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Simultaneous determination of donepezil (aricept®) enantiomers in human plasma by liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A rapid, sensitive and enantioselective LC–MS–MS method using deuterium-labeled internal standard was developed and evaluated for the simultaneous quantitative determination of donepezil enantiomers in human plasma without interconversion during clean-up process and measurement. The use of an avidin column allowed the separation of donepezil enantiomers, which were specifically detected by MS–MS without interference from its metabolites and plasma constituents. Evaluation of this assay method shows that samples can be assayed with acceptable accuracy and precision within the range from 0.0206 ng/ml to 51.6 ng/ml for both *R*-donepezil and *S*-donepezil. This analytical method was applied to the simultaneous quantitation of donepezil enantiomers in human plasma. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Donepezil; Aricept

1. Introduction

Donepezil, (±)-2-[(1-benzylpiperidin-4-yl)-methyl]-5,6-dimethoxyindan-1-one monohydrochloride, is the second drug approved by the FDA for the treatment of Alzheimer's disease; tacrine HC1, a centrally and peripherally active reversible cholinesterase inhibitor, was the first. Donepezil also is a reversible cholinesterase inhibitor, but differs from tacrine in its higher specificity for centrally active cholinesterases such as acetylcholinesterase [1–3]. Donepezil is a racemate (*R*-donepezil, *S*-donepezil) due to the presence of an asymmetric carbon atom and the enantiomer ratio is 0.97 to 1.03. The donepezil enantiomers have differing (and a little) extents of inhibition against acetylcholinesterase in vivo and in vitro; hence it was necessary to clarify the pharmacokinetic profiles of the individual isomers in a drug development program.

Chiral separations of drug enantiomers by high-performance liquid chromatography (HPLC) have progressed greatly in recent years [4–8] and many chiral separation phases have been developed [9–12]. Protein-conjugated columns, such as a bovine serum albumin-conjugated columns [13], α-acid glycoprotein-conjugated columns [14], ovomucoid-conjugated columns [15] and avidin-conjugated columns [16] separate drug enantiomers with a broad range [17] and can be used in the reversed-phase separation mode [18], which is well suited for the

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analysis of biological samples [19]. In these protein-conjugated columns the avidin-conjugated column is much more suitable for enantioselective separation of donepezil [20], therefore we tried to develop the enantioselective determination method of donepezil using this column. Since donepezil enantiomers interconvert in aqueous solutions and plasma via a keto-enol intermediate, we applied a simple and mild clean-up procedure to prevent the interconversion during extraction. We combined LC and MS to detect donepezil enantiomers with selected-ion monitoring without interference from the metabolites of donepezil and from plasma-constituents. Moreover, the use of the deuterium labeled internal standard (donepezil-d_7) allowed not only the improvement of the reproducibility of the ionization but the normalization the change of recovery of donepezil from plasma.

This analytical method was applied to the simultaneous quantitation of donepezil enantiomers in human plasma.

2. Experimental

2.1. Chemicals and reagents

(*R,S*)-Donepezil, *R*-donepezil, *S*-donepezil and donepezil-d_7 were synthesized at Eisai Co., Ltd., Japan and its chemical structure is shown in Fig. 1. (*R,S*)-Donepezil was a hydrochloride salt and *R* and *S*-donepezil were free bases, where 1.00 ng of the salt was equivalent to 0.912 ng of the free base. The enantiomeric ratio of (*R,S*)-donepezil was 0.97 to 1.03. The enantiomeric excess of *R*-donepezil and *S*-donepezil estimated by HPLC was 97.9 and 98.7%, respectively.

Other reagents and solvents used in this study were of analytical grade or HPLC grade and were obtained commercially.

2.2. Blank plasma

Blank human plasmas for the preparation of calibration curves and spiked samples were obtained commercially from Fuji Chemicals Co. Ltd. (Tokyo, Japan).

