Virginia Melis
Iris Usach
José-Esteban Peris

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Valencia, Spain

Received June 20, 2012
Revised August 6, 2012
Accepted August 12, 2012

Research Article

Determination of sertraline in rat plasma by HPLC and fluorescence detection and its application to *in vivo* pharmacokinetic studies

A simple, rapid, and sensitive HPLC method based on 9H-fluoren-9-ylmethyl chloroformate derivatization for the quantification of sertraline in rat plasma has been developed, requiring a plasma sample of only 0.1 mL, which was deproteinized and derivatized for 5 min in two single steps. The obtained derivative was stable at room temperature and was determined by HPLC using a fluorescence detector. The analytical column was a C(18) column and the mobile phase was acetonitrile and water (80:20, v/v). Calibration curves were linear in the range of 10–500 ng/mL. The limit of detection was approximately 3 ng/mL, and the lower limit of quantification was established at 10 ng/mL. The bias of the method was lower than 10%, and the within day as well as between day, relative standard deviations were lower than 12%. This analytical method was successfully applied to characterize sertraline pharmacokinetics in rats following intravenous ($t_{1/2}$ = 213 ± 48 min, Cl = 43.1 ± 8.7 mL/min, V_d = 11560 ± 1861 mL) and oral (C_{max} = 156 ± 76 ng/mL, t_{max} = 63.8 ± 16.3 min) administration of 2 and 5 mg, respectively.

Keywords: Fluorescence detection / Pharmacokinetics / Sertraline
DOI 10.1002/jssc.201200586

1 Introduction

Sertraline (SRT) is a potentenlrg selective serotonin reuptake inhibitor used in the treatment of depression, obsessive-compulsive and panic disorders, post-traumatic stress disorder, social anxiety disorder, and premenstrual dysphoric disorder [1]. Also, there is evidence of its efficacy in the treatment of hiccups [2]. Therapeutic daily dose of SRT is between 50 and 200 mg; the drug is slowly absorbed after oral administration and peak plasma concentrations occur approximately 4–8 h after administration of a single 100-mg dose [3]. SRT has a linear pharmacokinetic profile, with a half-life of about 26 h and a volume of distribution greater than 20 L/kg [1].

In the literature, several methods have been described for the analysis of SRT in pharmaceutical preparations, biological materials, and environmental samples. In biological samples, SRT has been determined by GC-MS, electron capture or nitrogen-selective detection, and also by HPLC with different types of detection, including UV-vis, fluorescence and MS. HPLC methods offer advantages of economy, simplified sample preparation and ease of measurement. Several analytical HPLC methods for SRT analysis in plasma samples have been published, but, in general, these methods involve excessively large plasma samples (0.5–2 mL) for their application in small animals such as rats [4–11], use long concentration processes [11–14] or expensive equipment (LC-MS/MS) [15–18].

Pharmacokinetic studies of SRT in animals, such as rats, are scarce. There are only two studies available about it [19,20], of which only one provides information about the bioavailability of this drug [20]. Knowledge on the disposition and absorption characteristics of SRT in animals can help to interpret results in humans, such as drug–drug interactions [21,22] and side effects [23–25], and to establish relationships between plasma concentrations and pharmacodynamics [26,27]. Thus, the potential hepatotoxicity of SRT has been associated with mitochondrial dysfunction in rats [28], and a rat model of Huntington's disease has been used to study the protective effect of orally administered SRT against induced cognitive dysfunction [29,30]. Pharmacokinetic studies relating administered doses with plasma levels obtained in rats could improve the extrapolation of results to humans, relating the observed effects to the SRT plasma levels rather than to the administered doses.

The aim of this study was to develop an HPLC analytical method for SRT in plasma samples having a low detection limit, using a small volume of the sample and straightforward treatment. The analytical method was applied to determine pharmacokinetics of SRT in rats after intravenous administration and also after oral dosing giving rise to plasma levels

Correspondence: Dr. José-Esteban Peris, Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Avda. V. Andrés Estellés, s/n 46100-Burjassot, Valencia, Spain
E-mail: jose.e.peris@uv.es
Fax: +34-96-3544911

Abbreviations: LLOQ, lower limit of quantification; **SRT**, sertraline

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

similar to those obtained in humans with therapeutic doses of SRT.

