# Quantitation of Donepezil in Human Plasma by HPLC-MS

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【ABSTRACT】 AIM and METHOD: A highly sensitive method for quantitation of donepezil in human plasma was established by using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS), with phenoprolamine hydrochloride as the internal standard (I.S.). After alkalizing with saturated sodium bicarbonate, plasma was extracted with ethyl acetate and the extracts were separated by HPLC on a $C_{18}$ reversed-phase column with methanol-water-acetic acid-triethylamine (70∶30∶0.3∶0.3, *v*/*v*) as mobile phase. LC-ESI-MS was performed in the selected ion monitoring (SIM) mode using target ions at *m*/*z* 380 for donepezil and *m*/*z* 344 for the I.S.. RESULT: Calibration curves, which were linear over the range of 0.2 to 20 ng/ml, were established contemporaneously with the analyses of each batch of samples, along with low (0.2 ng/ml), medium (2 ng/ml) and high (20 ng/ml) quality control samples. The intra and inter-assay variability ranged from 1.25% to 9.84% for the low, medium and high quality control samples. The extraction recovery of donepezil from plasma was in the range of 87.5% ~ 91.9%. CONCLUSION: The method has been used successfully to study donepezil pharmacokinetics in human plasma.

【KEY WORDS】 Donepezil; HPLC-MS; Human plasma; Determination of content

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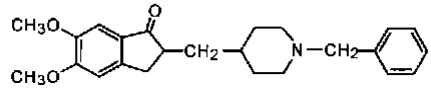
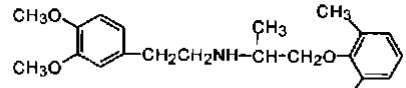
## 1 Introduction

Donepezil hydrochloride, the hydrochloride of (*R*, *S*)-1-benzyl-4-[(5, 6-dimethoxy-1-indanon)-2-yl] methylpiperidine, is a novel central-acting cholinesterase inhibitor. It has been demonstrated to be specific to brain cholinesterase (acetylcholinesterase) and to have a more potent inhibitory action than that of tetrahydroaminoacridine[1, 2]. The drug has been used clinically for the treatment of the symptoms of Alzheimer's disease. After administration of a single 2 mg dose the maximum plasma concentration of donepezil in adult human was about 3 ng/ml[3]. Few articles reported quantitation of donepezil in human plasma. Akihiro Ohnishi *et al*[3] reported a method for determination of donepezil by HPLC with ultraviolet detection, but the overall sample handling process was tedious and time-consuming. But the quantitation limit of 0.5 ng/ml in plasma and 2 ng/ml in urine for donepezil was not low enough for pharmacokinetic studies. Kenji Matsui *et al*[4] has reported a high-performance liquid chromatography-fast atom bombardment MS (HPLC-FAB-MS) method for the determination of donepezil enantiomers in the dog plasma, in which the limit of quantitation (LOQ) was 1.0 ng/ml and the spiked concentration was linear over the range of 1.0 ~ 502 ng/ml. In this study, we established a highly sensitive and rapid assay method using LC-ESI-MS with a LOQ of 0.1 ng/ml in plasma for donepezil. The method has been used successfully to study donepezil pharmacokinetics in human plasma.




Donepezil




I.S.

## 2 Experimental

### 2.1 *Materials and reagents*

Donepezil was supplied by Guizhou Shengjitang Pharmaceutical Company Limited. The internal standard (I.S.), phenoprolamine hydrochloride, was a gift from Organic Chemistry Laboratories of China Pharmaceutical University. Methanol was of HPLC grade. Sodium bicarbonate, ethyl acetate, acetic acid and triethylammine were of analytical grade. All the chemicals were purchased from Nanjing Chemical Reagent Co.

### 2.2 *Instrument and Conditions*

HPLC analyses were performed using a Hewlett-

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Packard HP1100 LC/DAD/MSD system (Hewlett-Packard, USA) with a Hypersil ODS-2 $C_{18}$ column (4.6 ×250 mm, 5 μm, Dalian Elite science Co. China). The mobile phase was methanol-water-acetic acid-triethylamine (70∶30∶0.3∶0.3, *v*/*v*), and the column temperature was maintained at 25 ℃. A constant flow-rate of 1.0 ml/min was employed throughout the analysis. LC-ESI-MS was carried out using nitrogen to assist nebulization. A quadrupole mass spectrometer equipped with an electrospray ionization source was used in positive ion selected ion monitoring (SIM) mode, set with a drying gas ($N_2$) flow of 10 L/min, nebulizer pressure of 40 psi, drying gas temperature of 350 ℃ and capillary voltage of 4 kV. The fragmentor voltage was 120 V. Target ions were monitored at *m*/*z* 380 for donepezil and *m*/*z* 344 for the I.S. in the SIM mode.

