Determination of Donepezil Hydrochloride (E2020) in Plasma by Liquid Chromatography–Mass Spectrometry and Its Application to Pharmacokinetic Studies in Healthy, Young, Chinese Subjects

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Abstract

A sensitive, simple, and specific liquid chromatographic method coupled with electrospray ionization-mass spectrometry for the determination of donepezil in plasma is developed, and its pharmacokinetics in healthy, male, Chinese is studied. Using loratadine as the internal standard, after extraction of the alkalized plasma by isopropyl alcohol-*n*-hexane (3:97, v/v), solutes are separated on a C_{18} column with a mobile phase of methanol–acetate buffer (pH 4.0) (80:20, v/v). Detection is performed with a time-of-flight mass spectrometer equipped with an electrospray ionization source operated in the positive-ionization mode. Quantitation of E2020 is accomplished by computing the peak area ratio (donepezil $[M+H]^+$ *m/z* 380–loratadine $[M+H]^+$ *m/z* 383) and comparing them with the calibration curve (r = 0.9998). The linear calibration curve is obtained in the concentration range 0.1–15 ng/mL. The limit of quantitation is 0.1 ng/mL. The mean recovery of E2020 from human plasma is 99.4% ± 6.3% (ranging 93.4–102.6%). The inter- and intraday relative standard deviation is less than 15%. After an oral administration of 5 mg E2020 to 20 healthy Chinese volunteers, the main pharmacokinetic parameters of E2020 are as follow: T_{max}, 3.10 ± 0.55 h; $t_{1/2}$, 65.7 ± 12.8 h; C_{max}, 10.1 ± 2.02 ng/mL; MRT, 89.4 ± 13.4 h; and CL/F, 9.9 ± 4.3 L/h.

Introduction

Donepezil·HCl(E2020),[(*R,S*)-1-benzyl-4[(5,6-dimethoxy-1-indanon)-2-yl]methyl-piperidine hydrochloride], a novel inhibitor of acetylcholinesterase, was developed for the treatment of Alzheimer's disease (1–3). Cholinergic deficit is one of the major pathological features of Alzheimer's disease. This deficit relates to the loss of cognition and memory, the primary symptoms of this disorder. For its longer plasma half-life and less side effects, E2020 is currently used for the symptomatic treatment of Alzheimer's disease in many countries. Several methods for E2020 determination, including high-performance liquid chromatography (HPLC)–UV (4–6) and HPLC with fluorescence detection (7), have been reported. However, for HPLC–UV methods, the sensitivity is rather low, and its limit of detection is only 0.5 ng/mL (6); thus, this method cannot meet the need of the pharmacokinetics and bioavailability studies of E2020. As for HPLC with fluorescence detection, a sophisticated column is required to separate the *R* and *S* enantiomers (7), which was not suitable for the routine analysis in clinical laboratory.

This paper describes a very simple, rapid, sensitive, and specific liquid chromatography (LC)–electrospray ionization (ESI)-mass spectrometry(MS) method for the determination of E2020 in the concentration range 0.1–15 ng/mL in human plasma. We used the protonated molecular ions of donepezil ($[M+H]^+$, *m/z* 380) and internal standard (loratadine) ($[M+H]^+$, *m/z* 383) as target ions and determined the concentration of donepezil by computing the peak area ratio (donepezil–loratadine) of the ions and then compared them with the calibration curve. The background components did not interfere with the determination, and the low quantitation of the method was 0.1 ng/mL. The mean recovery of E2020 from human plasma was 99.4% ± 6.3% (ranging 93.4–102.6%). The inter- and intraday relative standard deviation (RSD) was less than 15%, which can meet the need of the pharmacokinetics and bioavailability studies of E2020.

Experimental

Reagents and materials

E2020 (M_w = 415, 99.8% purity) (Figure 1A) and its tablet (5 mg) were all obtained from Eisai Company (Suzhou, China). The internal standard (loratadine, M_w = 382, 99.8% purity)

(Figure 1B) was obtained from Sigma Chemical (St. Louis, MO). Methanol was purchased from Tedia Company (Fairfield, OH). All solvents were of HPLC grade. Redistilled water was used for the preparation of the eluent and the sample solution.