2 Materials and methods

2.1 Reagents

Sertraline hydrochloride (SRT, Aremis®) was obtained from Dr. Esteve Laboratories (Barcelona, Spain) and Fmoc-Cl (9H-fluoren-9-ylmethyl chloroformate) was obtained from Sigma Chemical (Madrid, Spain). Acetonitrile was of HPLC grade and all other analytical reagents were of analytical grade.

2.2 Standard solutions

A main stock solution of SRT (0.5 mg/mL) was prepared in methanol. This stock solution was further diluted with acetonitrile to obtain an intermediate concentration solution of 5 μg/mL. Appropriate dilutions in water and rat plasma were prepared from this solution to obtain calibration standards in the range of 10–500 ng/mL. Although most experiments were carried out with rat plasma, blank human plasma was also assayed in order to check the absence of interference peaks from endogenous human plasma components. Dilutions for calibration standards were prepared daily, while quality control samples were prepared in plasma, divided in small aliquots and stored at −20°C until use.

2.3 Derivatization procedure

The following solutions were used for derivatization of SRT: Fmoc-Cl in acetonitrile (1 mg/mL), 0.1 M borate buffer (pH 7), and 1 M citric acid.

The derivatization of SRT was performed as follows: a sample volume of 100 μL was mixed with 200 μL of acetonitrile in a 1.5-mL Eppendorf tube and centrifuged (5000 × *g* for 5 min). Then, 200 μL of the supernatant was transferred to another 1.5-mL Eppendorf tube and mixed with 50 μL of borate buffer and 50 μL of Fmoc-Cl solution. This mixture was kept at room temperature for 5 min to allow the derivatization of SRT, and, after adding 20 μL of citric acid solution and centrifugation (5000 × *g* for 5 min), an aliquot was injected into the chromatograph.

2.4 Chromatographic conditions

SRT was quantified in rat plasma using an HPLC assay with fluorescence detection. The HPLC equipment consisted of a quaternary pump HP series 1050, a Rheodyne manual sample injector and an HP 1046A programmable fluorescence detector set at 260 nm (excitation) and 310 nm (emission). Chromatographic separation was performed on a Waters Nova-Pack C-18 (3.9 mm × 150 mm, 4 μm) at room temperature, using a mixture of acetonitrile/water (80:20, v/v) as mobile phase. The flow rate was 1 mL/min, and the injection volume was 25 μL.

2.5 Method validation

The calibration curves (peak area versus nominal concentration) were constructed using a least square linear regression analysis for the calculation of the slope, intercept, and correlation coefficient. The accuracy (bias) and precision (relative standard deviation; RSD) of the assay were determined from the analysis of plasma samples spiked with SRT at four concentrations (10, 100, 200, and 500 ng/mL). Within-day accuracy and precision were calculated from the six replicate analyses on the same day. Between-day accuracy and precision were calculated from the results obtained in six different analyses carried out within 2 wk. The LOD was estimated as the concentration of SRT in plasma giving rise to a peak whose height is three times the noise. The lower limit of quantification (LLOQ) was determined as the concentration of the lower standard with accuracy within 80–120% and RSD within 20%. The recovery of SRT from plasma was calculated by comparing the peak areas obtained with standards prepared in plasma to the peak areas obtained with standards prepared in water at the same SRT concentrations.

2.6 Drug administration and sampling

Male Wistar rats (280–310 g) were divided into two groups ($n = 5$) depending on the administration route of SRT (intravenous or oral). The experiments were performed in accordance with the authors' institutional rules for the use of animals in research (institutional committee on animal care at the University of Valencia), which are based on the European Union (EU) directives for the protection of animals used for scientific purposes (Directive 2010/63/EU). Rats were subjected to jugular vein cannulation on the day before drug administration [31] to facilitate intravenous drug administration and blood sampling with heparinized syringes. SRT was intravenously dosed at 2 mg/rat (approximately 6.7 mg/kg), and blood samples (0.2 mL) were collected at the following postdosing times: 5, 15, 30, 60, 120, 180, 240, and 300 min. The oral administration of SRT (5 mg/rat, approximately 16.7 mg/kg) was performed by gastric gavage, and the blood sampling times were: 30, 45, 60, 90, 120, 180, 240, 300, and 360 min. Blood was collected in the Eppendorf tubes, which were then centrifuged at 2000 × *g* for 5 min, and the supernatant plasma was separated and frozen at –20°C until SRT analysis.

