DOI 10.5012/bkcs.2010.31.6.1496

Enantioseparation and Determination of Sibutramine in Pharmaceutical Formulations by Capillary Electrophoresis

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Received December 7, 2009, Accepted April 7, 2010

Sibutramine enantiomers were separated successfully by capillary zone electrophoresis using substituted cyclodextrins as chiral selectors. The effects of cyclodextrin concentration, pH, voltage, buffer type, and electrolyte concentration on the migration time and resolution of enantiomers were examined. Separation of sibutramine enantiomers on an unmodified fused silica capillary (total length, 54 cm; effective length, 45 cm) was achieved using a mixed buffer of 20 mM phosphate/10 mM citrate containing either 5 mM methyl-β-cyclodextrin (pH 4.3) or 5 mM carboxymethyl-β-cyclodextrin (pH 6.5). Samples were injected with a pressure of 50 mbar for 5 s and were detected at a wavelength of 223 nm. The established method showed good precision and accuracy, with intra- and inter-day variations of less than 2.9 and 4.7%, respectively, and recoveries of 95.7 - 103.8%. The stability constants of (*R*)- and (*S*)-sibutramine demonstrated that the resolution of sibutramine enantiomers was attributable primarily to the difference in stability constants. When this optimized method was applied to the determination of sibutramine enantiomers in commercial drug formulations, it proved to be economical and convenient, affording sufficient accuracy, precision, and reproducibility as well as sensitivity and selectivity.

Key Words: Sibutramine, Enantioseparation, Chiral selector, Methyl-β-cyclodextrin, Capillary electrophoresis

Introduction

Sibutramine, *N*-(1-(1-(4-chlorophenyl)-cyclobutyl)-3-methylbutyl)-*N*,*N*-dimethylamine (Figure 1), a serotonin and noradrenalin reuptake inhibitor,[1] is currently used as an anti-obesity drug when weight loss is medically indicated. This drug shows dual actions by enhancing satiety to decrease food intake and enhancing thermogenesis to increase energy expenditure.[2] Sibutramine contains a chiral carbon and thus two enantiomers are available. Both pharmacodynamic and pharmacokinetic data have revealed the enantioselective behaviors of sibutramine: (*R*)-sibutramine decreases body weight and food uptake, whereas (*S*)-sibutramine increases body weight and food uptake.[3] In addition, (*R*)-sibutramine is biotransformed preferentially over (*S*)-sibutramine in rat liver microsomes and in primary cultures of rat hepatocytes.[4] Compared with (*S*)-sibutramine, (*R*)-sibutramine displays higher pharmacokinetic activity.[5] The active metabolites of sibutramine also exhibit enantioselective effects, and the (*R*)-sibutramine metabolites have dramatically higher potency than the (*S*)-sibutramine metabolites.[6] Until now, sibutramine has been marketed as a racemic mixture, and optically pure enantiomers have not been commercially available. However, for efficiency and safety reasons, sibutramine should be developed and administered as a single isomer drug. For the development of sibutramine as a chiral drug and for pharmacological and pharmacokinetic research, the enantioselective isolation and determination of sibutramine are needed.

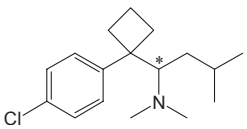

Figure 1. Chemical structure of sibutramine. The chiral carbon is identified by an asterisk.

There have been reports of the separation of sibutramine by HPLC methods, using Chiralcel OD[7] and chiral-AGP[5,8,9] as chiral stationary phases and β-cyclodextrin[10] as a chiral additive in the mobile phase. In one of only a few reports using capillary electrophoresis (CE) methods for the enantioselective separation of sibutramine, oversaturated 20 mM β-cyclodextrin was used as the chiral selector.[11] However, at this high concentration, the chiral selector caused blockage of the capillary, resulting in incomplete resolution of the enantiomers. A substituted β-cyclodextrin may be more soluble and thus more useful for the CE analysis of sibutramine.

In this study, chiral separation of sibutramine enantiomers was investigated by CE using carboxymethyl-β-cyclodextrin (CMCD) and methyl-β-cyclodextrin (MCD) as chiral selectors. This is the first demonstration that CMCD and MCD can be used as chiral selectors for the enantioselective analysis of sibutramine. We compared the separation efficiencies of CMCD and MCD as chiral selectors and developed an appropriate method for the separation of sibutramine enantiomers. The method was validated and applied to commercial sibutramine preparations. This study establishes an economical and convenient method for the stereoselective analysis of sibutramine and recommends effective chiral selectors.