2.3. Apparatus

The LC instrument consisted of a Waters 600-MS (Millipore Co., Ltd., Tokyo Japan). In this system, a 5μm Bioptick AV-1 column of 2.1 mm I.D.×150 mm length (GL Sciences Inc., Tokyo, Japan), was used as an analytical column, elution was with 75% 10 m*M* formic acid and 25% methanol at a flow-rate of 0.2 ml/min. The elution conditions (elute pH, methanol content and buffer concentration) were optimized to retain and resolve the donepezil enantiomers completely.

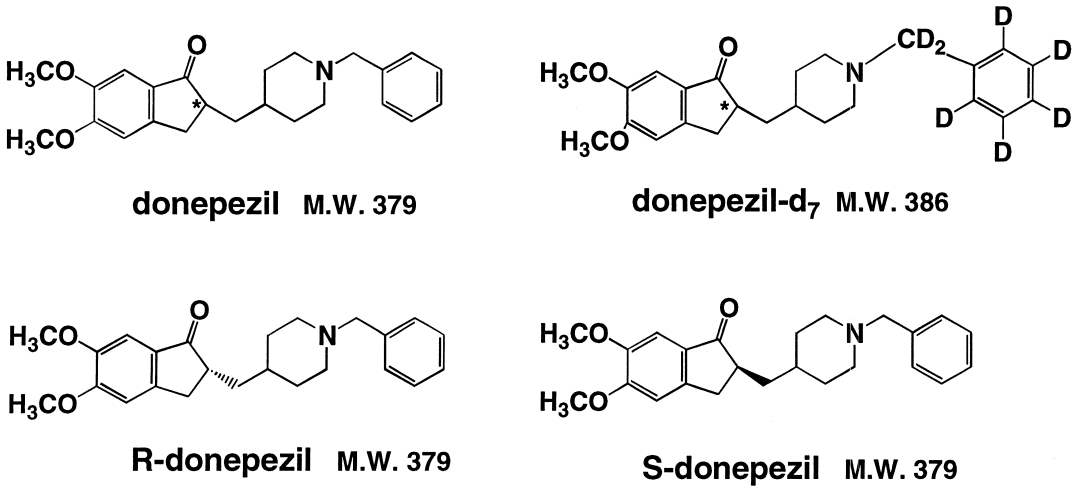

Fig. 1. Structures of the compounds measured in this assay. The asterisk indicates an asymmetric carbon.

ESI–MS–MS was carried out on a Finnigan MAT TSQ7000 (Finnigan MAT Instrument Inc., Tokyo, Japan) mass spectrometer equipped with the above LC system. The source was operated at 4.5 kV, capillary temperature 200°C, nitrogen sheath gas pressure 70 p.s.i., auxiliary nitrogen gas flow 10 units, and the spectrometer was set to admit the protonated molecules $[M+H]^+$ at m/z 380 (donepezil) and m/z 387 (donepezil-d_7) via the first quadrupole filter (Q1) with collision-induced fragmentation in Q2 (collision gas argon, 40 eV, 1.5 mTorr) and monitoring, via Q3, of the product ions at m/z 91 and m/z 98 for donepezil and donepezil-d_7, respectively.

2.4. Preparation of racemic donepezil ((R,S)-donepezil) standard solutions

A standard solution of (*R,S*)-donepezil was prepared by dissolving donepezil in 0.001 *M* hydrochloric acid and diluted with 0.001 *M* hydrochloric acid.

2.5. Preparation of R-donepezil and S-donepezil standard solutions

Each standard solution was prepared by dissolving the free base of *R*-donepezil or *S*-donepezil in 0.1 *M* hydrochloric acid and was diluted with 0.001 *M* hydrochloric acid.

2.6. Preparation of racemic donepezil-d_7 ((R,S)-donepezil-d_7) standard solution

(*R,S*)-donepezil-d_7 was used as the internal standard. A stock solution was prepared by dissolving donepezil-d_7 in 0.001 *M* hydrochloric acid at a concentration of 200 ng/ml.