2.7 Pharmacokinetic analysis

WinNonlin (version 5.1, Pharsight Corp., Mountain View, CA, USA) was used to estimate noncompartmental

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

pharmacokinetic parameters. The following parameters were estimated after intravenous SRT administration: half-life ($t_{1/2}$), area under the plasma concentration–time curve from zero to infinity (AUC_{∞}), area under the plasma concentration–time curve from time zero to 300 min (AUC_{300}), clearance (Cl), and volume of distribution (V_d). In the case of oral SRT administration, the estimated parameters were: $t_{1/2,}$ AUC_{∞}, AUC_{300}, maximum plasma concentration (C_{max}), and time to C_{max} (t_{max}). Oral bioavailabity was estimated from the ratio of mean AUC values, normalized for a 1-mg dose, obtained after oral and intravenous administrations.

2.8 Statistical analysis

Statistical comparisons were performed by means of one-way ANOVA. A P value of <0.05 was considered statistically significant.

3 Results and discussion

Fmoc-Cl is a reagent used as a UV/fluorescence tag for derivatizing amino acids for HPLC analysis and for preparing N-Fmoc amino acids for solid-phase peptide synthesis [32]. It is also used as an N-protecting reagent for peptide and oligonucleotide syntheses. Due to its high reactivity with amino groups, it is also used for the analysis of drugs containing primary or secondary amino groups. However, the practical uses of Fmoc-Cl are limited by the requirement that excess Fmoc-Cl must be removed before analysis, because the reagent itself is fluorescent. To avoid the extractive step separating the derivatized SRT from the excess of Fmoc-Cl reagent, the reagent concentration and the chromatographic conditions were carefully selected in this work to obtain an adequate separation of corresponding peaks in the chromatograms.

3.1 Chromatographic variables

To achieve the most effective chromatographic separation and analysis, the mobile phase was optimized by examining the effect of pH (in the range of 3–7 in steps of one pH unit) and varying the percentage of methanol and acetonitrile content (from 60 to 90%). Other chromatographic variables were investigated including flow rate and column type. Three C_{18} columns were examined: Waters Nova-Pack C-18 (3.9 mm id mm 150 mm length, 4 μm particle diameter), Kromasil C-18 (4.6 mm × 150 mm, 5 μm), and Waters Spherisorb ODS-2 (4.6 mm × 250 mm, 5 μm). The best chromatographic separation was obtained using Waters Nova-Pack C-18 and the chromatographic conditions described in Section 2.4.

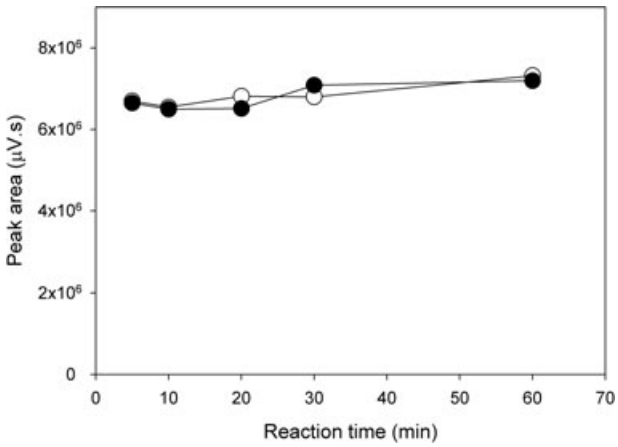

Figure 1. Peak area of SRT derivative as a function of reaction time at room temperature. (○) SRT standard prepared in water (500 ng/mL), (●) SRT standard prepared in plasma (500 ng/mL).

3.2 Optimization of the derivatization procedure

To study the time course of derivatization, several reaction times were tested: 5, 10, 20, 30, and 60 min. As can be seen in Fig. 1, the derivatization reaction of SRT with Fmoc-Cl is very fast at room temperature in the presence of borate buffer (pH 7). From the data in Fig. 1, a reaction time of 5 min was selected for analyzing plasma samples containing SRT.