2.3 *Preparation of standard solutions, calibration standards, LOQ and quality control samples*

Stock solutions of donepezil and the I.S. were prepared at 1 mg/ml in methanol, respectively and stored at −20 ℃. These solutions were stable for 6 months at least. Standard solutions containing 10, 1, 0.1 and 0.01 μg/ml donepezil were prepared by diluting the stock solution with methanol. A solution containing 0.4 μg/ml I.S. was also prepared using methanol.

Calibration standards of donepezil (0.2, 0.5, 1, 2, 5, 10, 20 ng/ml) and a LOQ sample at 0.1 ng/ml were prepared by spiking appropriate amount of the standard solutions in blank plasma obtained from healthy, non-smoking volunteers. Quality control (QC) samples were prepared using blank plasma at concentrations of 0.2, 2 and 20 ng/ml.

2.4 *Sample preparation*

To a 1 ml aliquot of plasma in a 10 ml centrifuge tube, 50 μl I.S. solution (0.4 μg/ml), 1 ml of saturated sodium bicarbonate solution and 5 ml of ethyl acetate were added. Then the tubes were vortexed for 3 min and centrifugated for 10 min. The organic phase was transferred and evaporated to dryness under a stream of nitrogen in 50 ℃ water bath. The residue was reconstituted in 100 μl of the mobile phase, and 35 μl aliquot was injected into the LC-ESI-MS system.

2.5 *Method validation*

2.5.1 *Linearity and LOQ* Calibration standards of donepezil ranged from 0.2 to 20 ng/ml and LOQ plasma sample of donepezil were extracted and determined. A calibration curve with a slope of 0.1342, an intercept of 0.0004023 and $r^2$ of 0.9996, was constructed by plotting the area ratios of donepezil to the I.S. against donepezil concentrations in plasma. LOQ for donepezil, established based on a S/N ratio of 10, was 0.1 ng/ml.

2.5.2 *Precision and accuracy* The precision of the assay was determined by the low, medium and high QC plasma samples by replicate analyses of the three different concentrations (0.2, 2, 20 ng/ml). Intra-day precision was determined by repeated run of each QC sample on one day ($n$=5), and inter-day precision and accuracy was determined by repeated run on five consecutive days ($n$=1 per day). The concentration of each sample was determined using calibration standards prepared on the same day. Accuracy is defined as the relative deviation in the found value ($E$) of a standard from that of its true value ($T$) expressed as a percentage (RE%). It was calculated according to RE%=[($E$−$T$)/$T$]×100%. Assay precision was defined as the relative standard deviation (RSD) from the mean (M), and calculated according to RSD%=(SD/M)×100%.

The intra- and inter-day ($n$=5) precision and accuracy, shown in Table 1, were satisfactory for our purpose. The intra-day precision expressed as relative standard deviation (RSD) for each QC sample (0.2, 2, 20 ng/ml) was 1.25%~3.69%, and the inter-day RSD for the same QC samples was 2.04%~9.84%.

Tab 1 Precision and accuracy of the assay for determination of donepezil in plasma ($\bar{x}\pm s$, $n$=5)

| Added to plasma (ng/ml) | Intra-assay measured concentration (ng/ml) | RE (%) | RSD (%) | Inter-assay measured concentration (ng/ml) | RE (%) | RSD(%) |
|---|---|---|---|---|---|---|
| 0.20 | 0.18±0.0066 | −9.47 | 3.69 | 0.18±0.018 | −9.87 | 9.84 |
| 2.00 | 2.09±0.051 | 4.50 | 2.44 | 2.01±0.069 | 0.50 | 3.43 |
| 20.04 | 20.15±0.251 | 0.55 | 1.25 | 19.53±0.398 | −2.54 | 2.04 |

2.5.3 *Extraction recovery* Ethyl acetate, ethyl ether and cyclohexane were evaluated as extraction solvents. Ethyl acetate was chosen as the extraction solvent for its higher extraction efficiency to the two target compounds than that of the other two solvents.

