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Short communication

Determination of nateglinide in animal plasma by micellar electrokinetic chromatography and on-line sweeping technique

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Abstract

A micellar electrokinetic chromatography (MEKC) method was developed for the determination of nateglinide in animal plasma by on-line sweeping technique, in which plasma samples were simply deproteinized with acetonitrile, and analyzed with 16 mmol/L NaH_2PO_4 + 6 mmol/L $Na_2B_4O_7$ + 60 mmol/L sodium dodecyl sulfate (SDS) (pH 7.14) as the running buffer, a fused-silica capillary as the separation tube, 21 kV as the running voltage and UV detection at 214 nm. Under these conditions, more than 100-fold enrichment of nateglinide was obtained with the good linear relation in the range of nateglinide plasma concentration 0.2–7 mg/L ($R = 0.998$). The method could be applied successfully to determine trace drugs in clinical analysis.

Keywords: MEKC; On-line sweeping; Nateglinide; Plasma

1. Introduction

Micellar electrokinetic chromatography (MEKC), one of the most widely used CE modes, provides the possibility of separating neutral as well as charged compounds in one single run. But just like other modes of CE, MEKC is hampered by the low concentration sensitivity associated with the limited optical path-length for on-capillary photometric detection and the minute volume of sample solution that can be injected. So it is difficult to run trace analysis. In order to improve the concentration detection limits of MEKC, many methods such as off-line sample concentration (e.g. solid-phase extraction and liquid–liquid extraction) [1,2], increasing the path-length for photometric detector [3,4], using high sensitivity detector [5,6] and on-line concentration [7–10] have been used.

On-line sweeping is one of the on-line sample concentration technique which is defined as a phenomenon where the analyze are picked up and concentrated by the pseudostationary phase that enters the sample zone containing no pseudostationary phase in MEKC. It occurs when the sample matrix is void of a charged carrier phase and it does not matter whether the sample matrix has a higher, similar, or lower conductivity compared to the background solution. In theory, it provides for almost unlimited detection sensitivity for analytes that have high affinities toward the pseudostationary phase. 5000-fold improvements have been reported [11,12], which is greater than any other reported technique.

About 20–30 years ago, the determination of plasma levels was performed by a microbiological method [13], however, this method was time-consuming and was affected by the presence of other antibiotics. Later, high-performance liquid chromatographic technology was the main method for determining drugs concentration in blood, which improved some of the problems such as the time needed, the accuracy and the sensitivity of the detection [14–18]. Because biofluid samples contain large amounts of proteins, it can generate interfering peaks and lead to disadvantageous matrix effects. So the sample preparing process of these methods was considered very complicating and time-consuming. If the sample preparing process is not suitable, HPLC often associated with peak tailing, low efficiency and the stationary phase can be fouled by irreversible sorption of matrix constituents. Compared with HPLC, CE is a promising technique for clinical analysis because of its excellent separation efficiency, high versatility, and low cost [19–23]. There is no stationary phase in MEKC and the separation electrolyte

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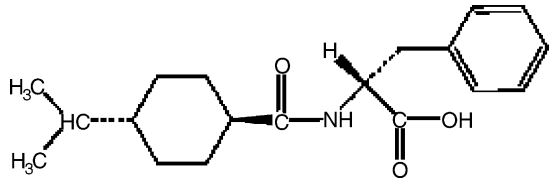

Fig. 1. Chemical structure of nateglinide.

is exchanged by rinsing procedures after each run. However, MEKC methods often exhibit detection limits that are at least one order of magnitude poorer than those of corresponding HPLC methods.

Nateglinide [*N*(trans-4-isopropylcyclohexylcarbonyl)-D-phenylalanine] is a novel oral mealtime glucose regulator, and was approved for the treatment of type II diabetes mellitus [24,25] recently (the chemical structure of nateglinide was shown in Fig. 1). Nateglinide can increase the insulin release from pancreatic β-cells through inhibition of potassium-ATP channels [26] after oral administration. It can be rapidly absorbed and peak plasma concentrations are reached after 0.5–1.0 h [27]. The elimination of the substance is also fast, with a half-life of approximately 1.4 h. Ono et al. [28] described the estimation of nateglinide and its main metabolites with column switching HPLC after solid-phase sample preparation. The method employed a special column that was not commercially available and required expensive column switching equipment. Bauer et al. [29] developed a HPLC method with UV detection for the analysis of nateglinide in human plasma with relatively short sample preparing time and analyzing time, but the problems for HPLC methods can also not be avoided.