3.3 Assay validation

Figure 2 shows two chromatograms corresponding to blank rat plasma and rat plasma spiked with SRT. Peaks corresponding to endogenous components extracted from blank plasma and excess of reagent eluted before the peak of the SRT derivative, but sufficiently apart to avoid interference with the analyte peak. Human blank plasma was also assayed and no interfering peaks were observed at, or near, the retention time of the SRT derivative.

The stability of the SRT derivative was checked at room temperature by comparing the peak areas obtained immediately after finishing the reaction and, again after 48 h at room temperature. Practically, the same peak area value was obtained, which indicates that the derivative is stable for at least 48 h at room temperature.

A linear relationship was found between the SRT peak area and the SRT concentration in standards, in the range of 10–500 ng/mL (10, 25, 50, 100, 200, and 500 ng/mL) (Fig. 3). The LOD was approximately 3 ng/mL, and the LLOQ was established at 10 ng/mL. The bias of the method was lower than 10%, the within-day as well as between-day RSD values were lower than 12% (Table 1), and the SRT recovery from plasma was between 82 and 99% for the four concentrations assayed (10, 100, 200, and 500 ng/mL). Although the analytical method was validated using rat plasma, the fact that human blank plasma did not show interfering peaks at the retention time of the SRT derivative suggests that this

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

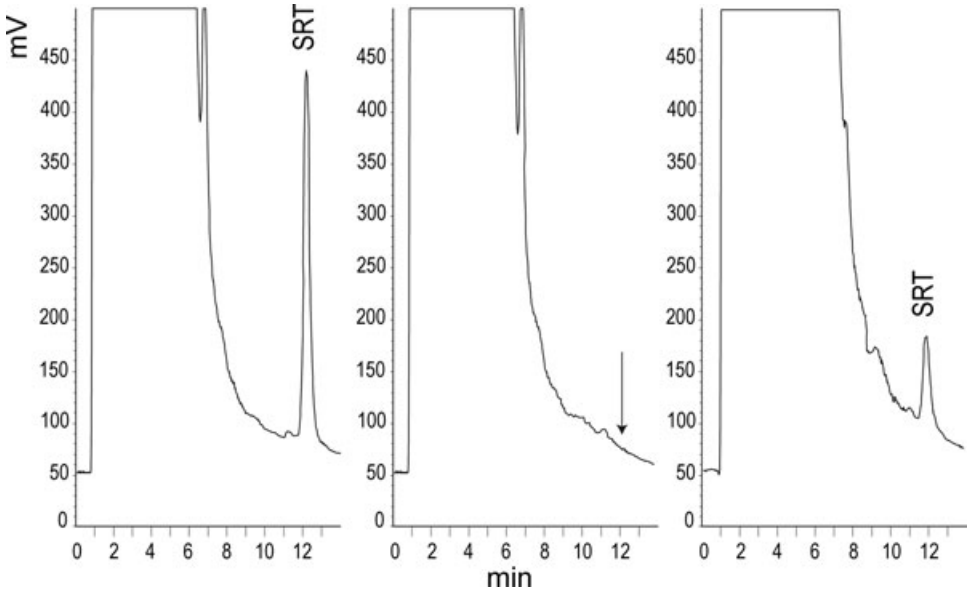

Figure 2. HPLC chromatograms corresponding to rat plasma spiked with 500 ng/mL of SRT (left), blank rat plasma (center), and a rat plasma sample obtained at 60 min after the intravenous dosing of 2 mg of SRT (right).

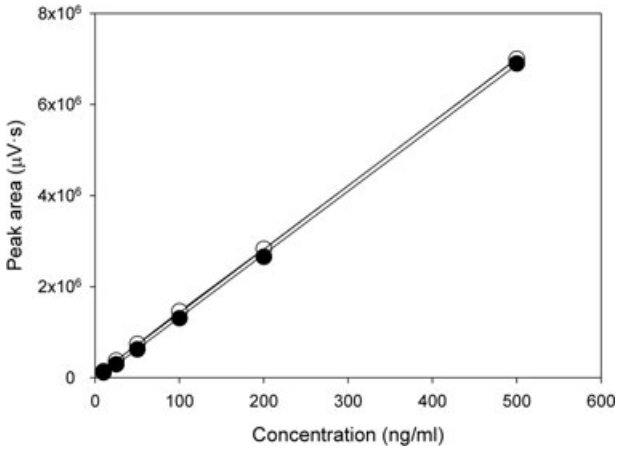

Figure 3. Calibration curves of SRT prepared in water (○) ($y = 13\,959x + 29\,617$) and rat plasma (●) ($y = 13\,876x - 12\,941$) showing a linear relationship between peak area and concentration ($r > 0.999$).

timely and cost-effective HPLC method can be useful for the therapeutic drug monitoring of SRT in humans.

