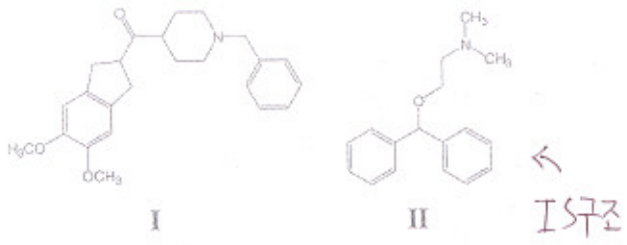

Figure 1. Structures of donepezil (I) and diphenhydramine (II), the internal standard.

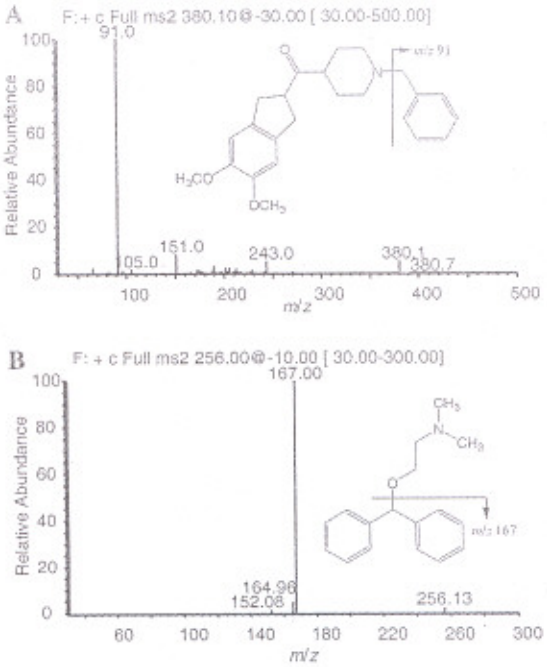

Figure 2. Product ion mass spectra of $[M+H]^+$ ions of (A) donepezil and (B) diphenhydramine (internal standard).

EXPERIMENTAL

Chemicals and reagents

Donepezil hydrochloride (purity ≥99.9%) and diphenhydramine hydrochloride (purity ≥99.8%) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol, acetonitrile and formic acid were purchased from Tedia Company Inc. (Beijing, China). All other reagents were of analytical grade. Blank human plasma was obtained from the Guangzhou Blood Donor Service (Guangzhou, China). Ultrapure water was obtained from a Milli Q-plus system (Billerica, MA, USA).

Equipment

The LC/MS/MS system consisted of a Surveyor MS pump, a Surveyor autosampler (ThermoFinnigan, USA) and a ThermoFinnigan TSQ Quantum triple quadrupole mass spectrometer (San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. Data acquisition was performed with Xcalibur 1.3 software (ThermoFinnigan, USA). Peak integration and calibration were performed using LCQuan software (ThermoFinnigan, USA).

Chromatographic conditions

Chromatographic separation was achieved using an Aquasil C_{18} column (150 mm × 2.1 mm i.d., 5 μm; Thermo Finnigan, USA) with a 4.0 mm × 2.0 mm i.d. Security Guard C_{18} (5 μm) guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of methanol/acetonitrile/1% formic acid (70:10:20, v/v/v), delivered at a flow rate of 0.3 mL/min. The column temperature was maintained at 25°C.

Mass spectrometric conditions

The mass spectrometer was operated in the positive mode. Quantification was performed using selected reaction monitoring (SRM) of the transitions of m/z 380 → 91 for donepezil and m/z 256 → 167 for diphenhydramine (IS), respectively, with a scan time of 0.3 s per transition. The tuning parameters were optimized for donepezil and IS by infusing a solution, containing 1 μg/mL of each analyte, at a flow rate of 10 μL/min into the mobile phase (0.3 mL/min) using a post-column 'T' connection. The optimal MS parameters obtained were as follows: the spray voltage was 3500 V with a source collision-induced dissociation (CID) voltage of 10 V, the heated capillary temperature was 350°C. Nitrogen was used as the sheath gas (50 psi) and auxiliary gas (12 psi). Argon was used as the collision gas at a pressure of approximately 1.2 mTorr (1 Torr = 133.3 Pa). The optimized collision energies chosen for donepezil and IS were 33 and 10 eV, respectively. Figure 2 shows the product ion mass spectra of the $[M+H]^+$ ion of donepezil and IS.

Preparation of stock and working solutions

The stock solutions of donepezil (400 μg/mL) and diphenhydramine (400 μg/mL) were prepared in methanol and serially diluted to produce a 4 μg/mL stock solution in methanol/water (50:50, v/v). The donepezil stock solution was then diluted to give working solutions of 1, 2, 8, 20, 50, 100 and 200 ng/mL in methanol/water (50:50, v/v). Diphenhydramine working solution (200 ng/mL) was also prepared by diluting the 400 μg/mL stock solution of diphenhydramine with methanol/water (50:50, v/v). All stock solutions and working solutions were stored at 4°C.

Preparation of calibration standards and quality control (QC) samples

Calibration curves were prepared by spiking 50 μL of the appropriate working solution to 500 μL blank human plasma. Effective donepezil concentrations in plasma samples were 0.1, 0.2, 0.8, 2.0, 5.0, 10.0, and 20.0 ng/mL. The QC samples used in the validation and during the pharmacokinetic (PK) study were prepared in the same way as the calibration standards. The nominal donepezil plasma concentrations of QC samples were 0.2, 2.0 and 20.0 ng/mL. The spiked plasma samples (standards and QC samples) were extracted in each analytical batch along with the unknown samples.

Sample preparation

To a 500-μL aliquot of plasma sample, 50 μL of IS (200 ng/mL diphenhydramine), 50 μL of methanol/water (50:50, v/v) and 0.5 mL of 10 mmol/L phosphate buffer (pH 14) were

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