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Validated LC–MS/MS method for quantitative determination of rasagiline in human plasma and its application to a pharmacokinetic study

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ABSTRACT

A highly sensitive liquid chromatography-tandem mass spectrometric (LC–MS/MS) method has been developed to determine rasagiline in human plasma. The analytical method utilized liquid–liquid extraction of plasma with *n*-hexane–dichloromethane–isopropanol (20:10:1, v/v/v). Separation of analyte and the internal standard (IS) pseudoephedrine was performed on a Zorbax Extend C_{18} column (150 mm × 4.6 mm, 5 μm) with a mobile phase consisting of acetonitrile–5 mM ammonium acetate–acetic acid (40:60:0.05, v/v/v) at a flow rate of 0.5 mL/min. The API 4000 triple quadrupole mass spectrometer was operated in multiple reaction monitoring mode via positive electrospray ionization interface using the transitions *m/z* 172.1 → *m/z* (117.1 + 115.1) for rasagiline, and *m/z* 166.0 → *m/z* 148.1 for the internal standard. The method was linear over the concentration range of 0.020–50.0 ng/mL. The intra- and inter-day precisions were less than 11.2% in terms of relative standard deviation (R.S.D.), and the accuracy was within ±6.4% in terms of relative error (RE). The lower limit of quantification (LLOQ) was identifiable and reproducible at 0.020 ng/mL with acceptable precision and accuracy. The mean extraction-efficiency at three concentrations was 95.6 ± 7.0%, 97.9 ± 3.0% and 95.3 ± 8.3%. The validated method offered increased sensitivity (10 times higher than those reported) and wide linear concentration range. This method was successfully applied for the evaluation of pharmacokinetics of rasagiline after single oral doses of 1, 2 and 5 mg rasagiline to 12 Chinese healthy volunteers.

1. Introduction

Rasagiline (*N*-propargyl-L-(*R*)-aminoindan), is a potent selective irreversible inhibitor of monoamine oxidase type B [1] and has been used for the treatment of idiopathic Parkinson's disease (PD) both as monotherapy in early disease and as adjunctive therapy for levodopa in advanced disease [2]. Rasagiline undergoes almost complete biotransformation through *N*-dealkylation and hydroxylation pathways in the liver, in which *N*-dealkylation metabolite, aminoindan, has demonstrated favourable pharmacological activity in vitro and in vivo [3]. However, unlike selegiline, rasagiline is not metabolized to amphetamine derivatives which have been shown to interfere with cardiovascular function or to have a neurotoxic effect [4]. The recommended dosage for initial monotherapy is 1 mg once daily. When rasagiline is used as adjunctive therapy with levodopa, the recommended initial dose is 0.5 mg/day and may be increased to 1 mg/day if the desired clinical effect is not achieved [5,6]. In patients with advanced PD and motor fluctuations, the maximum daily dosage could be increased to 5 mg. Due to extensive metabolism and wide distribution to tissues (V_d value ranges from 182 to 243 L) [6], the plasma concentrations of rasagiline were very low in humans. It was reported that in patients with PD, the mean rasagiline C_{max} observed after multiple administration of 1 mg for 12 weeks is 8.50 ± 2.20 ng/mL [6]. Therefore, to support clinical investigations, a reliable analytical method with adequate sensitivity is necessary.

In literature, only a precolumn derivatization GC–MS method with the lower limit of quantification (LLOQ) at 0.250 ng/mL was described to characterize the kinetics of rasagiline in human [7]. The study demonstrated that rasagiline treatment was safe and well tolerated in healthy volunteers at all doses studied (up to 20 mg once/day in the single-dose study and up to 10 mg once/day in the repeated-dose study). However, due to analytical difficulties arising from analyte concentrations, which were below the limit of detection, a full pharmacokinetic analysis for rasagiline 1 and 2 mg doses was not possible. Nowadays, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has proved to be an extremely sensitive and specific technique for the analysis of basic

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drugs. To our knowledge, several methods for the determination of selegiline or metabolites in biological matrixes have been reported including GC–MS [8,9] and LC–MS [10,11] or LC–MS/MS [12]. However, there was no publication on the determination of rasagiline by LC–MS/MS. The purpose of this study was to establish and validate a novel sensitive LC–MS/MS method to quantify rasagiline in human plasma. This method exhibited excellent performance with respect to high sensitivity and wide linear concentration range. It was successfully applied to the pharmacokinetic studies of rasagiline after oral administration of 1, 2 and 5 mg rasagiline mesylate tablets.