Experimental Section

Apparatus. A HP^{3D}CE system (Hewlett Packard, Germany) equipped with a diode array detector was employed. Instrument control and data acquisition were performed using HP^{3D}CE ChemStation software. Uncoated fused silica capillary (BGB Analytic, Germany) with a total length of 54 cm (effective length, 45 cm) and an inner diameter of 50 μm was used. The pH was adjusted using ATI 370 pH meter (Orion, MA, USA).

Chemicals. Racemic sibutramine hydrochloride, (*R*)-sibutramine and domperidone (internal standard) were kindly donated from College of pharmacy, Taegu Catholic University. All reagents used for the preparation of the separation buffer were of analytical grade. Citric acid, dibasic sodium phosphate, and phosphoric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). MCD and CMCD were from Wacker-Chemie GmbH (Germany). Purified water from an ultra filtration system (Sinhan, Korea) was used, and nylon membrane filters (0.2 μm) were obtained from Whatman (England).

Preparation of buffer and solutions. The running buffer consisted of 10 mM citrate and 20 mM phospate containing 5 mM MCD (pH 4.3) or 5 mM CMCD (pH 6.5). The pH of the buffer was adjusted with 1.0 M sodium hydroxide or 95% phosphoric acid. A stock solution of sibutramine (1.0 mg/mL) was prepared by dissolving racemic sibutramine hydrochloride in a 10 mL of buffer in volumetric flask. For a calibration curve, the stock solution was diluted to 25, 50, 75, 100, and 150 μg/mL with buffer. For quality control, the stock solution was diluted to 25, 75, and 150 μg/mL with buffer. The internal standard (domperidone) was dissolved in buffer to a concentration of 40 μg/mL. For analysis, each commercial sibutramine capsule was weighed individually, dissolved in 10 mL of buffer in a volumetric flask, and diluted with buffer. The sample solution was filtered through 0.2 μm membrane filters and injected into the CE instrument.

CE operation. An uncoated fused silica capillary (total length, 54 cm; effective length, 45 cm; inner diameter, 50 μm) was used for the analysis. The capillary temperature was maintained at 25 °C and the voltage was 25 kV throughout the analysis. The signal was monitored at a wavelength of 223 nm. Samples were injected at the anodic end using a pressure of 50 mbar for 5 s. Before its first use, the new capillary was rinsed with methanol for 5 min, with deionized water for 5 min, with 1.0 M sodium hydroxide for 30 min, with water for 5 min, and then with running buffer for 5 min. Between each run, the capillary was treated with 1.0 M sodium hydroxide for 3 min, followed by water for 3 min and running buffer for 5 min.

Method validation. Linearity was evaluated by comparing the ratio of peak area of each enantiomer and the internal standard against half of the corresponding concentration of racemate. The intra-day precision and accuracy were evaluated by analyzing QC samples in five replicates, performed by one operator within a day. The inter-day variability of the method was assessed by replicating the analysis of QC samples on 5 days. Precision was expressed as the intra-day and inter-day percent relative standard deviation. Stability was determined by analyzing standard stock solutions that had been stored for 2 weeks at room temperature or for 2 months at −4 °C. The migration order of the sibutramine enantiomers was confirmed by analyzing a spiked (*R*)-sibutramine to sample.

