Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 830 (2006) 114–119
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Stereoselective HPLC assay of donepezil enantiomers with UV detection and its application to pharmacokinetics in rats

Mahasen A. Radwan [a,*], Heba H. Abdine [b], Bushra T. Al-Quadeb [c], Hassan Y. Aboul-Enein [d,**], Kenichiro Nakashima [e]

[a] *Department of Clinical Pharmacy, College of Pharmacy, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia*
[b] *Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia*
[c] *Department of Pharmaceutics, College of Pharmacy, King Saud University, PO Box 22452, Riyadh 11495, Saudi Arabia*
[d] *Centre for Clinical Research (MBC-03-65), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia*
[e] *Department of Clinical Pharmacy, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*

Received 3 August 2005; accepted 20 October 2005
Available online 9 November 2005

Abstract

This investigation describes a new precise, sensitive and accurate stereoselective HPLC method for the simultaneous determination of donepezil enantiomers in tablets and plasma with enough sensitivity to follow its pharmacokinetics in rats up to 12 h after single oral dosing. Enantiomeric resolution was achieved on a cellulose tris (3,5-dimethylphenyl carbamate) column known as Chiralcel OD, with UV detection at 268 nm, and the mobile phase consisted of *n*-hexane, isopropanol and triethylamine (87:12.9:0.1). Using the chromatographic conditions described, donepezil enantiomers were well resolved with mean retention times of 12.8 and 16.3 min, respectively. Linear response ($r > 0.994$) was observed over the range of 0.05–2 μg/ml of donepezil enantiomers, with detection limit of 20 ng/ml. The mean relative standard deviation (R.S.D.%) of the results of within-day precision and accuracy of the drug were ≤10%. There was no significant difference ($p > 0.05$) between inter- and intra-day studies for each enantiomers which confirmed the reproducibility of the assay method. The mean extraction efficiency was 92.6–93.2% of the enantiomers. The proposed method was found to be suitable and accurate for the quantitative determination of donepezil enantiomers in tablets. The assay method also shows good specificity to donepezil enantiomers, and it could be successfully applied to its pharmacokinetic studies and to therapeutic drug monitoring.

Keywords: Donepezil; Stereoselective; HPLC; Enantiomers; Pharmacokinetics; Chiral stationary phase

1. Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by progressive loss of memory followed by complete dementia. It accounts for 50% of dementia cases [1]. Donepezil hydrochloride (DH), {2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one}hydrochloride (Fig. 1), is a reversible inhibitor of acetylcholinesterase. It is the second drug approved by the FDA for the treatment of mild to moderate dementia of the Alzheimer's type. DH was demonstrated to be a potent and selective inhibitor of brain acetylcholinesterase with less adverse effects than physostigmine and tacrine [2,3]. In clinical trials, significant correlations are demonstrated between plasma concentration of donepezil and percentage of acetylcholinesterase inhibition. A 50% inhibition of acetylcholinesterase activity is obtained at a plasma drug concentration of 15.6 ng/ml, and the inhibition plateaus at plasma concentration of donepezil higher than 50 ng/ml [4]. Therefore, plasma drug concentration can be a useful tool to predict clinical outcome of donepezil in the treatment of Alzheimer disease. It is characterised by a long plasma half-life (70 h), which is dose-independent [5], and a similar disposition in young and elderly subjects [6]. The elimination of donepezil occurs slowly and through both renal excretion of intact drug and biotransformation via the cytochrome P450 system.

* Corresponding author.
** Corresponding author. Tel.: +966 14427859; fax: +966 14427858.
E-mail addresses: mradwan@ksu.edu.sa (M.A. Radwan), hyaboulenein@yahoo.com (H.Y. Aboul-Enein).

1570-0232/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2005.10.031

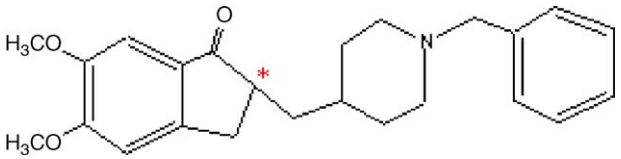

Fig. 1. Chemical structure of donepezil (the asterisk indicates the chiral center).