The absolute recovery (extraction efficiency) of donepezil was determined at low, medium and high concentrations by the external standard method. A known amount of donepezil was added to human plasma prior to extraction as described in section 2.4. The internal standard was added after extraction. Concentration of donepezil following extraction was calculated using the calibration curves prepared on the same day, and was compared with the nominal concentration to estimate extraction recovery.

The mean recovery of donepezil from human plasma with ethyl acetate was (89.16 ± 2.41)% (range: 87.50%~91.92%). The recovery data reported here are the average for the three QC standards shown in Table 2.

Tab 2 The extraction recovery of donepezil from human plasma ($\bar{x} \pm s$, $n = 5$)

| Added(ng/ml) | Measured(ng/ml) | Recovery(%) | RSD(%) |
|---|---|---|---|
| 0.20 | 0.175±0.0084 | 87.50 | 4.80 |
| 2.00 | 1.76±0.044 | 88.05 | 2.49 |
| 20.04 | 18.42±0.259 | 91.92 | 1.41 |

2.6 *Pharmacokinetics*

Each of twenty healthy male volunteers received a tablet containing 5 mg donepezil after overnight fasting. Blood samples were drawn at appropriate intervals and centrifuged to obtain plasma samples. A calibration curve was prepared contemporaneously with each batch of samples.

The maximum plasma concentrations ($c_{max}$) and the time to those ($t_{max}$) were noted directly.

The other pharmacokinetic parameters are calculated.

## 3 Result and discussion

3.1 *Conditions of chromatography*

Phenoprolamine hydrochloride was selected as an internal standard because its chemical properties were similar to those of donepezil. The selection of mobile phase components was a critical factor in achieving good chromatographic peak shape and resolution. A solvent system of acetic acid and triethylamine was selected as a buffer for its good volatility. Moreover, acetic acid could improve the ionization efficiency and triethylamine could inhibit the tailing problem of chromatographic peaks of donepezil and I.S.. Good separation of target compounds and short run time were obtained by using an elution system of methanol-water-triethylamine-acetic acid (70∶30∶0.3∶0.3, *v*/*v*). Representative chromatograms are shown in Fig. 1 in which the retention time was 4.1 min for donepezil and 5.5 min for I.S..

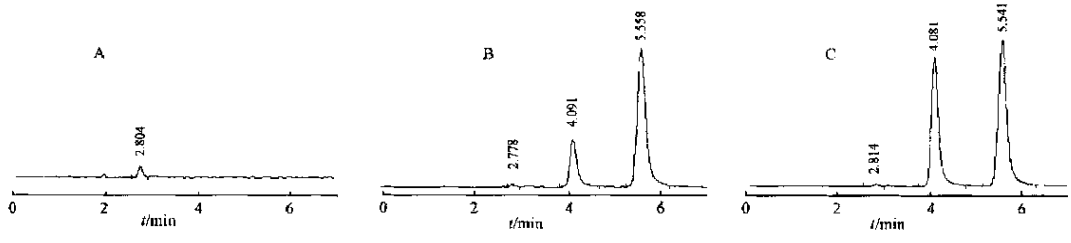


Fig 1. Typical chromatograms of blank plasma(A), plasma spiked with donepezil (2.00 ng/ml) and the I.S.(B) and Plasma obtained from a volunteer 6 h after donepezil administration(C), in which the retention time was 4.1 min for donepezil and 5.5 min for I.S.. The donepezil concentration of the plasma sample shown in (C) was estimated to be 5.12 ng/ml. Each plasma sample was extracted as described in the text and a 35-μl aliquot (of 100 μl total) was analyzed by LC-ESI-MS(C).