In this work, a MEKC method for determination of trace nateglinide in rabbit plasma samples was established by on-line sweeping concentration technique. By using a simple sample preparation method, the separation time was shortened and the detector responsive signal could be improved significantly. The proposed method could be applied successfully to determine trace drugs in clinical analysis.

2. Experimental

2.1. Apparatus

The experiment was performed on a Waters Quanta 4000E capillary electrophoresis system (Milford, MA, USA) with a built-in 0–30 kV high voltage power supply, a fixed wavelength UV absorbance detector near the cathode end and a forced-air cooling system. Uncoated fused-silica capillary (75 μm i.d. total length is 60 cm and the effective length is 52 cm) was purchased from Yongnian Optical Fiber Factory, Hebei Province, China. The UV detection wavelength was set at 214 nm. The temperature remained constant at 25 °C. Samples were introduced to the capillary by using gravity injection and the injection height was 12 cm. Data processing was carried out with a CKChrom chromatography data system. The sensitivity of the detector was set at 0.005 AUFS.

2.2. Reagents and solutions

Nateglinide was provided by Jiheng Pharmaceutical Group, Hebei Province, China. Sodium dodecyl sulfate (SDS) was purchased from Beijing Zhongxi Chemical Factory, China. HPLC grade acetonitrile was obtained from Merck (Germany). Phosphate and borate were purchased from Tianjin Chemical Reagent Factory (analytical grade; Tianjin, China). The other chemical reagents are all of analytical grade. The concentration of nateglinide stock solution was 1.0 g/L. All buffers and solutions were prepared with double deionized water (Milli-Q, Millipore).

All of the backgrounds (BGS) were prepared by mixing stock solutions of 200 mM SDS, 200 mM sodium phosphate, 100 mM sodium borate with double deionized water and filtered through 0.45 μm filters (Ruili Separation Instrument Factory, Shanghai, China) prior to use.

2.3. Procedures

Freshly installed capillaries were rinsed with 0.1 mol/L sodium hydroxide for 30 min, followed by methanol (10 min), double-distilled water (10 min) and running buffer (15 min). In order to ensure repeatability, the capillary was purged between consecutive analysis with 0.1 mol/L sodium hydroxide (5 min), double deionized water (3 min) and BGS (3 min). These steps were necessary to remove plasma contents from the capillary and to recondition the capillary surface. The run buffer was renewed every morning and evening.

2.4. Analysis of samples

The standard solutions were prepared as following: the stock solution of nateglinide were diluted with the drug-free plasma and then were centrifuged at 3600 rpm for 3 min. The upper layer serum was mixed with a same volume of acetonitrile. Vortex was mixed for 1 min and centrifuged at 10,000 rpm for 3 min, and then supernatants were used for analysis.

The blood samples were prepared by drawing blood from the edge of rabbits' ears before and after injecting nateglinide. The blood was centrifuged at 3600 rpm for 3 min. Then the upper layer serum was mixed with a same volume of acetonitrile. Vortex was mixed for 1 min and centrifuged at 10,000 rpm for 3 min, and then supernatants were used for analysis.

3. Results and discussion

3.1. Effects of buffer solution

In our experiment, the effects of acetate, tris, phosphate and borate as the running buffer were investigated. The results showed that the height of nateglinide peak and

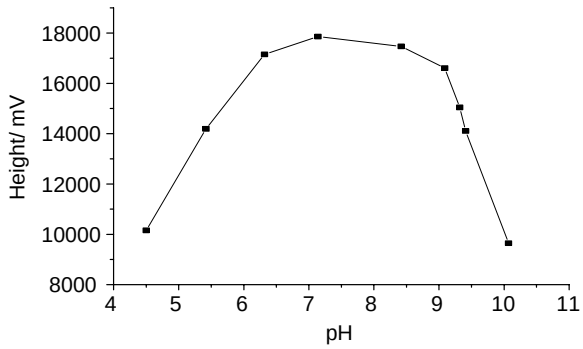

Fig. 2. Effect of background buffer solution pH on the concentration fold. Experimental conditions: applied voltage 21 kV, 60 cm/75 μm; effective length 52 cm; UV detection wavelength 214 nm.