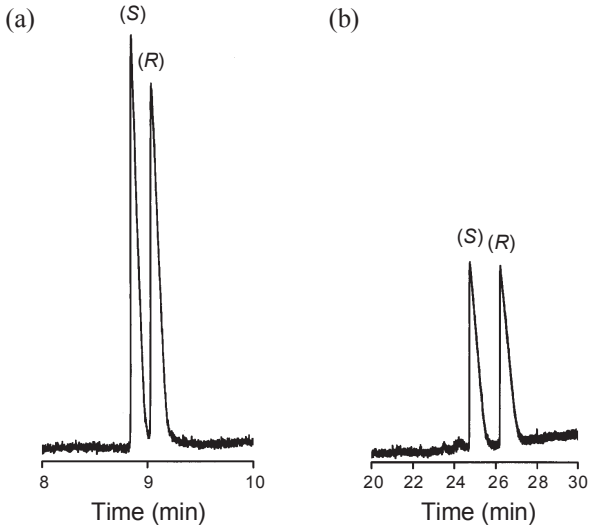

Figure 2. Effect of cyclodextrin type on the separation of sibutramine enantiomers separated with a mixed buffer of 20 mM phosphate/10 mM citrate containing (a) 5 mM of MCD (pH 4.3) and (b) 5 mM of CMCD (pH 6.5). Sample concentration, 0.1 mg/mL. Experimental conditions: uncoated fused-silica capillary, 50 μm i.d. × 45 cm (total length, 54 cm); injected by 50 mbar, 5 s; applied voltage, 25 kV; temperature, 25 °C; detection at 223 nm.

Results and Discussion

Selection of the chiral additive. The crucial step in achieving enantiomeric separation may be the choice of the chiral selector. Cyclodextrins are the preferred chiral selectors in CE, because of their high solubility in aqueous solvents, low toxicity, and low UV absorbance; they have been used to separate the enantiomers of a huge number of chiral drugs. The resolution of enantiomers depends on the hydrophobic cavity of the cyclodextrin type. The enantioselective analysis of sibutramine was performed with MCD and CMCD as chiral selectors, at concentrations 0 - 15 mM in buffer solution. Both of the cyclodextrins were used successfully to separate the sibutramine enantiomers, but there were some differences in resolution, peak shape, and migration time (Figure 2). CMCD, an anionic selector, gave longer migration times and broader peaks, owing to low electroosmotic flow (Figure 2b). With MCD, a neutral selector, the electrophoretic direction of the complexes between MCD and sibutramine was the same as the electroosmotic direction, resulting in a shorter migration time and sharper peak shape (Figure 2a). The resolution of sibutramine enantiomers was 1.6 using CMCD and 1.3 using MCD as chiral selectors, indicating that CMCD allowed higher enantiomer recognition, by hydrophobic as well as electrostatic interactions. Although CMCD gave higher resolution, MCD was chosen as the chiral selector in this study because of the shorter analysis time and better signal-to-noise (S/N) ratio.

Effect of buffer on peak shape and resolution. To evaluate the effect of buffer type on peak shape, buffer solutions made with acetate, phosphate, and citrate were used for CE separation. Citrate buffer showed good peak shape; phosphate buffer

provided a good S/N ratio. To achieve greater resolution and a lower S/N ratio, we tried buffers of phosphate and citrate mixed at various ratios and prepared at pH 4.3 and 6.5. The peak shape deteriorated at a high buffer concentration (20 mM phosphate/20 mM citrate) because of heating. A buffer of 20 mM phosphate/10 mM citrate was better than 30 mM phosphate buffer for the separation of sibutramine enantiomers (data not shown).

Effect of MCD concentration on resolution. The effect of the MCD concentration on the enantiomeric separation of sibutramine was investigated for 0 - 15 mM MCD. The relationship between resolution and MCD concentration is depicted in Figure 3. With an increase of the MCD concentration from 1 to 5 mM, the resolution of enantiomers increased from 0.78 to 1.3; however, beyond 5 mM MCD, the resolution decreased. Differences in migration behavior between the enantiomers became more significant at higher MCD concentrations, which shift the equilibrium of inclusion complexation toward formation rather than dissociation. At higher MCD concentrations, the number of complexes between MCD and sibutramine would be increased. The reduced resolution at excessive MCD concentrations (> 5 mM) could be ascribed to a reduction in the difference between enantiomers. Thus, a concentration of 5 mM MCD was selected for enantioseparation, as it provided a baseline separation of the enantiomers.

Effects of pH and voltage on resolution. The effect of buffer pH on the resolution of enantiomers was examined in the pH range of 3.7 to 6.4. With an increase from pH 4.3 to 6.4, both the resolution and migration time decreased. A higher pH value generates a higher electroosmotic flow and a shorter migration time of the analytes. Buffer pH values below 4.3 caused peak broadening and decreased resolution. The relationship between resolution and pH, illustrated in Figure 4, indicates that pH 4.3 is optimal. The effect of voltage on enantiomeric resolution was investigated at 15, 20, 25, and 30 kV. The resolution increased with a voltage increase from 15 to 25 kV; at 30 kV, no further improvement was observed. Thus, 25 kV was chosen as the working voltage. The appropriate CE condition for the sibutramine enantiomers deduced from the experimental results was 20 mM phosphate/10 mM citrate buffer containing 5 mM MCD (pH 4.3) at a voltage of 25 kV at 25 °C. At this condition (*S*)-sibutramine was eluted before (*R*)-sibutramine.