3.2 *Conditions for ESI-MS*

The ESI mass spectrum at fragmentor voltage of 70 V showed that the protonated molecule $[M+H]^+$ of donepezil was at *m*/*z* 380. The intensity of protonated molecule of donepezil at *m*/*z* 380 was compared at cone voltages of 70, 100, 120, 150, 180 and 200 V in order to determine the optimal collision energy. The result showed that the highest sensitivity was obtained by using a cone voltage of 120 V. Therefore, a cone voltage of 120 V was used to carry out LC-ESI-MS in the SIM mode. At this cone voltage the most intensive ion in the ESI mass spectrum of I.S. was at *m*/*z* 344, the proto-

nated molecule of I. S. Therefore, the protonated molecule of I.S. ($m/z$ 344) was selected as the target ion of I.S. in the SIM.

3.3 *Pharmacokinetic parameters*

The method described above was successfully applied to the pharmacokinetic study in which the plasma concentrations of donepezil in 20 healthy male volunteers were determined up to 192 h after receiving a single 5-mg oral dose of donepezil hydrochloride. The mean plasma concentration-time curve was shown in Fig. 2.

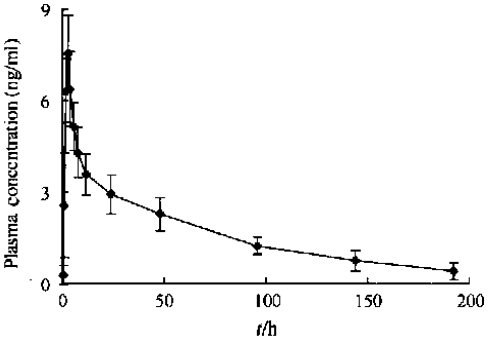


Fig 2. Mean donepezil plasma concentration-time profile in 20 healthy volunteers after a 5 mg oral dose.

The pharmacokinetic parameters were calculated. The maximum plasma concentration of 7.72 ± 1.01 ng/ml was achieved 3.0 ± 0.4 h after administration. The area under plasma concentration-time curve [$AUC(0-\tau)$] was 315.3 ± 69.6 $\mu g \cdot h \cdot L^{-1}$. The elimination half-life and mean residence time of donepezil in healthy human body were 61.1 ± 15.3 h and 83.5 ± 19.3 h respectively.

## 4 Conclusion

This method has high sensitivity and specificity for analysis of donepezil in human plasma. The LOQ of 0.1 ng/ml for donepezil was better than that obtained by HPLC-FAB-MS. No significant interference caused by endogenous compounds was observed. This simple and rapid method can be successfully used in pharmacokinetic study of donepezil in human.

## 【Reference】

[1] Yamanishi Y, Ogura H, Kosasa T, *et al*. Clinical and therapeutic aspect of alzheimer's and parkinson' diseases[J]. *Plenum Press*, 1990, (2): 409.

[2] Rogers SL, Yamanishi Y, Yamatsu K. *Cholinergic basis for alzheimer therapy*[M]. Boston: Birkhäuser, 1991. 314.

[3] Ohnishi A, Mihara M, Kamakura H, *et al*. Comparison of the pharmacokinetics of $E_{2020}$, a new compound for Alzheimer's disease, in healthy young and elderly subjects[J]. *J Clin Pharmacol*, 1993 (33): 1086-1090.

[4] Matsui K, Oda Y, Ohe H, *et al*. Direct determination of $E_{2020}$ enantiomersin plasma by liquid chromatography-mass spectrometry and column-swithing[J]. *J Chromatogr A*, 1995(694): 209.

# 人血浆中盐酸多奈哌齐的 HPLC-MS 测定法

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【摘 要】 目的: 建立人血浆中盐酸多奈哌齐的 HPLC-MS 法。方法: 血样经饱和 $NaHCO_3$ 碱化后用乙酸乙酯提取, 进行 HPLC-MS 分析, 色谱柱为 Hypersil ODS(5 μm, 250 mm × 4.6 mm), 流动相为甲醇-水-三乙胺-冰醋酸 (70∶30∶0.3∶0.3), 内标为盐酸非洛普, 检测离子为 $m/z$ 380(多奈哌齐)、$m/z$ 344(内标), 裂解电压为 120 V。结果: 在 0.2 ~ 20 ng/ml 范围内多奈哌齐与内标峰面积比值与浓度线性关系良好($r$ = 0.9996), 最低可定量浓度为 0.1 ng/ml, 提取回收率为 87.52% ~ 90.91%。结论: 本实验建立的分析方法灵敏、准确、简便。

【关键词】 盐酸多奈哌齐; HPLC-MS; 血浆; 含量测定