resolution of nateglinide with interferences were better with the mixture of borate and phosphate as the running buffer. When the concentration of SDS (40 mmol/L) and the total concentrations of borate and phosphate were kept unchanged, different buffer pH values ranging from 4.0 to 10.50 were also tested. The results were shown in Fig. 2. From Fig. 2 it could be seen that when the pH value changed from 4.0 to 7.14, the height of the nateglinide peak became higher and the resolution became better. One reason may be that the change of running buffer pH influenced the interaction of nateglinide molecule to its surrounding and it affected the UV sensitivity of nateglinide. On the other part, with the increase of buffer pH, the theoretical plate number of the peak increased, which made the width of nateglinide peak become narrower and height of nateglinide peak higher. When the pH value was above 7.14, the height of the nateglinide peak began to decrease, the current was too high and more Joule heat was produced. It was not favorable to separation and determination. So we chose 7.14 as the optimum pH value of the buffer solution.

3.2. Effect of SDS concentration

When the injection time was kept as 200 s and the other experiment conditions were the same as above, the concentrations of SDS were investigated with the range from 10 to 100 mmol/L. The experiment showed that nateglinide and other interferences could not be separated by ordinary CZE mode. The reason may be that these compounds have similar velocity. When 20 mmol/L SDS was added as the micellar phase in the buffer solution, the separation of nateglinide and other interferences was improved. From the experiment, it was seen that the effect of enrichment increased with the increase of SDS concentration. But when SDS concentration was above 60 mmol/L, the current was too high to run the separations and much Joule heat was produced, which resulted in bubbles (the bubbles were observed by spikes in the electropherogram) in the buffer and also an unstable baseline. The optimum SDS concentration for this system is thus, about 60 mmol/L.

3.3. Effect of the injection time

In the experiment, when the buffer solution (60 mmol/LSDS + 16 mmol/L NaH_2PO_4 + 6 mmol/L $Na_2B_4O_7$, pH 7.14) and the other conditions were maintained unchanged, the effect of different injection times on the enrichment of peaks was investigated in the range from 1 to 300 s. It was shown that the height of nateglinide peak increased with the increase of injection time. When the injection time was 300 s, about 260-fold concentration of the height of nateglinide peak could be obtained. However, separation of the sample peaks with other impurities decreased and the sample peak cluttered when the injection time was too long. When the injection time was 120 s, more than 100-fold concentration of the nateglinide could be obtained and separated completely with interference peaks. So we chose 120 s as the injection time.

3.4. Effect of applied voltage

As demonstrated by many other authors, the applied voltage is also an important factor affecting EOF as well as separation efficiency and resolution in CE analysis. In our experiment, different voltages from 10 to 23 kV had been applied to find an optimum value. It was observed in the experiment that the height of peaks and the resolution increased with the increase in voltage and reached a maximum value at about 21 kV. After that, with the increase of the voltage, the resolution decreased. So 21 kV was used in the following experiments.

3.5. Sample preparation

The biological samples contain protein and other interference substances, which interfere in the process of separation and detection. In this work, different sample pretreatment, including protein precipitation with trichloroactic acid, tungstenic acid, methanol and acetonitrile were investigated, respectively. The results showed that the deposition efficiency of protein with trichloroactic acid, tungstenic acid and methanol was low. The electropherogram was in pell-mell and the peak of nateglinide was not easy to spot and quantitatively analyzed. All these make against the detection of trace drugs in blood. However, it was found that the deposition efficiency of protein with acetonitrile was satisfactory. After the sample was treated by acetonitrile, the matrix peaks nearly disappeared; the peak of sample

Table 1
Recovery and relative standard deviation

Content (mg/L)	Added (mg/L)	Found (mg/L)	Recover (%)	%R.S.D ($n = 3$)
1.09	0.70	1.72	90.0	3.72
1.07	1.05	2.04	92.4	2.89
1.10	1.30	2.35	96.2	2.45
1.21	0.90	2.06	94.4	4.36
1.23	1.50	2.67	96.0	1.91

was symmetric and there were no other interferences. So acetonitrile was chosen in this work.