Instrumentation and chromatographic condition

The LC system (Waters 2690 HPLC, Milford, MA) consisted of a quaternary pump, mobile phase vacuum degassing unit, autosampler, temperature-controlled column compartment, and UV diode-array detector (DAD). In this system, a Kromasil-ODS column (5 µm, 250- × 4.6-mm i.d.) (Metachem, Lake Forest, IL) was used for all of the chromatographic separations. The mobile phase was methanol–acetate buffer (pH 4.0) [0.1% NH$_4$Ac, 0.1% HAc, and 0.01% (C$_2$H$_5$)$_3$N] (80:20, v/v) and run at a flow rate of 0.8 mL/min and 40°C.

The MS analyses were performed with a Mariner 5140 TOF mass spectrometer equipped with an ESI source (Applied Biosystem Company, Foster City, CA). The responses of donepezil and loratadine were measured in the positive ion mode with a spray tip potential of 5500 V, nozzle potential of 180 V, detector voltage of 2000 V, quadrupole temperature of 100°C, and nozzle temperature of 155°C. The system was operated in the full scanning and then selected-ion monitoring modes, in which the target ions were [M+H]$^+$ m/z 380 for donepezil and [M+H]$^+$ m/z 383 for loratadine.

Preparation of the stock solution

The standard stock solution was prepared by dissolving 10 mg E2020 in 100 mL methanol to obtain a nominal concentration of 0.1 mg/mL and then diluted with methanol to 5 µg/mL. The internal standard stock solution was prepared by dissolving 15 mg loratadine in methanol to obtain a nominal concentration of 0.15 mg/mL and diluted with methanol to 1.5 µg/mL. All the stock solutions were kept at 4°C before use.

Validation procedure

The calibration curves were constructed by least-squares linear regression analysis of the peak area ratio of donepezil–loratadine versus the concentration of E2020. The calibration curve equation was used to calculate the concentrations of E2020 in the samples and quality control standards (QC) from their peak areas ratios.
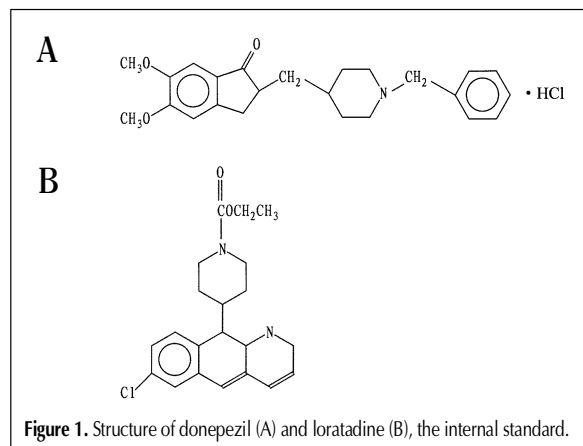
The calibration standard samples (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 15.0 ng/mL) were prepared by spiking blank human plasma with appropriate amounts of the mentioned stock solution. QCs to determine the recovery, accuracy, and precision were independently prepared at low (0.2 ng/mL), medium (5.0 ng/mL), and high (15.0 ng/mL) concentrations in the same manner. The internal standard was added to each standard and sample just prior to sample processing. All the samples were stored at –20°C before use.

Extraction procedure

Twenty microliters of the internal standard solution (1.5 µg/mL) was added into 1.0 mL of plasma (including QC and human plasma samples) then vortex mixed for 30 s. Afterwards, 200 µL of 0.1 mol/L NaOH and 5 mL of isopropyl alcohol-n-hexane (3:97, v/v) were added and then vortex mixed for 3 min. Following centrifugation at 3000 g for 10 min, 4 mL of the organic layer was transferred to a 10-mL tube and evaporated to dryness in a water bath at 40°C under a nitrogen stream. Before injection, 200 µL methanol was added to the dried human plasma extract. The extract was then vortex mixed for 60 s, and 40 µL was injected into the chromatograph.

Extraction efficiency

The absolute recovery (extraction efficiency) of E2020 through the extraction procedures was determined at 0.2, 5.0, and 15.0 ng/mL (low, medium, and high concentrations) by peak area ratio. A known amount of E2020 was added to human plasma prior to extraction as described in the Extraction procedure subsection. The internal standard was added after extraction to eliminate bias introduced by sample processing. The peak areas of E2020 (A$_s$) and the internal standard (A$_{is}$) were recorded. The same amount of E2020 and internal standard were mixed without human plasma and extraction. The peak area of E2020 (A'$_s$) and the internal standard (A'$_{is}$) were also recorded. The recovery was obtained by the equation:

$$R\% = A_s \times A'_{is}/(A'_s \times A_{is}) \times 5/4 \times 100\% \qquad \text{Eq. 1}$$