3.4 Application of the analytical method

The HPLC method was used to determine the SRT concentration in plasma samples obtained intravenously and orally challenged rats with doses of 2 and 5 mg of SRT, respectively. Mean plasma concentrations of SRT (Fig. 4) ranged from 363 ng/mL at 5 min postdose, to 55 ng/mL at 5-h postdose after intravenous administration, and from 62 ng/mL at 30 min postdose, to 65 ng/mL at 6-h postdose after oral administration.

3.5 Sertraline pharmacokinetics and bioavailability

The oral dose (5 mg/rat, approximately 16.7 mg/kg) was chosen with the aim of obtaining a plasma concentration

Table 1. Within- and between-day precision and accuracy for the determination of SRT in spiked rat plasma

Nominal concentration (ng/mL)	Within day			Between day		
	Observed concentration[a] (ng/mL)	RSD[b]	Bias (%)	Observed concentration (ng/mL)	RSD (%)	Bias (%)
10	10.7 ± 0.9	8.4	7	10.9 ± 1.2	11.0	9
100	104.2 ± 3.3	3.2	4	106.5 ± 6.2	5.8	7
200	192.4 ± 4.2	2.2	–4	190.2 ± 7.1	3.8	–5
500	530.1 ± 20.6	3.9	6	524.8 ± 25.6	4.9	5

a) Mean ± standard deviation, $n = 6$.
b) Relative standard deviation.

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 2. Pharmacokinetic parameters of SRT in rats after intravenous and oral administration ($n = 5$)

Parameter	Administration route		Statistically significant
	iv	Oral	
AUC_{300} (ng·min/mL)[a)]	15 685 ± 1921[b)]	5600 ± 2750[b)]	$P < 0.01$
AUC_{∞} (ng·min/mL)[c)]	24 221 ± 5043[b)]	11 440 ± 6117[b)]	$P < 0.01$
AUC_{extrap} (%)[d)]	34 ± 10	39 ± 9	NS
$t_{1/2}$ (min)	213 ± 48	245 ± 60	NS
C_{max} (ng/mL)	–	156 ± 76	–
t_{max} (min)	–	63.8 ± 16.3	–
V_d (mL)	11 560 ± 1861	–	–
Cl (mL/min)	43.1 ± 8.7	–	–

a) Area under the plasma concentration–time curve from time zero to 300 min.
b) Parameter value normalized for an 1-mg dose.
c) Area under the plasma concentration–time curve from time zero to infinity.
d) Percentage of area extrapolated from the last experimental sampling time (iv, 300 min; oral, 360 min) to infinity.
NS, not significant.

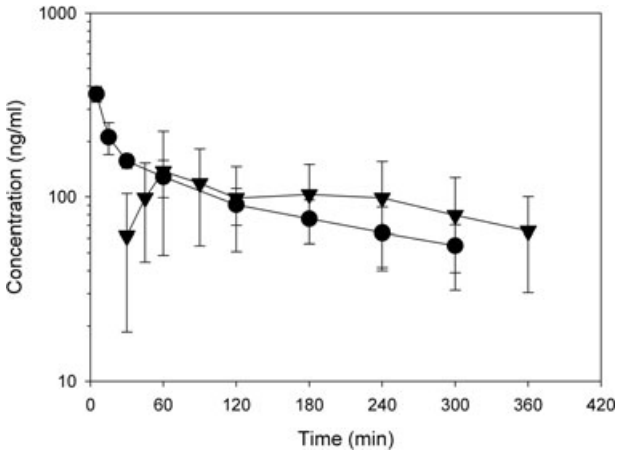

Figure 4. Plasma SRT concentrations (mean ± SD) following intravenous (●) and oral (▼) administration of 2 mg and 5 mg, respectively ($n = 5$).