2. Experimental

2.1. Materials

Rasagiline mesylate (99.8% purity) was obtained from Chongqing Pharmaceutical Research Institute Co., Ltd. (Chongqing, China). Pseudoephedrine hydrochloride (100% purity) for use as the internal standard (IS) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol (HPLC grade) were purchased from Sigma (St. Louis, MO, USA). Ammonium acetate and acetic acid of HPLC grade were purchased from Tedia (Fairfield, OH, USA). Other chemicals were of analytical reagent grade and purchased from commercial sources. Milli-Q water (18.2 mΩ and TOC ≤50 ppb) from Milli-Q system (Millipore SAS, Molsheim, France) was used throughout the study. Drug-free plasma for the preparation of calibration standards was obtained from Shanghai Shuguang Hospital (Shanghai, China).

2.2. Instrument

An Agilent 1100 system consisting of a G1311A quaternary pump, a G1379A vacuum degasser, a G1316A thermostatted column oven and a G1313A autosampler (Agilent, Waldbronn, Germany) was used. Mass spectrometric detection was performed on an API 4000 triple quadrupole instrument (Applied Biosystems, Concord, Ontario, Canada) in multiple reaction monitoring (MRM) mode. A TurboIonSpray ionization (ESI) interface in positive ionization mode was used. Data processing was performed with Analyst 1.4.1 software package (Applied Biosystems).

2.3. Chromatographic conditions

The chromatographic separation was achieved on a Zorbax Extend C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Agilent, Wilmington, DE, USA) with a SecurityGuard C_{18} guard column (4 mm × 3.0 mm i.d. Phenomenex, Torrance, CA, USA). A mixture of acetonitrile–5 mM ammonium acetate–acetic acid (40:60:0.05, v/v/v) was used as mobile phase at a flow rate of 0.5 mL/min. The temperatures of column and autosampler were both maintained at ambient temperature (25 °C). The chromatographic run time of each sample was 3.6 min, which separated rasagiline and IS from endogenous components. A divert valve was used and in the first 2 min of the chromatographic run, the eluate was directed to the waste container.

2.4. Mass spectrometric conditions

The mass spectrometer was operated using ESI source in the positive ion detection. In order to optimize all the MS parameters, standard solutions (10.0 ng/mL) of the analyte and IS were infused into the mass spectrometer at a flow-rate of 20 μL/min. Turbo spray voltage (IS) was set at 4000 V. Source temperature was maintained at 400 °C. Nitrogen was used as nebulizing gas (50 psi), auxiliary gas (40 psi) and curtain gas (10 psi). For collision activated dissociation (CAD), nitrogen was employed as the collision gas at a pressure of 4 psi. Quantification was performed using MRM mode with the following transitions: m/z 172.1 → m/z (117.1 + 115.1) for rasagiline, and m/z 166.0 → m/z 148.1 for IS, respectively, with a dwell time of 200 ms.

2.5. Preparation of calibration standards and quality control (QC) samples

A stock solution of rasagiline with a concentration of 400 μg/mL (calculated as base) was prepared by dissolving rasagiline mesylate in methanol. A series of standard working solutions with concentrations in the range of 0.200–500 ng/mL for rasagiline were obtained by further dilution of the stock solution with methanol–water (50:50, v/v). Effective concentrations in plasma samples were 0.020, 0.050, 0.100, 0.500, 2.00, 5.00, 20.0 and 50.0 ng/mL. The quality control (QC) samples were similarly prepared at concentrations of 0.050, 2.00, 45.0 ng/mL with blank human plasma, by a separate weighing of the reference standard. A 100 ng/mL internal standard working solution was prepared by diluting the stock solution of pseudoephedrine with methanol–water (50:50, v/v). All the solutions were stored at 4 °C and were brought to room temperature before use.

2.6. Sample preparation

A 50-μL aliquot of the IS solution (pseudoephedrine, 100 ng/mL), 50 μL of methanol–water (50:50, v/v) and 100 μL of pH 7 phosphate buffer (the mixture of 39.0 mL 2 M monobasic sodium phosphate and 61.0 mL 2 M dibasic sodium phosphate) were added to 500 μL of plasma sample. The sample was vortex-mixed and extracted with 4 mL of *n*-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) by shaking (240 times/min) for 10 min. The organic and aqueous phases were separated by centrifugation at 2000 × *g* for 5 min. The upper organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen in the TurboVap evaporator (Zymark, Hopkinton, MA, USA). The residue was dissolved in 150 μL of the mobile phase and vortex-mixed for 1 min. A 20 μL aliquot of the reconstituted extract was injected onto the LC–MS/MS system for analysis.

2.7. Method validation

To ensure the selectivity, accuracy, reproducibility and sensitivity, the method was validated on the items described as follows.