Calibration, precision and accuracy. The standard curves of each enantiomer were linear over the concentration range examined (12.5 - 75 μg/mL), with correlation coefficients of 0.9998 and 0.9996 for (*R*)- and (*S*)-sibutramine, respectively. The precision for both intra- and inter-day as well as the accuracy for the method are listed in Table 1. The precisions for intra- and inter-day assays were 0.8 - 2.9% and 1.6 - 4.7%, respectively, and the accuracy was 99.4%. Sibutramine was stable for at least 2 weeks at room temperature and for 2 months at −4 °C. The criteria for calibration, precision, and accuracy indicated that this validated method was suitable.

Stability constants and separation mechanism. As shown in Figure 3, the concentration of MCD had a profound effect on the migration and resolution of sibutramine enantiomers. With increasing MCD concentration, the host-guest complex formation between sibutramine and MCD may be enhanced. The extent of complex formation could be explained by the stability

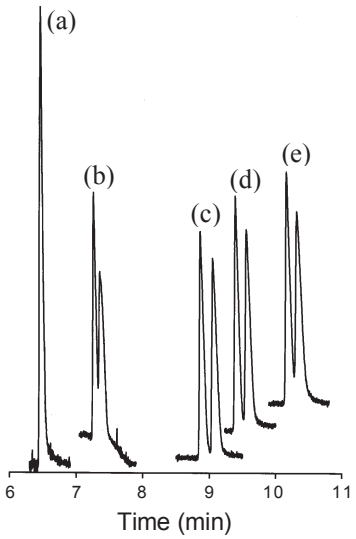

Figure 3. Effect of MCD concentration on the resolution of sibutramine enantiomers separated using a buffer containing (a) 0, (b) 1.0, (c) 5.0, (d) 10.0 and (e) 15 mM of MCD at pH 4.3. Other conditions were the same those noted in Figure 2.

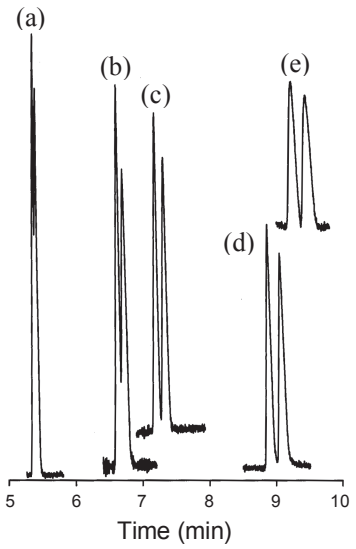

Figure 4. Effect of pH on the resolution of sibutramine enantiomers separated using a buffer containing 5 mM MCD at pH of (a) 6.4, (b) 5.3, (c) 4.8, (d) 4.3 and (e) 3.7. Other conditions were the same those noted in Figure 2.

constant of the complex (*K*), which might have affected the resolution and migration order of the enantiomers. The stability constant can be calculated using Eq. 1 with the mobility of the enantiomeric analytes (Eq. 2) and the complexes at a given concentration of chiral selector.[12-15]

$$\frac{1}{\mu_{eff}-\mu_f} = \frac{1}{(\mu_c-\mu_f)K}\frac{1}{[M]} + \frac{1}{\mu_c-\mu_f} \quad (1)$$

$$\mu_{eff} = \left(\frac{1}{t} - \frac{1}{t_0}\right)\frac{Ll}{V} \quad (2)$$

Table 1. Precision and accuracy of the analysis method for sibutramine enantiomers