similar to that described in humans when therapeutic doses of SRT are used. In fact, the mean C_{max} value obtained in rats (Table 2), 156 ng/mL, was close to the steady-state C_{max} reported in humans (55–250 ng/mL) for daily doses ranging from 50 to 200 mg (approximately 0.7–2.9 mg/kg) [33]. The intravenous dose (2 mg/rat, approximately 6.7 mg/kg) was lower than the oral dose in order to obtain SRT plasma levels during the slow disposition phase similar to the levels obtained with the oral administration (Fig. 4). By comparing the mean AUC values obtained after oral administration with the mean AUC value corresponding to the intravenous route, an SRT bioavailability of 36% or 47% can be estimated depending on the use of AUC_{300} or AUC_{∞}.

Following the intravenous administration of 2 mg (approximately 6.7 mg/kg), the elimination half-life for SRT in rats was 3.55 h. This value is very similar to that reported in the literature (4.5 h) after the intravenous administration of a 5 mg/kg dose [20]. Other pharmacokinetic parameters of SRT calculated after intravenous administration were V_d (11560 mL) and Cl (43.1 mL/min). These values differ from those obtained by Tremaine et al. [20] (6900 mL and 17.7 mL/min, respectively), and could be attributed to the fact that different strains of rats were used (Wistar versus Sprague-Dawley) [34], and also that rats were of different age (approximately 9.5 wk old in our study versus 6.5 wk old [20]).

The analytical methodology described in this study is simpler and faster than the methodology of Tremaine et al. [20] based on the use of GC and a long processing time (extraction into ether, partial evaporation, obtention of a derivative of SRT at 50°C, evaporation to dryness and re-dissolution into ethyl acetate before injection on the chromatograph). On the other hand, in that work, rats ($n = 4$) were sacrificed at each time point to estimate pharmacokinetic parameters from the mean plasma concentrations, whereas in the present work individual pharmacokinetic profiles have been obtained and the mean pharmacokinetic parameter values have been obtained, as usual, averaging the individual values, which allows the estimation of inter-individual variation in the pharmacokinetic parameters (standard deviations in Table 2) and the use of a significant lower amount of animals (10 versus 80).

4 Conclusion

In the present study, a simple, specific, and sensitive HPLC method based on Fmoc-Cl derivatization for the quantification of SRT in plasma was developed. The method provides significant advantages with regard to other related methods previously published. It requires only 100 μL of plasma sample, which was deproteinized and derivatized in two single steps. Moreover, the obtained derivative was stable at room temperature during, at least, 48 h. The LLOQ of the method was 10 ng/mL, and no interference with endogenous plasma

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

peaks was observed. The proposed method is suitable for the determination of SRT in plasma samples for pharmacokinetic studies in rats.

The authors have declared no conflict of interest.

5 References

[1] Murdoch, D., McTavish, D., *Drugs* 1992, *44*, 604–624.

[2] Vaidya, V., *Psychosomatics* 2000, *41*, 353–355.

[3] van Harten, J., *Clin. Pharmacokinetics* 1993, *24*, 203–220.

[4] Vatassery, G. T, Holden, L. A., Hazel, D. K., Dysken, M. W., *Clin. Biochem.* 1997, *30*, 565–568.

[5] Frahnert, C., Rao, M. L., Grasmader, K., *J. Chromatogr. B* 2003, *794*, 35–47.

[6] Chaves, A. R., Silva, S. M., Queiroz, R. H., Lancas, F. M., Queiroz, M. E., *J. Chromatogr. B* 2007, *850*, 295–302.

[7] Esrafili, A., Yamini, Y., Shariati, S., *Anal. Chim. Acta* 2007, *604*, 127–133.

[8] Malfara, W. R., Bertucci, C., Costa Queiroz, M. E., Dreossi Carvalho, S. A., Bianchi Mde, P. L., Cesarino, E. J., Crippa, J. A., Costa Queiroz, R. H., *J. Pharm. Biomed. Anal.* 2007, *44*, 955–962.

[9] Silva, B. J., Lancas, F. M., Queiroz, M. E., *J. Chromatogr. B* 2008, *862*, 181–188.