DH has a chiral center adjacent to a carbonyl group. It is commercially available as the hydrochloride salt and is used as a racemic mixture in dosage forms. The donepzil enantiomers have differing extents of inhibition against acetylcholinestrerase in vivo and in vitro [7]; hence it was necessary to clarify the pharmacokinetics of each isomer during drug development course. The importance to develop new chiral discrimination separation systems to determine enantiomers in bulk substance, drug formulations and in biological fluids increases. This is because stereoisomers may have different pharmacological effects and also show differences in pharmacokinetic and pharmacodynamic properties [8].

Several pharmacokinetic studies [6,7,9–11] including drug interactions with donepezil have been reported using high performance chromatographic (HPLC) method with UV detection [8], but methodology was not described. It should be mentioned that Matsui et al. [7] were the only group addressed the pharmacokinetics of DH enantiomers in human using liquid chromatography–mass spectrometry, which was sensitive, but complicated. To our knowledge, no data on the pharmacokinetics of the enantiomers of DH in rats is available; also no method has been published for their determination in tablets using this type of column with UV detection. The present study describes a stereospecific, isocratic, normal-phase HPLC method for the determination of the enantiomers of DH in tablets and plasma and its application to pharmacokinetic studies in rats.

2. Experimental

2.1. Materials

Racemic donepezil HCl was extracted from tablets and purity was confirmed by HPLC and mass spectrometry. All other reagents and chemicals were analytical grade, and used as received.

2.2. Chromatographic system and conditions

An HPLC Waters' system was used in this study. It was equipped with Waters 484 variable UV absorbance detector and Waters 717 Plus autosampler. Waters 515 solvent delivery system was used to operate the isocratic flow. The data were collected with an Empower Pro Chromatography Manager Data Collection System, utilizing a Pentium 4 computer connected to Inkjet HP 895 Cxi printer. The used analytical column (250 mm × 4.6 mm i.d., 10 μm particle size) was a Chiralcel OD column (Daicel Industries, Tokyo Japan). The mobile phase consisted of *n*-hexane, isopropyl alcohol and triethylamine (87:12.9:0.1, v/v/v), filtered through 0.22 μm millipore filter and degassed by sonication. Separation was carried out isocratically, at ambient temperature (22 ± 1 °C), and a flow rate of 1.0 ml/min, with ultraviolet (UV) detection at 268 nm. The injection volume was 75 μL.

2.3. Preparation of standard solutions

Stock solution of DH was prepared by dissolving 1 mg/ml of racemic DH in methanol and stored in 4 ml amber glass vials at −20 °C until used. Calibration standards were prepared daily by dilution with the mobile phase to give DH concentrations in the range of 0.5–5 and 0.05–2 μg/ml for in vitro and in vivo work, respectively.

The solutions were stable for at least 3 months when stored at −20 °C, and no evidence of degradation of the analytes was observed on the chromatograms during this period.

2.4. Determination of donepezil in the pharmaceutical dosage forms

Two commercially available formulations (Aricept® tablets) labeled to contain either 5 or 10 mg donepezil hydrochloride were analyzed. Six tablets of each formulation were used. One tablet was grinded and the content was suspended in methanol and transferred to a 100 ml volumetric flask with the aid of methanol. The content of the flask was vortexed for 2 min and sonicated for 15 min, the flask was made up to volume with methanol. Aliquot of the content was filtered and a specified volume of the filtrate was further diluted with the mobile phase to obtain final sample solutions which contained about 5 μg/ml of DH. The content of each enantiomer was determined by using external standard working solutions from pure reference compound run simultaneously.

2.5. Determination of donepezil in spiked rat plasma

Two hundred microlitres of rat plasma samples were spiked with 20–50 μL of DH stock solutions in 1.8 ml disposable polypropylene microcentrifuge tube. The tube was vortexed for 30 s. The solution was mixed with 600 μL of acetonitrile, vortexed at high speed for 1 min, and centrifuged at 20,000 rpm for 15 min. The supernatant was transferred to a 5 ml centrifuge tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 100 μL of the mobile phase. Each concentration was prepared at least in triplicate.

The absolute recovery of each enantiomer was assessed by extracting plasma specimens spiked to contain drug concentrations from 0.05 to 2 μg/ml. The area under the peak (AUP) was then compared with the AUP for mobile phase standards containing equivalent amounts of the drug without extraction.

Intra- and inter-day accuracy and precision were evaluated by assaying quality controls with different concentrations of DH (0.05–2 μg/ml). Intra- and inter-day accuracy and precision were assessed by analyzing six quality control samples at each concentration on the same day and mean values of six replicates with the same concentrations on three separate days within a month,

respectively. Accuracy was presented as percent relative error [(measured concentration − spiked concentration)/spiked concentration] × 100, while precision was quantitated by calculating intra- and inter-relative standard deviation values (R.S.D.%). Two different serial numbered Chiralcel OD columns were used to confirm the precision of the method (serial number 20-20-00418 and 50-20-30318).

2.6. Pharmacokinetics of donepezil in rats after oral administrations

The animal handling was fully complied with our institutional policies. Twelve male Wistar rats (164 ± 15 g) were used in this study. Rats were randomly divided into two groups ($n = 6$) for different sampling time. Each group was marked and housed in one cage. Water was available ad libitum at all times during the experiment. The content of one tablet was suspended in sterile water for injection (SWFI) containing acacia mucilage. Each rat received an oral dose of 3 mg/kg. Immediately before each administration the suspension was vortexed for few seconds. Blood samples (0.5 ml) were collected from the orbital venous plexus, under light halothane anesthesia, in microtainer, containing lithium heparin, at 0, 1, 2, 3 h and at 4, 8 and 12 h from the first and the second groups, respectively, after drug administrations. Each data point is the mean of six replicates. Plasma samples were separated by centrifugation at 4000 rpm for 15 min and stored at −20 °C till assayed.

2.7. Solution stability and mobile phase stability

The concentrations of DH enantiomers in spiked samples were determined immediately following their preparation and after storage at −20 °C for 1 month. The concentrations in the stored samples were compared with those found immediately after preparation.

Mobile phase stability was carried out by evaluating the concentration of DH enantiomers in solutions prepared freshly at 6 h intervals for 2 days. Same mobile phase was used during the study period.

2.8. Data and statistical analysis

All results were expressed as the mean ± S.D. of three to six replicates. The results were calculated by linear regression without weighing, using the formula: $Y = a + bX$, where Y is the AUP of the drug, a the intercept, b the slope, and X is the concentration of DH. The R.S.D.% was calculated for all values. Since no internal standard was available for the above mentioned conditions, the AUP was used for each enantiomer. Pharmacokinetic parameters were estimated using model-independent methods [12]. The terminal elimination rate constant (λ_n) was estimated by linear regression analysis of the terminal portion of the log-linear blood concentration-time profile of a drug. The terminal elimination half-life ($t_{1/2}$) was calculated from the terminal elimination rate constant using the formula $t_{1/2} = 0.693/\lambda_n$. The mean peak drug concentration (C_{max}) and the time to reach C_{max} (T_{max}) were derived directly from the individual blood levels. The area under each drug concentration time curve (AUC, μg ml^{-1} h) to the last data point were calculated by the linear trapezoidal rule and extrapolated to time infinity by the addition of C_{Last}/λ_n where, C_{Last} is concentration of the last measured blood sample. The apparent oral clearance (Cl/F) was calculated from dose/AUC.

The Student t-test was used to examine the concentration difference at each day, and one-way analysis of variance (ANOVA) was employed to evaluate the reproducibility of the assay. The level of confidence was 95%.

3. Results and discussion

3.1. Analytical parameters

3.1.1. Chromatography and selectivity

In recent years, the direct separation of enantiomers by chiral chromatography has been the target of intense research. Enantiomeric selectivity is usually achieved through the appropriate choice of a chiral stationary phase and the mobile phase conditions. In this study a stereoselective HPLC method was developed and validated for the rapid quantitative determination of donepezil enantiomers. Enantiomer resolution of DH and baseline separation in rat plasma was achieved using the Chiralcel OD column. There were no interfering peaks co-eluted with the compound of interest (Fig. 2A and B), which could originate from endogenous substances in rat plasma. This has indicated appropriate selectivity of the elaborated procedure. The order of elution was determined by using an optical detector Chiralycer (IBZ Messtechnik GmbH, Hannover, Germany), the retention times of (*R*)- and (*S*)-DH were approximately 12.9 and 16.9 min, respectively (Fig. 2B). The performance of the HPLC assay was assessed using the following parameters, namely, selectivity, linearity, limit of quantitation (LOQ), limit of detection (LOD), stability, precision, accuracy and recovery.

Various percentages of *n*-hexane, isopropyl alcohol and triethylamine in the mobile phase were tested to achieve the optimum resolution between DH enantiomers. The enantioselectivity increased by decreasing the concentration of isopropyl alcohol. However, lowering the isopropyl alcohol contents resulted in an increase in the chromatographic run time.

3.1.2. Linearity, LOQ and LOD

Excellent linear relationships ($r > 0.994$) were demonstrated between AUP of (−)*R*- and (+)*S*-DH and the corresponding plasma concentrations over a range of 0.05–2.0 μg/ml for both DH enantiomers. The mean linear regression equation of the peak area ratios (Y) versus drug concentrations (X) of DH was typically of the form $Y = (a \pm \text{S.D.}) + (b \pm \text{S.D.})X$ and it was $Y = (850 \pm 130) + (36528.3 \pm 3067)X$ for (*R*)-DH and $Y = (164 \pm 16.4) + (40076.7 \pm 3178)X$ for (*S*)-DH with mean correlation of 0.996 ± 0.002 and 0.997 ± 0.003, respectively. The LOQ of this assay was 0.05 μg/ml in rat plasma with the corresponding relative standard deviation of 4.8 and 4.0% for (−)*R*- and (+)*S*-DH, respectively. The LOD was 20 ng/ml for each enantiomer at a signal-to-noise ratio of >3.

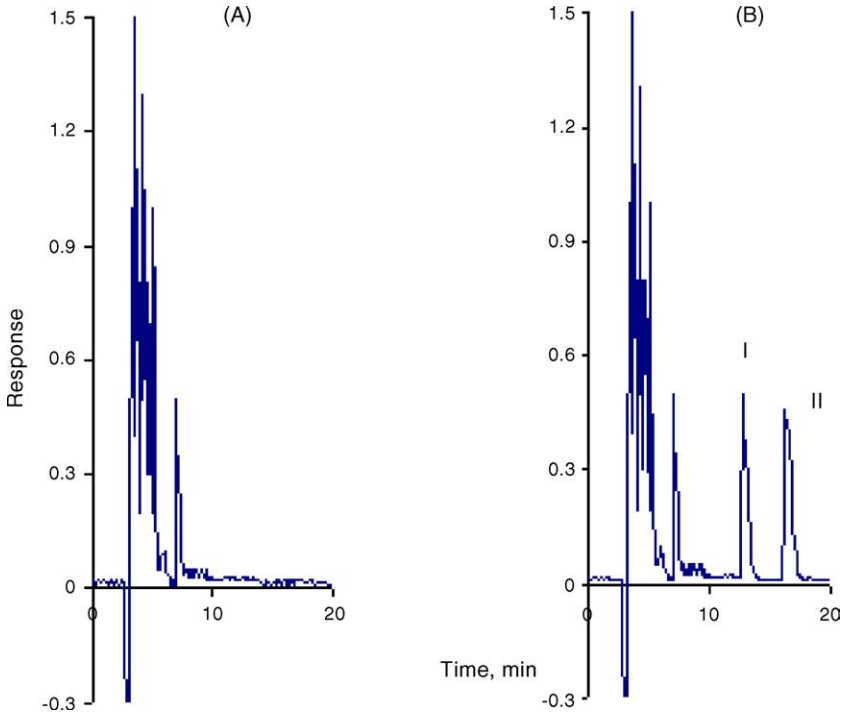

Fig. 2. Representative chromatograms, analyzed on Chiralcel OD column, of extracted drug-free plasma (A), plasma samples spiked with 200 ng/ml of donepezil HCl (B). I: (−)*R*-DH and II: (−)*S*-DH

3.1.3. Recovery, accuracy, and precision

The mean percentage recovery of 0.05–2 μg/ml of DH was 93.2 ± 4.9 for (−)*R*-DH and 92.6 ± 5.5 for (+)*S*-DH with R.S.D.% of ≤10 for both enantiomers. It should be mentioned that the extraction efficacy of the present assay was found not to significantly vary among different concentrations of DH enantiomers.

Within-day precision and accuracy of the method were determined from replicate analysis ($n = 6$) of four spiked plasma test standards at concentrations within the linear range of the assay for each drug (Table 1).

The reproducibility of the assay was evaluated by comparing the linear regressions of three standard plots prepared at three different days over a 3-week period. The mean correlation coefficient was >0.994 with R.S.D.% of the slopes of the three lines was 8.4 and 7.9% for (*R*)- and (*S*)-DH, respectively. Analysis of variance of the data indicated no significant difference ($p > 0.05$) in the slopes, intra- and inter-day, of the calibration curves. The results confirmed the reproducibility of the assay method.

The present method offers significant advantages over those previously reported methods, one of the reported methods, although more sensitive, but was complicated [7]. Another method, was used for the determination of DH in tablets [13], but was unable to differentiate between both (*R*)- and (*S*)-DH. A capillary electrophoresis (CE) was also reported [14] for the determination of DH enantiomers in tablets, but CE is not available in all laboratories. It should be mentioned that using two different serial numbered of Chiralcel OD columns showed no significant difference in the precision and accuracy of the assay.

3.1.4. Stability of DH samples

DH enantiomers were stable in spiked plasma samples (>90%) for at least 1 month for all the tested concentrations. Also there was no observed significant change in the DH enantiomers' concentrations during solution stability and mobile phase stability experiments. Hence, DH sample solution and mobile phase are stable for at least 3 months.

Table 1
Precision (R.S.D.%), accuracy (relative error, %) and recovery for determination of donepezil (−)*R*- and (+)*S*-DH enantiomers in spiked rat plasma samples ($n = 6$)

Nominal concentration (ng/ml)	Recovery (%)		Intra-day				Inter-day			
			Precision (%)		Accuracy (%)		Precision (%)		Accuracy (%)	
	(−)*R*-DH	(+)*S*-DH	(−)*R*-DH	(+)*S*-DH	(−)*R*-DH	(+)*S*-DH	(−)*R*-DH	(+)*S*-DH	(−)*R*-DH	(+)*S*-DH
50	95.7	93.9	5.9	6.4	5.1	4.6	4.8	4.0	4.3	5.1
100	89	92	6.5	5.4	5.3	3.7	7.9	8.6	9.7	8
1000	90.7	88.9	7.1	4.4	3.9	2.9	5.7	8.1	9.3	10
2000	97.3	95.5	4.3	3.1	1.2	2.8	6.5	7.1	2.7	4.5

Table 2
Determination of the mean (−)*R*- and (+)*S-donezepil* enantiomers content in pharmaceutical dosage forms ($n = 6$)

Dosage form	(−)*R*-DH		(+)*S*-DH	
	Content* (%)	R.S.D. (%)	Content* (%)	R.S.D. (%)
Aricept® 5 mg	95.6	8.3	96.2	9.6
Aricept® 10 mg	96.9	3.2	97.3	3.4

* There is no significant difference ($p > 0.05$) of DH enantiomers content among tablets for each for each formulation.

Table 3
Pharmacokinetic parameters of donepezil after 0.3 mg/kg PO dose in the rat ($n = 6$)

Parameter	(−)*R*-DH	(+)*S*-DH
C_{max}[a] (ng/ml)	143 ± 66	148 ± 110
T_{max}[a] (h)	5.2 ± 4	3.3 ± 2.7
AUC (mg h/L)	3.94	2.91
Cl/F (L/h kg)	0.038	0.051
$t_{1/2}$ (h)	27.2	16.8

[a] Calculated from the actual data.

3.2. Analysis of donepezil tablets

The validity of the proposed method in chiral analysis was studied by assaying DH enantiomers in two commercially available tablets (Aricept® 5 and 10 mg). Six replicate determinations were made. Compared with the content of DH declared by the manufacturer, the recoveries were in the range of 95–105 % (Table 2) for the two formulations.

3.3. Pharmacokinetics in rats

A 50% inhibition of acetylcholinesterase activity is obtained at a plasma drug concentration of 15.6 ng/ml, and the inhibition plateaus at plasma concentration of donepezil higher than 50 ng/ml [15]. Therefore, plasma drug concentration can be a useful tool to predict clinical outcome of donepezil in the treatment of Alzheimer disease. The worked out HPLC method has been successfully applied to the quantification of DH enantiomers for the pharmacokinetic study in rats. Following oral administration of 3 mg/kg of racemic DH, a rapid absorption and stereoselective disposition was observed for both enantiomers (Fig. 3). The pharmacokinetic parameters of DH (−)*R*- and (+)*S*-enantiomer are summarized in Table 3. It was reported [7] that the mean plasma levels of *S*-donepezil were found to be higher than those of *R*-donepezil in human. Although, in rats, C_{max} was not significantly different between the two enantiomers ($p > 0.05$), their T_{max} was significantly different ($p < 0.05$). The (−)*R*-enantiomer showed a slower elimination than its antipode which resulted in a longer $t_{1/2}$ of the (−)*R*- than (+)*S*-enantiomer.

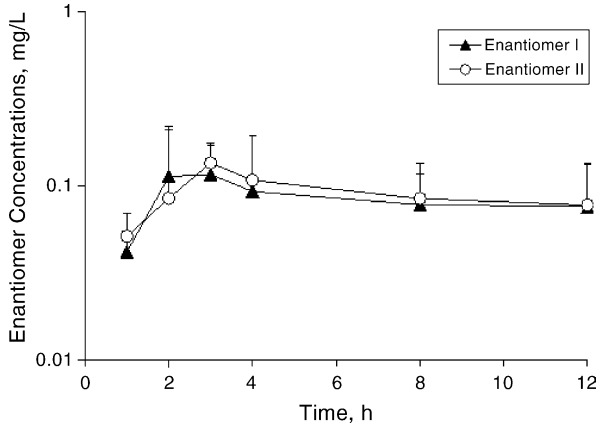

Fig. 3. Mean plasma concentrations (+S.D.) time profile of (−)*R*- and (+)*S-donezepil* enantiomers in rats after receiving 3 mg/kg oral dose of racemic donepezil.

4. Conclusion

A new simple, stereospecific, reproducible, accurate and sensitive HPLC method has been successfully developed and applied to the determination of DH enantiomers in rats and in its commercial tablets. The results were used to generate profiles and to estimate the pharmacokinetic parameters of DH enantiomers up to 12 h after oral administration in rats. The disposition of the two enantiomers seems to be different.

Acknowledgments

The first author wishes to express her gratitude to Dr. Ahmed Mitwalli Awad for his endless support during this study. Professor Aboul-Enein wishes to express his gratitude to the administration of King Faisal Specialist Hospital and Research Centre for its support.

References

[1] S.A. Areosa, F. Sherriff, Memantine for dementia. The Cochrane Database of Systematic Reviews 3, CD003154, 2003.
[2] H. Sugimoto, Y. Yamanishi, Y. Iimura, Y. Kawakami, Curr. Med. Chem. 7 (2000) 303.
[3] E. Scarpini, P. Scheltens, H. Feldman, Lancet Neurol. 2 (2003) 539.
[4] S.L. Rogers, R.S. Doody, R.C. Mohs, L.T. Friedhoff, Arch. Intern. Med. 158 (1998) 1021.
[5] S.L. Rogers, E.J. Walters, L.T. Friedhoff, Neurobiol. Aging 13 (1992) S125.
[6] A. Ohnishi, M. Mihara, H. Kamakura, Y. Tomono, J. Hasegawa, K. Yamazaki, N. Morishita, T. Tanaka, J. Clin. Pharmacol. 33 (1993) 1086.
[7] K. Matsui, Y. Oda, H. Nakata, T. Yoshimura, J. Chromatogr. B 729 (1999) 147.
[8] J.P. Mason, A.J. Hutt, in: H.Y. Aboul-Enein, I.W. Wainer (Eds.), The Impact of Sterochemistry in Drug Development and Use, John Wiley & Sons Inc., New York, USA, 1997, pp. 45–105 (Chapter 3).
[9] J.W. Lee, S.L. Rogers, L.T. Friedhoff, M.R. Stiles, N.M. Cooper, Pharm. Res. 9 (1992) S350.
[10] P.J. Tiseo, S.L. Rogers, L.T. Friedhoff, Clin. Pharmacol. Ther. 61 (1997) 184 (PII-75).
[11] N. Yasui-Furukori, R. Furuya, T. Takahata, T. Tateishi, J. Chromatogr. B 768 (2002) 261.

[12] M. Gibaldi, D. Perrier, Pharmacokinetics, second ed., Marcel Dekker, New York, 1982, pp. 407–417.
[13] H. Pappa, R. Farru, P.O. Vilanova, M. Palacios, M. Pizzorno, J. Pharm. Biomed. Anal. 27 (2002) 177–182.
[14] R. Gotti, V. Cavrini, R. Pomponio, V. Andrrisano, J. Pharm. Biomed. Anal. 24 (2001) 863–870.
[15] S.L. Rogers, R.S. Doody, R.C. Mohs, L.T. Friedhoff, Arch. Intern. Med. 158 (1998) 1021.