3.6. The linearity and detection limit

Plasma standards of 0.2, 0.5, 1.0, 2.0, 5.0 and 7.0 mg/L were prepared by diluting the standard nateglinide solutions of various concentrations with blank plasma. These standards were pretreated (Section 2.4) and then, measured by MEKC. The concentration with which signal-to-noise ratio was detected as 3:1 was determined to be the minimum limit of detection. The test data showed that the good linear relation was obtained in the range of 0.2–7 mg/L ($R = 0.998$). The linear regression equation was $Y = 131.42 + 6927.36X$. (In the equation, Y was the height of nateglinide peak (mV) and X was the concentration of the

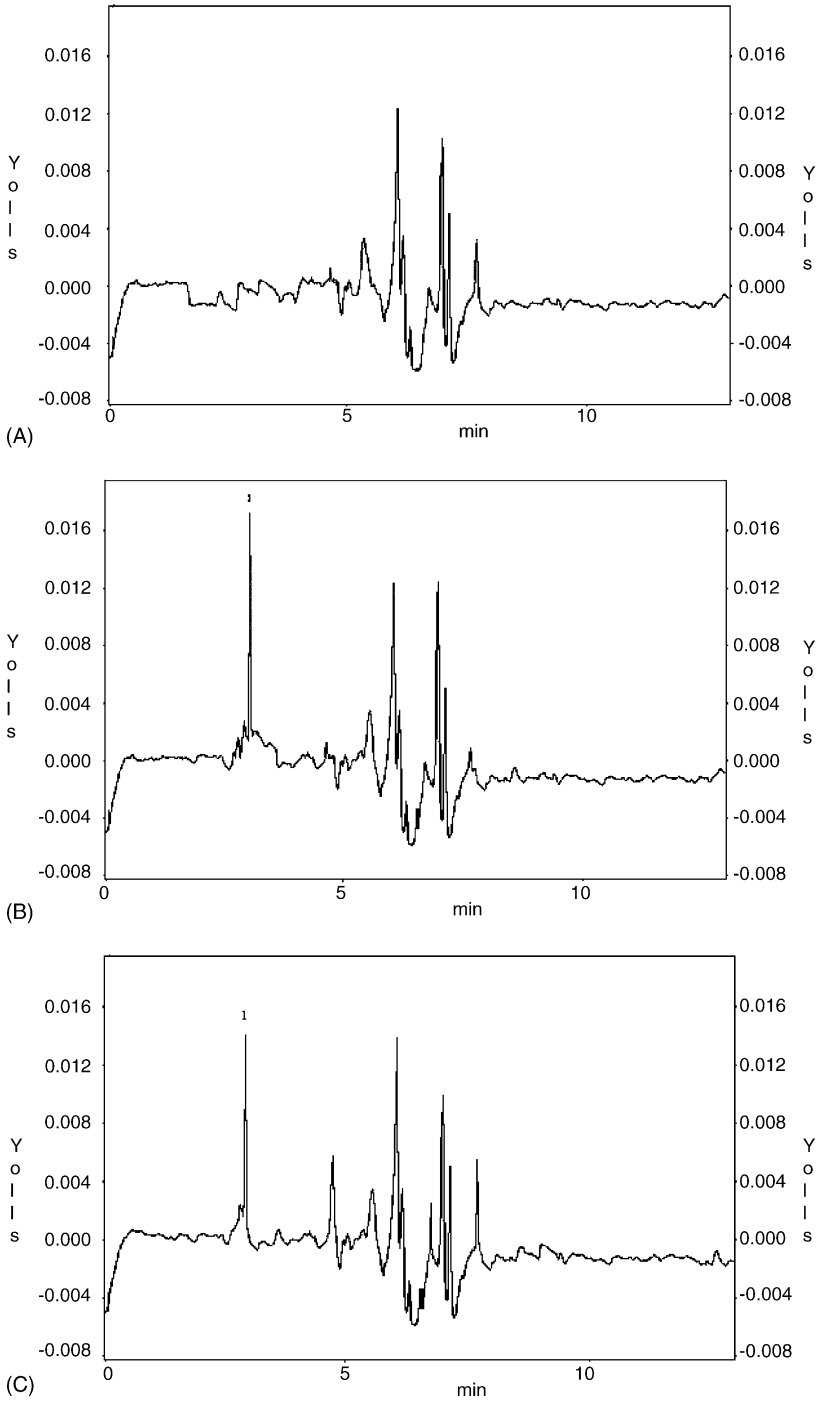

Fig. 3. Electropherogram of nateglinide in plasma: (A) blank plasma sample; (B) quality control standard (0.5 mg/L); (C) plasma sample 4.0 h post administration of 5 mg nateglinide (peak 1, nateglinide). Experimental conditions: buffer solution (16 mmol/L NaH_2PO_4 + 6 mmol/L $Na_2B_4O_7$ + 60 mmol/L SDS, pH 7.14); applied voltage 21 kV, 60 cm/75 μm; effective length 52 cm; UV detection wavelength 214 nm.