Pharmacokinetic studies in healthy, young, Chinese subjects

Twenty healthy, young, male volunteers age 19 to 23 years and weighing from 55 to 70 kg gave their written consent to participate in the study. They were each judged healthy on the basis of their medical history and the results of a physical examination,
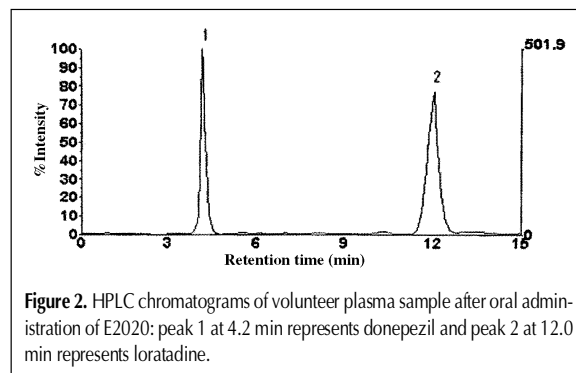

Figure 1. Structure of donepezil (A) and loratadine (B), the internal standard.

Figure 2. HPLC chromatograms of volunteer plasma sample after oral administration of E2020: peak 1 at 4.2 min represents donepezil and peak 2 at 12.0 min represents loratadine.

including the results of routine laboratory tests such as urinalysis, electrocardiogram, hematology, and serum biochemistry. All volunteers received a single 5-mg oral dose of E2020 in an open study design. Four milliliters of venous blood were drawn before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 144, and 192 h after dosing for determination of the plasma levels of E2020. Plasma samples separated by centrifugation were stored at –20°C until they were assayed.

Pharmacokinetic data analysis and statistics

The pharmacokinetic parameters of E2020 were calculated by using 3P97 program (the Chinese Society of Mathematical Pharmacology), and a two-compartmental open linear pharmacokinetic model was fitted to the plasma concentration data. Values for area under the curve (AUC), oral clearance (Cl/F), the plasma elimination half-life of the β-phase (T1/2), and mean residence time (MRT) were calculated by the following equations:

$$AUC_{0'192} = \Sigma(C_i + C_{i-1}) \times (t_i - t_{i-1})/2 \qquad \text{Eq. 2}$$

$$AUC_{0'\infty} = \Sigma(C_i + C_{i-1}) \times (t_i - t_{i-1})/2 + C_n/\lambda_n \qquad \text{Eq. 3}$$

$$Cl/F = Dose/AUC_{0'\infty} \qquad \text{Eq. 4}$$

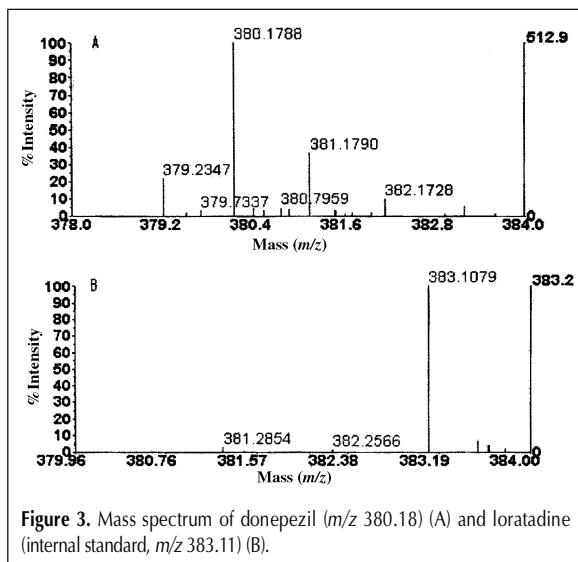$$T_{1/2} = 0.693/\lambda_n \qquad \text{Eq. 5}$$

Figure 3. Mass spectrum of donepezil (*m/z* 380.18) (A) and loratadine (internal standard, *m/z* 383.11) (B).

Table I. Precision of the Method for Determination of Donepezil (*n* = 5)

Concentration (ng/mL)	Within-day		Between-day	
	$x \pm s$	%RSD	$x \pm s$	%RSD
0.2	0.19 ± 0.02	10.4	0.20 ± 0.02	11.2
2.0	2.04 ± 0.07	3.2	2.00 ± 0.06	3.0
10.0	10.18 ± 0.30	2.8	10.49 ± 0.50	4.8

$$MRT = \int_0^\infty t.Cpdt/AUC_{0'\infty} \qquad \text{Eq. 6}$$

where λ_n = elimination rate constant of terminal phase. Data were expressed as mean ± SD of sample.