The selectivity was assessed by comparing the chromatograms of six different blank human plasma with the corresponding spiked plasma. Peak areas of endogenous compounds coeluting with the analytes should be less than 20% of the peak area of the LLOQ standard according to international guidelines [13]. And the deviation of the nominal concentrations for the LLOQ in these 6 plasma batches should be within ±20%.

The matrix effect was evaluated at two concentrations (0.050 and 45.0 ng/mL in plasma). Two groups of samples were prepared: group 1 was prepared to evaluate the MS/MS response for a pure standard of rasagiline dissolved in the mobile phase (A); group 2 was prepared in plasma originating from six different donors and submitted to the sample purification process and spiked with rasagiline after processing (B). The value (B/A × 100) was considered as an absolute matrix effect. The assessment of the relative matrix effects, which was expressed as R.S.D. (%), was made by a direct comparison of B values between six different lots of plasma. The same evaluation was performed for IS (10 ng/mL in plasma).

The inter-subject variability of matrix effect at every concentration level should be less than 15% [14].

The linearity was assessed by assaying calibration curves in human plasma in duplicate in three separate runs. In addition, a blank and a zero plasma sample (only spiked with IS) were run to discard the presence of interferences. The calibration curves were fitted by a weighted ($1/x^2$) least squares linear regression method through the measurement of the peak-area ratio of the analyte to IS. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value [13]. The lower limit of quantification (LLOQ) is the lowest concentration of the calibration curve, which could be measured with acceptable accuracy and precision. It was determined in six replicates in three validation days. The precision should be equal or less than 20% and accuracy between 80% and 120% of the nominal concentration for both within and between-assay [13].

To evaluate the precision and accuracy of the method, QC samples at three concentration levels (0.050, 2.00 and 45.0 ng/mL) were measured in six replicates on three consecutive validation days. The assay precision was calculated by using the relative standard deviation (R.S.D.) and a one-way analysis of variance (ANOVA) [15]. It separates out the sources of variance due to within- and between-run factors. The assay accuracy was expressed as relative error (RE), i.e. (observed concentration − nominal concentration)/(nominal concentration) × 100%. The intra- and inter-day precisions were required to be below 15%, and the accuracy to be within ±15% [13].

The extraction recovery was estimated for rasagiline at three levels (low, 0.050 ng/mL; medium, 2.00 ng/mL; and high, 45.0 ng/mL) by comparing two groups of control samples: (B) drug spiked after extraction of blank plasma (post-extraction). (C) Drug spiked to plasma and prepared normally (pre-extraction). Extraction recovery was calculated as the response ratio of C/B. The reproducibility of the extraction procedure was determined as R.S.D.%. The extraction recovery of the IS was determined in a similar way using the QC samples at medium concentration as a reference.

The stability of rasagiline in human plasma was evaluated by analysing replicates ($n = 3$) of plasma samples at the concentrations of 0.050 and 45.0 ng/mL, which were exposed to different conditions (time and temperature). The short-term stability was determined after the exposure of the spiked samples at 25 °C for 2 h, and the ready-to-inject samples (after extraction, in the mobile phase) to the autosampler rack (25 °C) for 24 h. The long-term stability was assessed after storage of the standard spiked plasma samples at −20 °C for 9 days. The freeze/thaw stability was evaluated after three complete freeze/thaw cycles (−20 °C to 25 °C) on consecutive days. All stability tests samples at two concentration levels (0.050 and 45.0 ng/mL) were analysed in triplicate and the deviations were determined in relation to freshly prepared samples. The stability of stock solution in methanol was also evaluated at 4 °C for 9 days. The analytes are considered to be stable when the precisions are below 15% and the accuracies are in the range of 85–115% respectively for both levels.

2.8. Application to pharmacokinetic study

The developed LC–MS/MS method was applied to determine the plasma concentrations of rasagiline from a clinical trial study in which 36 (18 males and 18 females) healthy Chinese subjects were enrolled. The pharmacokinetic study was approved by the Medical Ethics Committee of Shanghai Shuguang Hospital. Informed consent was obtained from all subjects after explaining the aims and risks of the study. The doses of rasagiline chosen for this study were based on findings from preclinical and early clinical studies. Thirty-six volunteers were randomized into three groups. They were orally administered rasagiline mesylate tablets (Chongqing Pharmaceutical Research Institute Co., Ltd., Chongqing, China) which equivalent to rasagiline base at 1, 2 or 5 mg, respectively. Venous blood samples were collected into heparinized tubes before and 0.17, 0.33, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12 and 24 h post-dosing, and centrifuged at 2000 × g (4 °C) for 10 min to separate the plasma fractions. The collected plasma samples were stored at −20 °C until analysis.