Table 2
Intra- and inter-assay accuracy and precision results for nateglinide

Nominal concentration (ng/mL)	Observed concentration (ng/mL)	Accuracy (%DEV)	Precision (%R.S.D) ($n = 7$)	
			Inter-assay	Intra-asay
0.45	0.43	4.44	5.87	8.06
1.10	1.07	2.73	2.91	4.73
1.85	1.72	7.03	4.62	7.56

nateglinide (mg/L).) The minimum limit of detection was 0.05 mg/L.

3.7. Recovery of the nateglinide

Recoveries were determined by the standard addition method. The plasmas that contained nateglinide were divided into ten units, five of which were analyzed directly according to the procedure as detailed in Section 2.4. The others were added into some nateglinide standards exactly and analyzed at the same conditions. All samples were processed and analyzed by MEKC as described above. Recovery was calculated by comparing the corrected peak areas of the samples with the corrected peak areas of the control samples, providing the 100% values. The results were listed in Table 1.

3.8. Intra- and inter-assay accuracy and precision

The intra- and inter-asay accuracy and precision of the assay were determined by assaying the samples (0.45, 1.10, 1.85 ng/mL) in seven replicates in three different days. The results of the quality control samples at three different concentrations of nateglinide were given in Table 2. The data in Table 2 showed that the intra- and inter-asay precision values (%R.S.D) at the various concentrations for nateglinide were ≤8.06%. The accuracy (percentage deviation, %DEV) for all three concentrations deviated by ≤7.03% from the corresponding nominal concentration.

3.9. Application to biological samples

This method was applied to determine the levels of nateglinide in rabbits following the oral administration of a single 5 mg dose of nateglinide. Serial blood samples were drawn from the edge of rabbits' ears before and after nateglinide was given. The blood was analyzed directly according to Section 2.4. Concentration of the nateglinide in blood was calculated based on the linear regression equation. The results were shown in Figs. 3 and 4. The electropherograms of a blank plasma sample (A), a quality control standard with a nateglinide concentration of 0.5 mg/L (B), and plasma samples 4.0 h post administration of 5 mg nateglinide (C) were shown in Fig. 3. Fig. 4 was the plasma concentration-time curve of nateglinide.

From the Fig. 4 one could see that after oral administration, nateglinide was rapidly absorbed and peak concentrations were reached after 40–50 min, with a half-life of approximately 85 min. Nateglinide undergoes extensive biotransformation resulting in hydroxylated and carboxylated derivatives. Certain metabolites are subsequently conjugated with glucuronic acid or hepatic cytochrome P450 isoenzymes such as CYP2C9 and CYP3A4 [30].

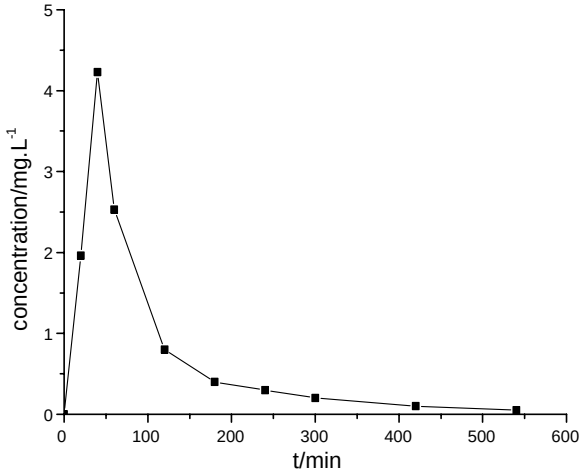

Fig. 4. Plasma concentration-time curve of nateglinide. Experimental conditions: buffer solution (16 mmol/L NaH_2PO_4 + 6 mmol/L $Na_2B_4O_7$ + 60 mmol/L SDS, pH 7.14); applied voltage 21 kV, 60 cm/75 μm; effective length 52 cm; UV detection wavelength 214 nm.