2.7. Preparation of spiked samples of R-donepezil and S-donepezil

Spiked samples of *R*-donepezil and *S*-donepezil were prepared by adding 0.1 ml of *R*-donepezil or *S*-donepezil standard solution to 1.0 ml of blank plasma.

The prepared samples were frozen and kept at −20°C.

2.8. Preparation of calibration curves

Racemic donepezil was used as the standard for both *R*-donepezil and *S*-donepezil. Calibration standards were freshly prepared for each extraction, by adding 0.1 ml of (*R,S*)-donepezil standard solution and 0.1 ml of donepezil-d_7 standard solution to 1.0 ml of plasma

2.9. Procedure for extraction of R-donepezil and S-donepezil from plasma

This simple and mild clean-up procedure was developed and optimized to prevent interconversion during extraction. To determine the plasma concentration of *R*-donepezil and *S*-donepezil, 0.1 ml of donepezil-d_7 solution and 5.0 ml of 3% isopropanol/*n*-hexane were added to 1.0 ml of plasma sample, then the samples were shaken for 5 min to extract the donepezil. After centrifugation (1800 *g*, 1 min), the organic phase was collected, then 0.2 ml of 0.001 *M* hydrochloric acid was added and the mixture shaken for 1 min to transfer donepezil to the aqueous phase. The solution was centrifuged (1800 *g*, 1 min), the organic layer removed, and 75 μl of the aqueous solution was subjected to LC–ESI–MS–MS. In this extraction method, the extraction recovery of donepezil from spiked plasma was more than 90%.

2.10. Intra-day variability

Standard solutions of (*R,S*)-donepezil were used for intra-day variability. Intra-day variability was determined for five replicates of spiked samples at each calibration concentration, which were then assayed against a single calibration curve.

2.11. Inter-day variation

Standard solutions of (*R,S*)-donepezil were used for inter-day variation. Inter-day variation was determined by analysis of the spiked samples on three separate occasions, relative to calibration samples which were prepared freshly each time.

2.12. Stability of R-donepezil and S-donepezil during the extraction procedure

Standard solutions of *R*-donepezil and *S*-donepezil were used to assess the stability of *R*-donepezil and *S*-donepezil during the extraction procedure.

The concentrations of *R*-donepezil and *S*-donepezil were determined immediately following preparation of samples for analysis, and were compared with the added amount.

2.13. Stability of R-donepezil and S-donepezil in the injection solvent

The concentrations of *R*-donepezil and *S*-donepezil in spiked samples were determined immediately following extraction, and after the samples had been kept in the extraction layer of hydrochloric acid at 4°C for 8, 16, and 30 h. The concentrations of *R*-donepezil and *S*-donepezil after storage were compared with their respective initial values.

2.14. Stability of R-donepezil and S-donepezil in frozen plasma

The concentrations of *R*-donepezil and *S*-donepezil were determined in aliquots of the spiked samples, immediately following their preparation and after storage at −20°C for 15 days, 1, 3 and 6 months. The concentrations in the stored samples of *R*-donepezil and *S*-donepezil were compared with those found immediately after preparation.

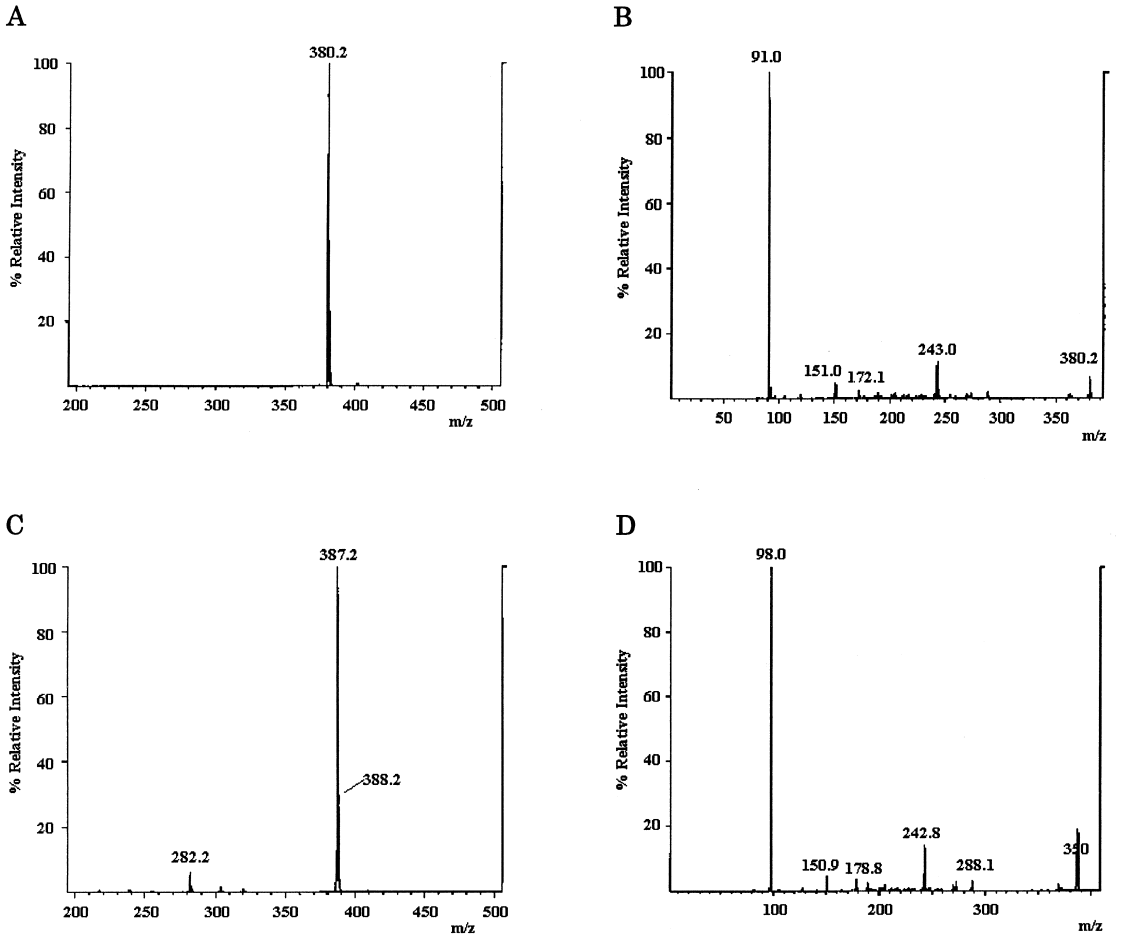

Fig. 2. Typical MS chromatograms. (A) and (B): MS and MS–MS chromatograms of donepezil (m/z 380→m/z 91.0). (C) and (D): MS and MS–MS chromatograms of donepezil-d_7 (m/z 387→m/z 98.0).

2.15. Calculation of concentrations

Peak area ratios (PAR) of the *R*-donepezil and *S*-donepezil to *R*-donepezil-d_7 and *S*-donepezil-d_7 were calculated and the results for calibration standards were then fitted to the following equation:

$$PAR = A \times Cp + B$$

where *Cp* is the added concentration.

2.16. Plasma samples

(*R,S*)-donepezil was orally administered to sixteen volunteers at 5.0 mg/man.

Venous blood samples of 4.5 ml were collected in heparinized tubes from eight of these volunteers, before and at 30 min, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 120 and 168 h after administration and immediately stored in ice.

Within 15 min after blood collection, blood plasmas were separated by centrifuging (1800 *g*) for 10 min, then 1.0 ml of plasma was taken, immediately frozen in liquid N_2 and stored at −20°C until analysis.