Results and Discussion

Separation and specificity

The typical chromatograms and mass spectrum from the study of E2020 in human plasma are shown in Figures 2 and 3. Shown in Figure 2 are the chromatograms for donepezil and loratadine from a sample of a volunteer 4 h after the oral administration of 5 mg E2020. The retention times of donepezil and loratadine were approximately 4 and 12 min, respectively. For both the drug and internal standard, the chromatograms were free of interfering peaks at their respective retention times. In mass spectra (Figure 3), the major ion was the protonated molecular ions as the base peaks (*m/z* 380), and only a few unidentified fragment ions were

Table II. Recovery of Donepezil from Plasma (*n* = 5)

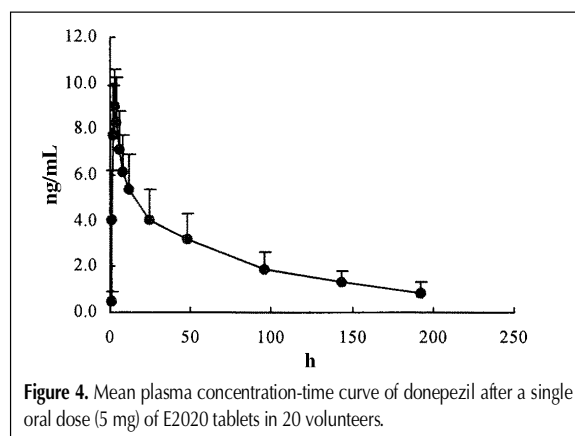| Added (ng/mL)	Recovery (%)	%RSD
0.2	94.3 ± 9.9	10.5
2.0	102.2 ± 3.3	3.2
10.0	101.6 ± 3.0	2.9

Figure 4. Mean plasma concentration-time curve of donepezil after a single oral dose (5 mg) of E2020 tablets in 20 volunteers.

Table III. Main Pharmacokinetic Parameters of Donepezil in 20 Volunteers after a Single Oral Dose (5 mg) of Donepezil HCl Tablets

Parameter	Values
$t_{1/2}$ (h)	65.7 ± 12.8
T_{max} (h)	3.10 ± 0.55
C_{max} (ng/mL)	10.1 ± 2.02
$AUC_{0'192}$ (ng.h/mL)	485 ± 150
$AUC_{0'\infty}$ (ng.h/mL)	564 ± 176.
MRT (h)	89.4 ± 13.4
CL/F (L/h)	9.9 ± 4.3

observed. The ion at m/z 380 for donepezil was not detected in the mass spectrum of loratadine (m/z 383). Therefore, the ions at m/z 380 and 383 were monitored by single ion monitoring as the target ions.

Linearity, accuracy, and precision

Calibration curves were plotted as the peak area ratio (drug–internal standard) versus drug concentration. Results for the calibration curve ($n = 9$) showed good linearity ($r = 0.9998$) over the concentration range 0.1–15 ng/mL with an equation of:

$$f = 0.0137 + 0.1056c \qquad \text{Eq. 7}$$

where c = donepezil concentration in ng/mL and f = donepezil area/loratadine area. The limit of quantitation (LOQ) was 0.1 ng/mL, with a precision (RSD) less than 15%.

Accuracy and precision were determined by calculating the intra- and interassay variabilities at three concentrations (0.2, 5.0, and 15.0 ng/mL) for five replicates. The results are shown in Table I. These results indicate that the method was reliable within the analytical ranges, and the use of the internal standard was very effective for reproducibility by LC–MS.

Recovery

The mean recovery of E2020 from human plasma was 99.4% ± 6.3% (ranging 93.4–102.6%). This data is the average of the three QC standards shown in Table II. The stability of E2020 in plasma was ascertained through two freeze–thaw cycles of QC samples. The results indicate that E2020 is very stable in plasma.

Application to healthy Chinese subjects

Figure 4 shows a profile of mean plasma concentrations ($n = 20$) for E2020 versus time. The main pharmacokinetic parameters of E2020 in 20 volunteers after a single oral dose (5 mg) of E2020 are listed in Table III. These results were somewhat different from what was obtained in previous studies (4). Things leading to the difference deserve thorough investigation.

Conclusion

In conclusion, the use of LC–MS provides an accurate, precise, and reliable method for the measurement of E2020 concentrations in human plasma for up to 192 h after the oral administration of 5 mg E2020 in healthy volunteers. The assay proved to be fast, simple, sensitive, and specific because of the inherent selectivity of MS.

Acknowledgments

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