4. Conclusions

In this work, a method of determination trace nateglinide in plasma was established by the micellar electrokinetic chromatography on-line sweeping technique. The results obtained above showed that on-line sweeping technique was a good sample concentration approach. It's a fast, effective, and easy way to concentrate neutral and anionic substances inside the capillary. The detector responsive signal can be improved significantly. On-line sweeping method makes it better for capillary electrophoresis to analysis trace components such as trace drugs in blood, urine, etc.

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References

[1] J.W. Luo, H.W. Chen, Q.H. He, J. Chromatogr. 53 (2001) 295–300.

[2] K.V. Penmetsa, R.B. Leidy, D. Shea, J. Chromatogr. A 766 (1997) 225–231.
[3] S.E. Moring, R.T. Reel, R.E. Soest, Anal. Chem. 65 (1993) 3454–3459.
[4] N.M. Djordjevic, R. Widder, M. Kuhnn, J. High Resolut. Chromatogr. 20 (1997) 189–192.
[5] C.J. Smith, J. Grainger, J.D.G. Patterson, J. Chromtogr. A 803 (1998) 241–247.
[6] J.C. Olsson, A. Dyemark, B. Karlberg, J. Chromatogr. A 765 (1997) 329–335.
[7] M. Hernandez, F. Borrull, M. Calull, J. Chromatogr. 5 (2002) 585–589.
[8] J.B. Kim, K. Otsuka, S. Terabe, J. Chromatogr. A 912 (2001) 343–352.
[9] X. Cahous, Y. Daali, S. Cherkaou, J.L. Veuthey, J. Chromatogr. 5 (2002) 211–216.
[10] J.B. Kim, K. Otsuka, S. Terabe, J. Chromatogr. A 932 (2001) 129–137.
[11] J.P. Quirino, S. Terabe, J. Chromatogr. 53 (2001) 285–289.
[12] J.P. Quirino, S. Terabe, Anal. Chem. 71 (1999) 1638–1644.
[13] O. Shigeyuki, M. Yasuyoshi, J. Chromatogr. B 686 (1996) 205–210.
[14] J. Xie, Y.L. Li, F.H. Zhang, Anal. Labor. 17 (1998) 58–61.
[15] W. Ma, W.R. Klemin, Alcohol 14 (1997) 469–472.
[16] T. Yasuda, K. Tanaka, K. Iba, J. Mass Spectrum. 31 (1996) 879–884.
[17] K.H. Bannefeld, H. Stass, G. Blaschke, J. Chromatogr. B 692 (1997) 453–459.
[18] G.J. Krol, G.W. Beck, T. Benham, J. Pharm. Biomed. Anal. 14 (1995) 181–190.
[19] J.W. Luo, H.W. Chen, Q.H. He, J. Chromatogr. 53 (2001) 295–300.
[20] M. Sabine, S. Michael, J. Chromatogr. A 730 (1996) 297–303.
[21] M. Hernandez, F. Borrull, M. Calull, J. Chromatogr. B 742 (2000) 255–265.
[22] H.K. Seong, W. Jong, C. Soo, J. Pharm. Biomed. Anal. 15 (1997) 1435–1441.
[23] H. Lamparezyk, P. Kowalski, D. Rajzer, J. Nowakowska, J. Chromatogr. B 692 (1997) 483–487.
[24] S. Fujitani, K. Okazaki, T. Yada, J. Pharmacol. 120 (1997) 1191–1198.
[25] A.H. Karara, B.E. Dunning, J.F. McLeod, J. Clin. Pharmacol. 39 (1999) 172–179.
[26] S. Fujitani, T. Yada, Endocrinology 134 (1994) 1395–1400.
[27] S. Choudhury, Y. Hirschberg, R. Filipek, K. Lasseter, J.F. McLeod, J. Clin. Pharmacol. 40 (2000) 634–640.
[28] I. Ono, K. Matsuda, S. Kanno, J. Chromatogr. B: Biomed. Sci. Appl. 678 (1996) 384–387.
[29] S. Bauer, E. Störmer, J. Kirchheiner, C. Michael, J. Brockmöller, I. Roots, J. Pharm. Biomed. Anal. 31 (2003) 551–555.
[30] M.L. Weaver, B.A. Orwig, L.C. Rodriguez, E.D. Graham, J.A. Chin, M.J. Shapiro, J.F. McLeod, J.B. Mangold, Drug Metab. Dispos. 29 (2001) 415–421.