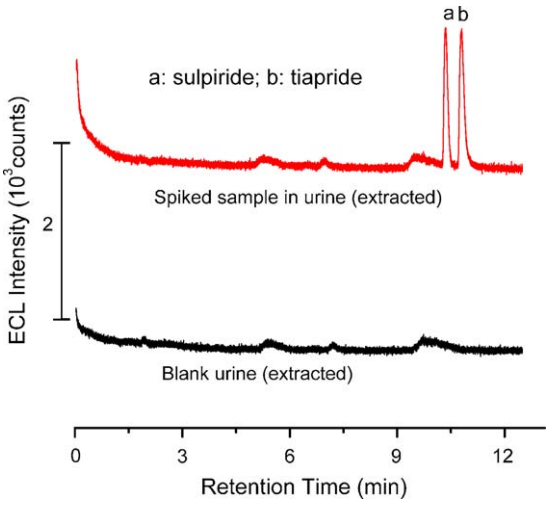

Fig. 8. CE–ECL electropherograms of blank urine sample and the urine sample spiked with 1.0 μM sulpiride and 1.5 μM tiapride under optimal conditions.

organic compounds in urine to obtain a clear electrophoretic sample profile, high detection sensitivity and good reproducibility. During extraction process, the pH value of urine sample was adjusted to about 11.0 [24] by adding 100 μl of saturated sodium carbonate solution into 200 μl urine sample. To prevent analytes from decomposing, the process of evaporating was endured at 35 °C for 10 min. The residue was dried by nitrogen gas stream. Table 1 shows the recoveries of sulpiride and tiapride spiked in blank urine samples. The recoveries of sulpiride and tiapride in urine samples at different concentrations were 84–91% and 87–95%, respectively. The R.S.D. of ECL peak intensity was less than 7%.

The developed method was applied to the detection of sulpiride and tiapride extracted from the urine samples, and the electrophoregrams were shown in Fig. 8. The analytes could be separated from the noise of the urine. Spiked in the urine matrix, the linear range for sulpiride and tiapride were from 1.0×10^{-7} to 1.0×10^{-4} M ($n=6$) with correlation efficient more than 0.995 with the same the limits of detection as those for standard solution.

4. Conclusions

This work proposes a rapid, sensitive and highly selective CE–ECL method for the simultaneous determination of sulpiride and tiapride. To obtain a good separation of sulpiride and tiapride with analogous structures, β-cyclodextrin is used as running buffer additives. The optimal conditions are an applied detection potential of +1.25 V, a separation voltage of 12 kV, a sample injection of 10 s at 10 kV, a running buffer of 20 mM pH 5.0 PBS containing 8.0 mM β-CD and a detection solution of 5.0 mM $Ru(bpy)_3^{2+}$ in 50 mM pH 6.0 PBS. This method is practical and valuable in clinical and biochemical laboratories for the determination for sulpiride and tiapride.

Acknowledgments

We gratefully acknowledge the financial support of the Distinguished Young Scholar Fund to HX Ju (20325518), the National Natural Science Foundation of China (20275017, 20535010) and the Specialized Research Fund for the Excellent Young Teachers from Ministry of Education of China.

References

[1] The British Pharmacopoeia, Her Majesty's Stationery Office, London, UK, 2001. p. 1566, 1611.
[2] J.E.F. Reynolds (Ed.), Martindale: The Extra Pharmacopoeia, 32nd ed., Royal Pharmaceutical Society of Great Britain, London, 1999, p. 692, p. 696.
[3] E. Distrutti, S. Fiorucci, S.K. Hauer, M.O. Pensi, M. Vanaisa, A. Morelli, Aliment. Pharmacol. Ther. 16 (2002) 613.
[4] F.A. Wiesel, G. Alfredsson, M. Ehrnebo, G. Sedvall, Eur. J. Clin. Pharmacol. 17 (1980) 385.
[5] G.R. Gorazza, M. Tonini, Clin. Drug Invest. 19 (2000) 151.
[6] F. Bressolle, J. Bres, M.D. Blanchin, R. Gomeni, J. Pharm. Sci. 73 (1984) 1128.
[7] S. Staveris, L. Jung, G. Jamet, J.C. Koffel, J. Chromatogr. 338 (1985) 79.
[8] F. Bressolle, J. Bres, J. Chromatogr. 341 (1985) 391.
[9] P. Nicolas, F. Fauvelle, A. Ennachachibi, H. Merdjan, O. Petitjean, J. Chromatogr. 381 (1986) 393.
[10] H. Tokunaga, K. Kudo, N. Jitsufuchi, Y. Ohtsuka, T. Imamura, J. Chromatogr. B 691 (1997) 203.
[11] M.C. Huang, H.O. Ho, G.C. Yeh, W.T. Ke, L.C. Lin, T.M. Hsu, C.C. Kao, M.T. Sheu, J. Chromatogr. B 763 (2001) 157.
[12] H.Y. Cho, Y.B. Lee, J. Chromatogr. B 796 (2003) 243.
[13] N. Jitsufuchi, K. Kudo, H. Tokunaga, T. Imamura, J. Chromatogr. B 690 (1997) 153.
[14] I.B. Paek, Y. Moon, H.Y. Ji, H.H. Kim, H.W. Lee, Y.B. Lee, H.S. Lee, J. Chromatogr. B 809 (2004) 345.
[15] J.F. Liu, W.D. Cao, H.B. Qiu, X.H. Sun, X.R. Yang, E.K. Wang, Clin. Chem. 48 (2002) 1049.
[16] X.H. Xu, J.T. Stewart, J. Pharm. Biomed. Anal. 23 (2000) 735.
[17] V. Ascalone, M. Ripamonti, B. Malavasi, J. Chromatogr. B 676 (1996) 95.
[18] R. Chiba, A. Ogasawara, T. Kubo, H. Yamazaki, M. Umino, Y. Ishizuka, Anal. Sci. 19 (2003) 785.
[19] K. Verleysen, P. Sandra, Electrophoresis 19 (1998) 2798.

[20] S. Terabe, K. Otsuka, H. Nishi, J. Chomatogr. A 666 (1994) 295.
[21] I.S. Lurie, J. Chromatogr. A 792 (1997) 297.
[22] J.F. Liu, J.L. Yan, X.R. Yang, E.K. Wang, Anal. Chem. 75 (2003) 3637.
[23] W.D. Cao, J.F. Liu, X.R. Yang, E.K. Wang, Electrophoresis 23 (2002) 3683.
[24] W.D. Cao, J.F. Liu, H.B. Qiu, X.R. Yang, E.K. Wang, Electroanalysis 23 (2002) 1571.
[25] S. Kumar, D.W. Rurak, K.W. Riggs, J. Mass Spectrom. 33 (1998) 1171.
[26] M.T. Chiang, M.C. Lu, C.W. Whang, Electrophoresis 24 (2003) 3033.