[10] Lucca, A., Gentilini, G., Lopez-Silva, S., Soldarini, A., *Ther. Drug Monit.* 2000, *22*, 271–276.

[11] Titier, K., Castaing, N., Scotto-Gomez, E., Pehourcq, F., Moore, N., Molimard, M., *Ther. Drug Monit.* 2003, *25*, 581–587.

[12] Duverneuil, C., de la Grandmaison, G. L., de Mazancourt, P., Alvarez, J. C., *Ther. Drug Monit.* 2003, *25*, 565–573.

[13] Patel, J., Spencer, E. P., Flanagan, R. J., *Biomed. Chromatogr.* 1996, *10*, 351–354.

[14] Mandrioli, R., Saracino, M. A., Ferrari, S., Berardi, D., Kenndler, E., Raggi, M. A., *J. Chromatogr. B* 2006, *836*, 116–119.

[15] Chen, X., Duan, X., Dai, X., Zhong, D., *Rapid Commun. Mass Spectrom.* 2006, *20*, 2483–2489.

[16] He, L., Feng, F., Wu, J., *J. Chromatogr. Sci.* 2005, *43*, 532–535.

[17] Jain, D. S., Sanyal, M., Subbaiah, G., Pande, U. C., Shrivastav, P., *J. Chromatogr. B* 2005, *829*, 69–74.

[18] Patel, B. N., Sharma, N., Sanyal, M., Shrivastav, P. S., *J. Chromatogr. B* 2009, *877*, 221–229.

[19] Rao, R. N., Kumar, K. N., Shinde, D. D., *J. Pharm. Biomed. Anal.* 2010, *52*, 398–405.

[20] Tremaine, L. M., Welch, W. M., Ronfeld, R. A., *Drug Metab. Dispos.* 1989, *17*, 542–550.

[21] Go, A. C., Golightly, L. K., Barber, G. R., Barron, M. A., *Drug Metab. Drug Interact.* 2010, *25*, 41–47.

[22] Schwartz, A. R., Pizon, A. F., Brooks, D. E., *Clin. Toxicol.* 2008, *46*, 771–773.

[23] Wang, J. H., Liu, Z. Q., Wang, W., Chen, X. P., Shu, Y., He, N., Zhou, H. H., *Clin. Pharmacol. Ther.* 2001, *70*, 42–47.

[24] Chander, W. P., Singh, N., Mukhiya, G. K., *J. Indian Med. Assoc.* 2011, *109*, 36–37.

[25] Kocabay, G., Yildiz, M., Eksi Duran, N., Ozkan, M., *Clin. Cardiol.* 2010, *33*, E114–E115.

[26] Hirano, K., Kimura, R., Sugimoto, Y., Yamada, J., Uchida, S., Kato, Y., Hashimoto, H., Yamada, S., *Br. J. Pharmacol.* 2005, *144*, 695–702.

[27] Rollema, H., Wilson, G. G., Lee, T. C., Folgering, J. H., Flik, G., *Neurochem. Int.* 2011, *58*, 78–84.

[28] Li, Y., Couch, L., Higuchi, M., Fang, J. L., Guo, L., *Toxicol. Sci.* 2012, *127*, 582–591.

[29] Kumar, P., Kalonia, H., Kumar, A., *Behav. Pharmacol.* 2010, *21*, 217–230.

[30] Kumar, P., Kumar, A., *Indian J. Exp. Biol.* 2009, *47*, 715–722.

[31] Torres-Molina, F., Aristorena, J. C., Garcia-Carbonell, C., Granero, L., Chesa-Jimenez, J., Pla-Delfina, J., Peris-Ribera, J. E., *Pharm. Res.* 1992, *9*, 1587–1591.

[32] Jámbor, A., Molnár-Perl, I., *J. Chromatogr. A* 2009, *1216*, 3064–3077.

[33] Bosch, M. E., Sanchez, A. J., Rojas, F. S., Ojeda, C. B., *J. Pharm. Biomed. Anal.* 2008, *48*, 1290–1302.

[34] Kishida, T., Muto, S., Hayashi, M., Tsutsui, M., Tanaka, S., Murakami, M., Kuroda, J., *J. Toxicol. Sci.* 2008, *33*, 447–457.

© 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim