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Determination of donepezil, an acetylcholinesterase inhibitor, in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection

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Abstract

A simple and sensitive high-performance liquid chromatographic (HPLC) method with UV absorbance detection is described for the quantification of donepezil, a centrally and selectively acting acetylcholinesterase inhibitor, in human plasma. After sample alkalinization with 0.5 ml of NaOH (0.1 *M*), the test compound was extracted from 1 ml of plasma using isopropanol–hexane (3:97, v/v). The organic phase was back-extracted with 75 μl of HCl (0.1 *M*) and 50 μl of the acid solution was injected into a C_{18} STR ODS-II analytical column (5 μm, 150×4.6 mm I.D.). The mobile phase consisted of phosphate buffer (0.02 *M*, pH 4.6), perchloric acid (6 *M*) and acetonitrile (59.5:0.5:40, v/v) and was delivered at a flow-rate of 1.0 ml/min at 40 °C. The peak was detected using a UV detector set at 315 nm, and the total time for a chromatographic separation was ~8 min. The method was validated for the concentration range 3–90 ng/ml. Mean recoveries were 89–98%. Intra- and inter-day relative standard deviations were less than 7.3 and 7.6%, respectively, at the concentrations ranging from 3 to 90 ng/ml. The method shows good specificity with respect to commonly prescribed psychotropic drugs, and it could be successfully applied for pharmacokinetic studies and therapeutic drug monitoring.

Keywords: Donepezil; Acetylcholinesterase

1. Introduction

Donepezil, (±)-2-[(1-benzylpipereidin-4-yl)-methyl]-5,6-dimethoxy-indan-1-one monohydrochloride (Fig. 1), is a centrally and selectively acting acetylcholinesterase inhibitor. It has been reported that donepezil is effective in the treatment of cognitive impairment and memory loss in patients with Alzheimer disease, and is well tolerated when 5 mg daily of the drug is prescribed [1].

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 [2]. Therefore, plasma drug concentration can be a useful

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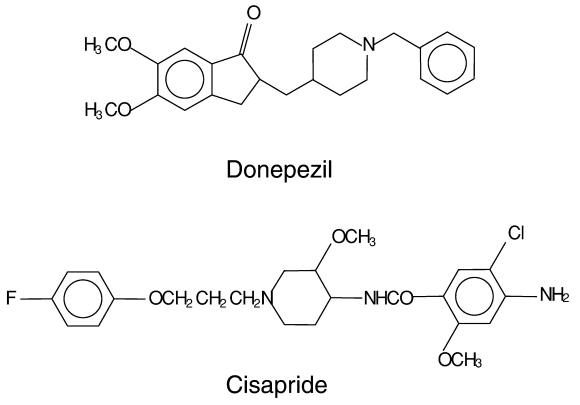

Fig. 1. Chemical structure of donepezil and cisapride.

tool to predict clinical outcome of donepezil in the treatment of Alzheimer disease.

Several pharmacokinetic studies including drug interactions with donepezil have been reported using high-performance chromatographic (HPLC) method with UV detection [3], but methodology was not described. Another study on determination of enantiomers of donepezil was performed using liquid chromatography–mass spectrometry [4], which was sensitive, but complicated.

In the present study, we describe a new, simple, rapid and sensitive HPLC method for determination of donepezil in plasma using liquid–liquid extraction. The assay involves a short chromatographic run and fulfills the requirements for use in therapeutic drug monitoring.

2. Experimental

2.1. Chemicals

Donepezil, (±)-2-[(1-benzylpipereidin-4-yl)-methyl]-5,6-dimethoxy-indan-1-one monohydrochloride, was kindly provided by Eizai (Tokyo, Japan). Cisapride, the internal standard (I.S.), was kindly donated by Welfide (Osaka, Japan). Potassium phosphate monobasic, acetonitrile, perchloric acid, *n*-hexane, and isopropanol were purchased from Wako (Osaka, Japan). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

Stock solutions of donepezil and I.S. for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 1.5 mg/ml. Working standard solutions of donepezil (1.5 μg/ml) and I.S. (3.0 μg/ml) were obtained by diluting 1000 and 500 times, respectively, each stock solution with 0.001 *M* HCl. Stock solutions were stable at 4 °C for at least 3 months. Drug-free plasma from healthy donors was used for validation studies. Calibration curves were prepared by spiking 10–300 μl of working solutions in 1 ml of blank plasma (final volume) to yield the final concentrations of 3, 7.5, 15, 30, 60 and 90 ng/ml for each analysis. Standard curves were prepared daily and constructed by linear regression analysis of the donepezil/internal standard peak-height ratio versus the respective concentration of donepezil. Another stock solution of donepezil was separately prepared for quality control. The 17.3 mg of donepezil was diluted with 57.5 ml of methanol to yield a concentration of 301 μg/ml. Working plasma solution was obtained by 1000 times dilution of stock solution with blank plasma (301 ng/ml). Quality control samples were obtained by spiking 10–300 μl of working plasma solutions in 1 ml of blank plasma (final volume) to yield the final concentrations of 3, 7.5, 15, 30, 60 and 90 ng/ml, and stored at at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.3. Blood sampling

A single oral dose (5 mg) of donepezil was given to two healthy volunteers and blood was obtained before dosing and at 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after dosing. Steady-state trough plasma concentrations of donepezil were obtained in samples just before the daily dose in seven Alzheimer's patients. The patients received 5 mg of donepezil once daily for at least 4 weeks. Blood samples were collected in heparinized tubes and centrifuged imme-

diately at 1710 *g* for 10 min. The plasma was stored at −20 °C until analysis.

2.4. Extraction procedure

I.S. 100 μl of 3 μg/ml and 0.5 ml NaOH (0.1 *M*) were added to 1 ml of plasma. The tubes were vortex-mixed for 10 s and 4 ml of *n*-hexane–isopropanol (97:3, v/v) was added as extraction solvent. After 10 min of shaking, the mixture was centrifuged at 620 *g* for 10 min at 4 °C, and the organic phase was transferred to new tubes containing 75 μl of 0.1 *M* HCl. The samples were mixed for 10 min at 100 cycles/min, and centrifuged at 1710 *g* for 5 min at 4 °C. The upper organic layer in each tube was carefully aspirated and the remaining organic solvent was evaporated under a stream of air at room temperature. A 50-μl aliquot of the remaining acid solution was injected into the HPLC system.

2.5. Apparatus

The chromatographic system consisted of a Tosoh CCPM high-pressure pump, AS-8000 autosampler and UV-8000 UV detector (Tokyo, Japan), a Shimadzu CTO-6A column oven and a C-R6A chromatography integrator (Kyoto, Japan).

2.6. Chromatographic conditions

A STR ODS-II analytical column (5 μm, 150×4.6 mm I.D.; Shinwa Chemical Industry, Kyoto, Japan) was used. The mobile phase consisted of phosphate buffer (0.02 *M*), perchloric acid (6 *M*) and acetonitrile (59.5:0.5:40, v/v) and was delivered at a flow-rate of 1.0 ml/min at 30 °C. Total run-time was 8 min, and the retention times for donepezil and cisapride (I.S.) were 5.1–5.2 and 7.1–7.3 min, respectively.

3. Results

3.1. Chromatography

A representative chromatogram of an unextracted working aqueous solution containing donepezil and cisapride (internal standard) is shown in Fig. 2A. The chromatogram of an extracted blank plasma sample is shown in Fig. 2B, while the chromatogram of an extracted sample spiked with 3 ng/ml of donepezil (lowest quality control) and cisapride (300 ng/ml) is shown in Fig. 2C. Both compounds were well separated from each other and from the front of the solvent peaks. The chromatogram of an extracted plasma sample obtained from a patient (43.2 ng/ml) receiving 5 mg/day of donepezil did not show interference peaks (Fig. 2D).

3.2. Recovery and linearity

Recovery from plasma was calculated by comparing the peak heights of pure standards prepared in 0.001 *M* HCl and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compound ($n=6$ each). Recoveries were determined at six different concentrations ranging from 3 to 90 ng/ml (Table 1). Calibration curves were linear over the concentration range of 3–90 ng/ml ($r=0.9987\pm0.0012$).

3.3. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with different concentrations of donepezil. Intra- and inter-day precisions were assessed by analyzing six quality control samples at each concentration on the same day and mean values of two quality controls for 6 days, respectively (Table 1). Intra- and inter-day relative standard deviations were less than 7.3 and 7.6%, respectively, in the concentration range of 3–90 ng/ml. Accuracy was presented as percent error (relative error), [(measured concentration−spiked concentration)/spiked concentration]×100 (%), while precision was quantitated by calculating intra- and inter-C.V. values.

3.4. Specificity

Potential interference from co-administered drugs was investigated by determining their retention times in this system. The retention times of nitrazepam,

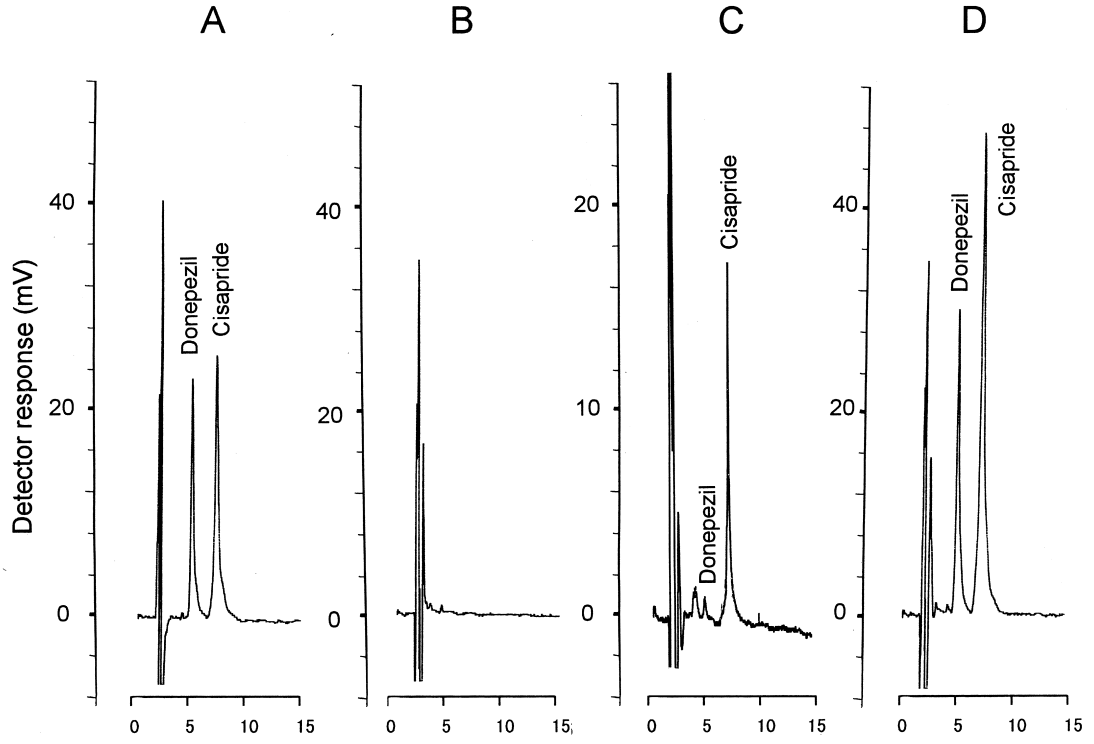

Fig. 2. Representative chromatograms of (A) pure standard of donepezil and cisapride in 0.001 *M* HCl injected without extraction, (B) blank plasma sample, (C) plasma spiked with 3 ng/ml of donepezil (the lowest quality control) and 300 ng/ml of cisapeide, and (D) plasma from a patient treated with 5 mg of donepezil per day. Calculated concentration was 43.2 ng/ml for donepezil.

diazepam, alprazolam, chlorpromazine, and levomepromazine were 3.2, 3.9, 4.4, 16.0 and 12.4 min, respectively. No peaks were observed until 60 min after injections of haloperidol or risperidone.

3.5. Donepezil concentration in human plasma

Fig. 3 shows concentration versus time curves obtained after oral administration of a tablet of donepezil (5 mg) to two volunteers. The pharmacokinetic parameters are summarized in Table 2. Plasma concentrations of donepezil during the elimination phase (12–72 h) were low, but successfully measured. Steady-state trough plasma concentrations of donepezil in patients treated with 5 mg/day of donepezil are shown in Table 3. The mean steady-state trough concentrations were higher than C_{max} after a single oral dose. No interfering peaks were observed, despite the fact that various other drugs were co-administered with donepezil.

Table 1
Precision (C.V.), accuracy (relative error) and recovery for determination of donepezil in spiked plasma ($n=6$)

Concentration (ng/ml)	C.V. (%)		Recovery (%)	Relative error (%)
	Within-day	Between-day		
3	7.3	7.6	94.6	9.0
7.5	6.3	6.4	89.5	−3.3
15	4.6	4.3	90.7	−2.8
30	3.6	4.7	92.8	−2.7
60	4.0	5.1	98.4	1.0
90	1.9	2.4	92.7	0.0

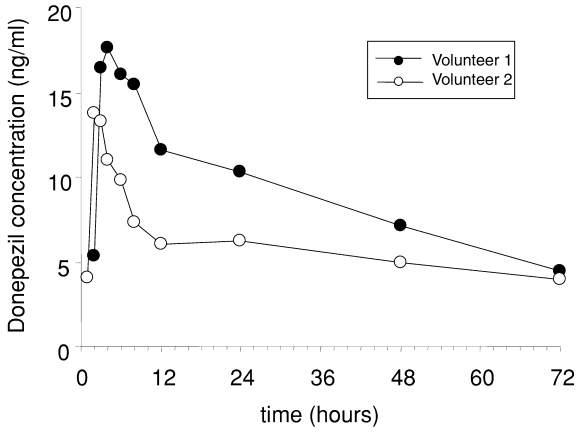

Fig. 3. Plasma concentration versus time curves of donepezil after a single oral dose of 5 mg to two volunteers.

Table 2
Pharmacokinetic parameters in volunteers taking a single 5-mg oral dose of donepezil

Parameters	Volunteer 1	Volunteer 2
C_{max} (ng/ml)	17.6	13.7
t_{max} (h)	4	2
$AUC_{(0-72)}$ (ng*h/ml)	628	416
CL/F (l/h per kg)	0.142	0.097
$t_{1/2}$ (h)	39.4	80.8

4. Discussion

To separate donepezil from endogenous interference peaks, the mobile phase was adjusted to pH 2. The retention time of donepezil was highly dependent on the percentage of perchloric acid.

Cisapride, a prokinetic agent, has been used for the treatment of a number of gastrointestinal disorders, but marketing of the drug has now been discontinued. As there is no possibility that cisapride would be administered with donepezil, cisapride was chosen as an internal standard.

Compared with donepezil, the recovery of cisapride using the condition described above was low (15–20%). Recovery should be improved, but then the recovery of donepezil was reduced. However, cisapride is considered to be acceptable as an internal standard because the recovery of cisapeide was consistent and calibration curves constructed from the peak-height ratio were linear ($r>0.99$) in all experiments.

In conclusion, the HPLC procedure described for donepezil determination is suitable for routine analysis. Satisfactory validation data were achieved for linearity, precision and recovery. The limit of quantification obtained allows measurement of therapeutic concentration of donepezil.

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Table 3
Clinical profiles of seven patients treated with 5 mg/day of donepezil and the drug concentrations in plasma

Age	Sex	Diagnosis	Plasma concentration (ng/ml)	Concomitant medications (mg/day)
85	F	AD	15.9	Valproate 300, zopiclone 10
83	F	AD	33.5	Fluvoxamine 150, nicergolin 10, enalapril 5, nilvadipine 4
62	M	AD	43.2	Nicergolin 10, nicorandil 10, diltiazem 60, kallidinogenase 100 IU
69	M	AD	39.2	Zopiclone 7.5, nifedipine 40
67	M	AD	17.7	Tiapride 75, trapidil 150, trandolapril 2
73	M	AD	32.6	Nicergolin 10, kallidinogenase 100 IU, fluvastatin 20
65	F	AD	17.4	Risperidone 2, biperiden 6, brotizolam 0.25, vinpocetine 15

AD, Alzheimer disease.