3. Results and discussion

The typical ESI–MS spectrum of donepezil is shown in Fig. 2A. The major ion was the protonated molecular ion (*m/z* 380) and few fragment ions were observed. The product fragment ion, which was generated by collision-induced fragmentation of the molecule at *m/z* 380, is shown in Fig. 2B. Fig. 2C and D show the corresponding spectra for donepezil-d_7. The ESI–MS–MS was, therefore, performed by monitoring at *m/z* 91 and *m/z* 98.

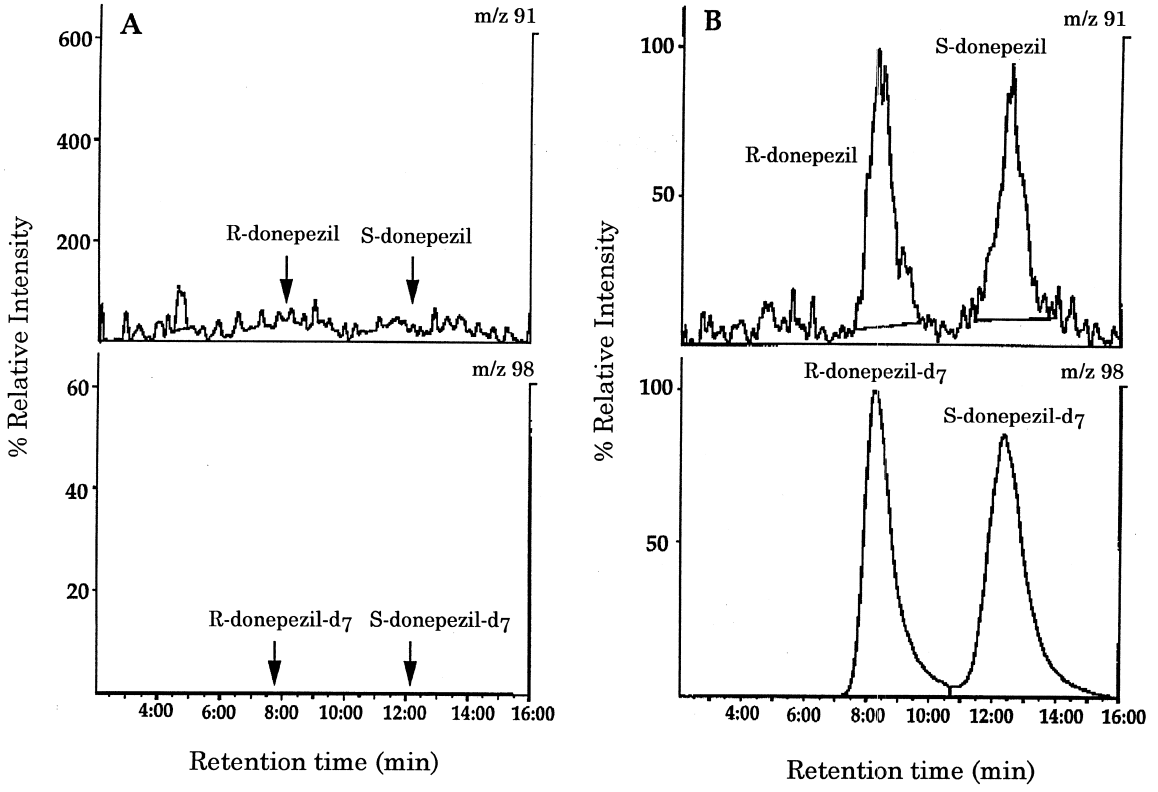

Fig. 3. Typical LC–MS–MS chromatograms. (A): blank plasma, (B): plasma sample spiked with 0.020 ng/ml of *R*- and *S*-donepezil and 20 ng/ml of (*R,S*)-donepezil-d_7

Table 1
Intra-assay validation of *R*-donepezil and *S*-donepezil[a]

Added amount (ng/ml)[b]	*R*-donepezil[c]				*S*-donepezil[d]			
	Mean (ng/ml)	SD (ng/ml)	C.V. (%)	Accuracy (%)	Mean (ng/ml)	SD (ng/ml)	C.V. (%)	Accuracy (%)
0.0206	0.0195	0.000817	4.2	−5.1	0.0195	0.000453	2.3	−5.4
0.0516	0.0502	0.00306	6.1	−2.8	0.0557	0.00435	7.8	7.9
0.103	0.103	0.00597	5.8	0.2	0.109	0.00577	5.3	5.4
0.206	0.212	0.00545	2.6	2.8	0.209	0.00832	4.0	1.3
0.516	0.554	0.0168	3.0	7.4	0.538	0.0124	2.3	4.3
1.03	1.08	0.0158	1.5	4.9	1.07	0.0200	1.9	3.9
2.06	2.16	0.0406	1.9	4.9	2.16	0.0458	2.1	4.9
5.16	5.28	0.0432	0.8	2.3	5.3	0.0377	0.7	2.7
10.3	10.1	0.0447	0.4	−1.7	10.1	0.1240	1.2	−1.6
20.6	19.4	0.217	1.1	−5.9	19.3	0.1870	1.0	−6.3
51.6	44.2	0.464	1.0	−14.3	44.6	0.3560	0.8	−13.6

[a] Values were obtained by five replicates.
[b] (*R,S*)-donepezil was added to plasma, at double the concentration shown.
[c] Calibration curve of *R*-donepezil: Found = (PAR − 0.000977772) ÷ 0.102977.
[d] Calibration curve of *S*-donepezil: Found = (PAR − 0.00037966) ÷ 0.102518.

3.1. Limit of detection

There were no significant interference peaks in the chromatogram of blank plasma (Fig. 3). The detection limit for pure substance was less than 7.7 pg per injection.

3.2. Linearity of calibration curve

The responses of both *R*-donepezil and *S*-donepezil were linear ($r \geq 0.997$) with respect to the concentration of (*R,S*)-donepezil within the range of 0.0206 ng/ml to 50.0 ng/ml.

3.3. Intra-assay validation

Intra-assay variability was determined for five replicates of spiked samples at each calibration concentration, which were then assayed against a single calibration curve. The data for *R*-donepezil and *S*-donepezil are shown in Table 1

Table 2
Inter-assay validation of *R*-donepezil[a]

Added amount (ng/ml)[b]	*R*-donepezil				*S*-donepezil			
	Mean (ng/ml)	SD (ng/ml)	C.V. (%)	Accuracy (%)	Mean (ng/ml)	SD (ng/ml)	C.V. (%)	Accuracy (%)
0.0206	0.0194	0.000872	4.5	−5.8	0.0196	0.000987	5.0	−4.7
0.0516	0.0531	0.00283	5.3	3.0	0.0532	0.00340	6.4	3.1
0.103	0.111	0.00379	3.4	7.5	0.108	0.00351	3.3	4.6
0.206	0.222	0.00751	3.4	7.6	0.223	0.00737	3.3	8.1
0.516	0.557	0.0137	2.5	7.9	0.553	0.0245	4.4	7.2
1.03	1.11	0.0208	1.9	7.4	1.11	0.0252	2.3	8.1
2.06	2.18	0.0764	3.5	6.0	2.21	0.0700	3.2	7.3
5.16	5.28	0.0321	0.6	2.4	5.28	0.0451	0.9	2.8
10.3	10.1	0.0577	0.6	−2.2	10.1	0.0577	0.6	−1.6
20.6	19.2	0.404	2.1	−6.7	19.3	0.200	1.0	−6.3
51.6	45.1	1.36	3.0	−12.7	45.7	1.15	2.5	−11.4

[a] Values were obtained by three replicates.
[b] (*R,S*)-donepezil was added to plasma, at double the concentration shown.

Table 3
Stability of *R*-donepezil and *S*-donepezil during extraction

Added amount (ng/ml)[a]		Found (%)[b]		
		Mean		SD
R-donepezil	0.125	90.7	±	4.11
	1.18	94.4	±	3.41
	10.4	97.7	±	1.10
S-donepezil	0.119	95.5	±	3.99
	1.21	95.6	±	0.98
	10.7	96.9	±	1.04

[a] Concentrations are expressed as the hydrochloride salt form.
[b] Found (%): Found amount/Added amount×100.

In the range from 0.0206 ng/ml to 51.6 ng/ml, the C.V.s of *R*-donepezil and *S*-donepezil ranged within 7.8% and accuracies ranged from −14.3% to +7.9%.

3.4. Inter-assay validation

Inter-assay variation was determined by analysis of the spiked plasma samples on three occasions, relative to the calibration samples, which were freshly prepared, each time. The data for *R*-donepezil and *S*-donepezil are shown in Table 2.

In the range from 0.0206 ng/ml to 51.6 ng/ml, the C.V.s of *R*-donepezil and *S*-donepezil ranged within 6.4%, and the accuracies ranged from −12.7% to +8.1%.

3.5. Limits of quantification

Based on the results of the assay validation, the lower limit of quantitation (LOQ) for both *R*-donepezil and *S*-donepezil was 0.020 ng/ml. The upper limit of reliable quantification was considered to be 50 ng/ml.

3.6. Stability of R-donepezil and S-donepezil during extraction

The concentrations of *R*-donepezil and *S*-donepezil in samples spiked by each enantiomer were 90.7 to 97.7% and 95.5 to 96.9% of the corresponding standard solutions, respectively, therefore decomposition and/or interconversion of the analyte during the preparation of samples accounted for less than 10% (Table 3).

3.7. Stability of R-donepezil and S-donepezil in the injection solvent

The concentrations of *R*-donepezil and *S*-donepezil in spiked samples were determined after storage in the injection solvent at 4°C for 8, 16 and 30 h, and were 96.5 to 104.5% and 96.6 to 103.4%, respectively, of those measured immediately following to the extraction (Table 4). Therefore, *R*-donepezil and *S*-donepezil were shown to be stable under the conditions mentioned above.

Table 4
Stability of *R*-donepezil and *S*-donepezil in the injection solvent

Added amount[a] (ng/ml)		Found (%)[b]								
		8 h			16 h			30 h		
		Mean		SD	Mean		SD	Mean		SD
R-donepezil	0.110	96.0	±	2.8	101.6	±	3.0	99.1	±	1.5
	1.10	100.6	±	1.4	100.9	±	4.1	104.0	±	5.5
	11.0	100.7	±	1.2	99.4	±	1.5	103.3	±	1.2
S-donepezil	0.110	98.2	±	7.0	101.0	±	6.5	103.1	±	2.1
	1.10	96.9	±	1.7	101.5	±	2.5	99.2	±	3.7
	11.0	99.4	±	2.4	98.1	±	1.0	98.8	±	2.0

[a] Concentrations are expressed as the hydrochloride salt form.
[b] Found (%): Found amount/Initial amount×100.

Table 5
Stability of *R*-donepezil and *S*-donepezil in the frozen plasma

Added amount[a] (ng/ml)		Found (%)[b]										
		15 day			30 day			90 day			180 day	
		Mean		SD	Mean		SD	Mean		SD	Mean	SD
R-donepezil	0.110	96.9	±	5.6	109.3	±	5.3	100.9	±	3.6	93.8	± 5.5
	1.10	94.0	±	3.8	89.6	±	2.1	84.4	±	1.2	83.1	± 3.3
	11.0	93.7	±	5.4	93.7	±	7.0	85.5	±	7.2	89.0	± 4.9
S-donepezil	0.110	95.2	±	4.2	94.8	±	5.9	76.4	±	6.2	90.7	± 10.8
	1.10	96.6	±	3.0	95.1	±	0.6	82.5	±	2.5	88.2	± 2.0
	11.0	97.8	±	0.5	98.1	±	2.9	85.3	±	0.3	95.0	± 1.4

[a] Concentrations are expressed as the hydrochloride salt form.
[b] Found (%): Found amount/Initial amount×100.

3.8. Stability of R-donepezil and S-donepezil in frozen plasma

The concentrations of *R*-donepezil and *S*-donepezil in spiked samples after storage at −20°C for 15 days, 1, 3 and 6 months were compared with those analyzed immediately following preparation (Table 5). The results indicated that both *R*-donepezil and *S*-donepezil were stable for up to 1 month, but after 3 months the 'found' concentrations of *R*-donepezil and *S*-donepezil in some samples had decreased by more than 10%.

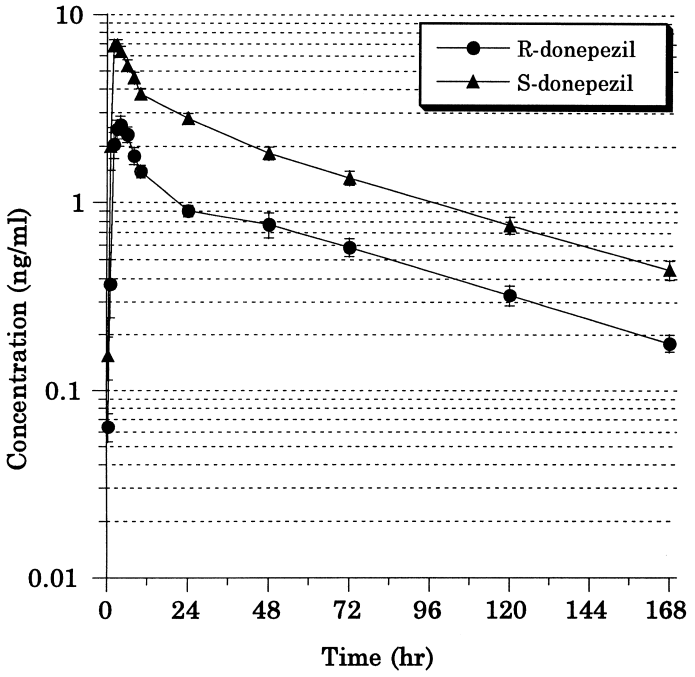

Fig. 4. Mean plasma levels of *R*-donepezil and *S*-donepezil after a single oral administration of donepezil (5.0 mg/man) to volunteers. Each point represents the mean with SEM of eight volunteers.

3.9. Plasma samples

The mean plasma levels of *R*-donepezil and *S*-donepezil, after a single oral administration of (*R,S*)-donepezil to healthy volunteers, are shown in Fig. 4.

The mean (±SEM) plasma levels of *R*-donepezil reached a peak of 2.588±0.291 ng/ml at 4.0 h after administration, followed by a biphasic exponential decline. The mean levels at 24 and 168 h were 0.907±0.062 and 0.181±0.019 ng/ml, respectively.

The mean plasma levels of *S*-donepezil were found to be higher than those of *R*-donepezil and reached a peak of 6.858±0.486 ng/ml at 3.0 h followed by a biphasic exponential decline almost in parallel with *R*-donepezil. The mean levels at 24 and 168 h after administration were 2.804±0.174 and 0.447±0.052 ng/ml, respectively.

4. Conclusion

The LC–ESI–MS–MS method described in this paper is sensitive, reproducible, precise, and stable for the measurement of *R*-donepezil and *S*-donepezil in human plasma. The method was considered sufficiently reliable to study the pharmacokinetics of donepezil enantiomers in humans. By using this method, plasma concentration-time profiles of *R*-donepezil and *S*-donepezil after oral administration of (*R,S*)-donepezil to humans were clarified, where *S*-donepezil showed higher plasma levels than *R*-donepezil and declined almost biphasically in parallel with *R*-donepezil.

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