QC concentration (mg/mL)	Accuracy (%)		Precision (% RSD)			
			Intra-day		Inter-day	
	(*S*)-isomer	(*R*)-isomer	(*S*)-isomer	(*R*)-isomer	(*S*)-isomer	(*R*)-isomer
12.5	101.1	103.8	0.8	0.8	1.6	3.3
37.5	96.6	95.7	1.4	1.0	4.0	4.6
75.0	100.5	98.7	2.9	1.5	4.7	4.0

where K is the stability constant; [M] is the total CD concentration in buffer; μ_{eff} is the effective electrophoretic mobility for enantiomer; μ_c and μ_f are the effective electrophoretic mobilities of the enantiomers in the complex and the free (without CD) forms, respectively; t and t_0 are the migration times of enantiomers and neutral marker, respectively. According to Eq. 1, the reciprocal of $\mu_{eff} - \mu_f$ and the reciprocal of [M] showed a linear relationship, and the linear equations of (*R*)- and (*S*)-sibutramine were $y = -0.0008x - 0.1681$ and $y = -0.001x - 0.1674$, with R^2 values of 0.9975 and 0.9976, respectively. The stability constant of inclusion complexes formed by sibutramine with MCD can be obtained by dividing the intercept by the slope to give 211 M^{-1} for (*R*)-sibutramine and 167 M^{-1} for (*S*)-sibutramine, indicating that the (*R*)-sibutramine complexes were more stable than the (*S*)-sibutramine complexes. The mobilities of sibutrmine enantiomers complexed with MCD calculated from nonlinear plotting method were the same indicating that the separation of enantiomers was mainly responsible for the different stability constants. The theoretical separation factor (ratio of the stability constant of each enantiomer) was 1.3, which is the same as the enantiomeric resolution obtained by the experiment with phosphate-citrate mixed buffer. The theoretical concentration of MCD for the maximal mobility difference of enantiomers can be calculated from the equation $C_{opt} = (K_R K_S)^{-1/2}$, where K_R and K_S represent the stability constants of (*R*)- and (*S*)-sibutramine, respectively, to give 5.3 mM; this value is in good agreement with the experimental concentration of 5 mM MCD. The separation factor and optimal concentration calculated from stability constants are consistent with those observed experimentally, demonstrating that the resolution of sibutramine enantiomers was attributable primarily to the difference in stability constants. In general, compounds are separated by CE based on their different mobilities. The separation of sibutramine enantiomers in a buffer containing MCD can be explained by the difference in stability constants, i.e., $K_R \neq K_S$, and by the difference in mobilities between free and complexed sibutramine, i.e., $\mu_f \neq \mu_c$.

Application to pharmaceutical formulation. The amounts of the enantiomers and racemate from two commercial pharmaceutical formulations were analyzed (Table 2). Formulation SI-01 contained 13.01 mg of sibutramine per capsule, comprising 6.61 and 6.40 mg of (*S*)- and (*R*)-sibutramine, respectively. Formulation SI-02 contained 12.72 mg of sibutramine per capsule, composed of 6.58 and 6.14 mg of (*S*)- and (*R*)-sibutramine, respectively. The sibutramine contents for SI-01 and SI-02 were 101.2% and 101.4%, respectively, of the labeled amount. There were no significant differences between the concentration of (*R*)- and (*S*)-sibutramine in the formulations. The electropherogram of a standard mixture and a diluted solution of SI-02 (corresponding to 0.06 mg/mL sibutramine) shown in Figure 5, demonstrated that this method is suitable for the determination of sibutramine enantiomers in commercial formulations.

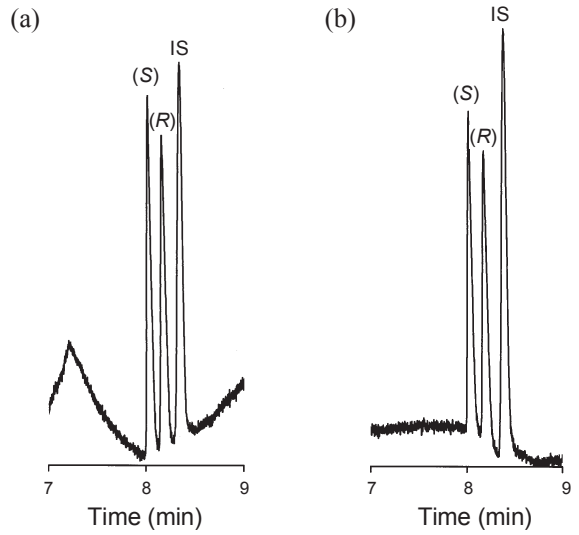

Figure 5. Electropherograms of (a) standard mixture and (b) commercial formulation. Experimental conditions: uncoated fused-silica capillary, 50 μm i.d. × 45 cm (total length, 54 cm); injected by 50 mbar, 5 s; applied voltage, 25 kV; temperature, 25 °C; buffer, 20 mM phosphate/10 mM citrate containing 5 mM MCD, pH 4.3; detection at 223 nm. Peaks: (*R*)-sibutramine, (*S*)-sibutramine and domperidone (IS).

Table 2. Content of sibutramine enantiomers and total sibutramine determined in commercial pharmaceutical formulations (mg/capsule)

Formulation ID	(*S*)-isomer	(*R*)-isomer	Total found	Total labeled
SI-01	6.61 ± 0.34	6.40 ± 0.19	13.01 ± 0.53	12.85
SI-02	6.58 ± 0.15	6.14 ± 0.36	12.72 ± 0.47	12.55

Conclusion

A CE method for the separation of sibutramine enantiomers using a mixed buffer of 20 mM phosphate/10 mM citrate containing 5 mM MCD or 5 mM CMCD as a chiral selector was developed and applied successfully to the enantiomeric determination of (*R*)- and (*S*)-sibutramine in commercial pharmaceutical formulations. During the method development, the mixed buffer showed superior separation ability compared with single component buffers. The stability constants between

sibutramine and MCD were 211 M^{-1} and 167 M^{-1} for (*R*)- and (*S*)-sibutramine, respectively, indicating that the resolution of the sibutramine enantiomers was attributable mainly to the difference in stability constants.

Acknowledgments. This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815).

References

1. Heal, D. J.; Cheetham, S. C.; Prow, M. R.; Martin, K. F.; Buckett, W. R. *Br. J. Pharmacol.* **1998**, *125*, 301.
2. Stock, M. J. *Int. J. Obes. Relat. Metab. Disord.* **1997**, *21*, S25.
3. Bodhankar, S. L.; Thakurdesai, P. A.; Singhal, S.; Gaur, V. *Indian J. Physiol. Pharmacol.* **2007**, *51*, 175.
4. Link, M.; Novotna, R.; Suchanova, B.; Skalova, L.; Wsol, V.; Szotakova, B. *J. Pharm. Pharmacol.* **2005**, *57*, 405.
5. Bae, K.; Noh, K.; Jang, K.; Kim, S.; Yong, C. S.; Choi, H. G.; Kang, J. S.; Chen, J. B.; Ma, E. S.; Lee, M. H.; Shin, B. S.; Kwon, K.; Kang, W. K. *J. Pharm. Biomed. Anal.* **2009**, *50*, 267.
6. Glick, S. D.; Haskew, R. E.; Maisonneuve, I. M.; Carlson, J. N.; Jerussi, T. P. *Eur. J. Pharmacol.* **2000**, *397*, 93.
7. Radhakrishna, T.; Narayana, C. L.; Rao, D. S.; Vyas, K.; Reddy, G. O. *J. Pharm. Biomed. Anal.* **2000**, *22*, 627.
8. Pan, Y.; Yu, L.; Yang, X.; Yang, L.; Guo, X. *Shenyang Yaoke Daxue Xuebao* **2006**, *23*, 521.
9. Singh, A. K.; Pedro, L. G.; Gomes, F. P.; Yano, H. M.; Auricchio, M. T.; Kedor-Hackmann, E. R.; Santoro, M. I. *J. AOAC Int.* **2008**, *91*, 572.
10. Xiao, S.; Liu, Y.; Chen, H.; Chen, Z.; Xia, Z. *Fenxi Huaxue* **2005**, *33*, 1761.
11. Huang, F.; Chen, H.; Xia, Z. *Yaowu Fenxi Zazhi* **2004**, *24*, 113.
12. Tanaka, Y.; Terabe, S. *J. Chromatogr. B* **2002**, *768*, 81.
13. Hsiao, J. Y.; Wu, S. H.; Ding, W. H. *Talanta* **2006**, *68*, 1252.
14. Phuong, N. T.; Lee, K. A.; Kim, K. H.; Choi, J. K.; Kim, J. M.; Kang, J. S. *Arch. Pharm. Res.* **2004**, *27*, 1290.
15. Park, K. L.; Kim, K. H.; Jung, S. H.; Lim, H. M.; Hong, C. H.; Kang, J. S. *J. Pharm. Biomed. Anal.* **2002**, *27*, 569.
16. Uselová-Vceláková, K.; Zusková, I.; Gas, B. *Electrophoresis* **2007**, *28*, 2145.