Calculation of the pharmacokinetic parameters was performed by non-compartmental assessment of data using the computer program WinNonlin (v5.0.1, Pharsight, Mountain View, CA, USA). The maximum plasma concentrations (C_{max}) and their time of occurrence (T_{max}) were both obtained directly from the measured data. The area under the plasma concentration–time curve from time zero to the time of the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal method. The terminal elimination rate constant (k_e) was estimated by log-linear regression of concentrations observed during the terminal phase of elimination, and the corresponding elimination half-life ($t_{1/2}$) was then calculated as $0.693/k_e$.

3. Results and discussion

3.1. Optimization of the mass spectrometric condition

Rasagiline is a low molecular weight compound, containing a secondary amine in its structure. Due to the presence of basic nitrogen in the molecule, rasagiline exhibited favourable sensitivity in positive ion mode detection. Electrospray ionization (ESI) was found to be more sensitive than atmospheric pressure chemical ionization (APCI).

Under (+) ESI conditions, the analyte and pseudoephedrine (internal standard) formed predominantly protonated molecule $[M+H]^+$ at m/z 172.1 and m/z 166.0 in Q1 full scan mass spectra, respectively. The corresponding product ion mass spectra are depicted in Fig. 1, where $[M+H]^+$ of each compound was selected as precursor ion. Rasagiline gave fragment ions at m/z 117.1 and 115.1. In order to increase the sensitivity of rasagiline, the transitions m/z 172.1 → 117.1 and m/z 172.1 → 115.1 were simultaneously chosen for quantification analysis. The collision energy in the product MS/MS mode was investigated from 5 to 50 eV to optimize the sensitivity, and the optimal values were found to be 17 and 40 eV for the two chosen product ions, respectively. For IS, the product ion spectrum of the $[M+H]^+$ ion showed a major fragment ion at m/z 148.1, which derived from the lost of a water. The optimum collision energy (24 eV) was determined by observing the maximum response obtained for m/z 148.1.

3.2. Optimization of the chromatographic condition

Chromatographic conditions, especially the composition of mobile phase, were optimized to achieve good sensitivity and peak shape for rasagiline, as well as a short run time. In our experiment, it was observed that acetonitrile can give better peak shape than methanol and was chosen as the organic phase. Good peak shape could be achieved by adding 5 mM ammonium acetate (containing 0.1% acetic acid) into the mobile phase. Finally, a mobile phase consisting of acetonitrile–5 mM ammonium acetate–acetic acid (40:60:0.05, v/v/v) was used in our experiment.

3.3. Sample preparation

Sample preparation is a critical step for accurate and reliable LC–MS/MS assays. The most widely employed biological sample

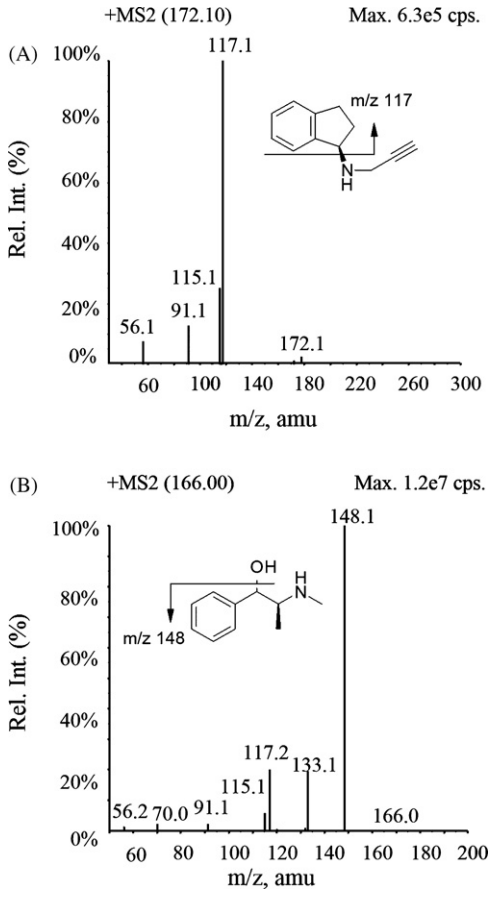

Fig. 1. Product ion spectra of $[M+H]^+$ of rasagiline (A) and pseudoephedrine (B).

preparation methodologies currently are liquid–liquid extraction (LLE), protein precipitation (PPT), and solid-phase extraction (SPE). In the early stage of method development, a PPT method was employed to separate rasagiline from plasma samples, but strong ion suppression from the endogenous substances in plasma occurred. Although it could be decreased by chromatographic separation, the run time would be sacrificed.

Finally, liquid–liquid extraction (LLE) procedures were used to prepare rasagiline plasma samples in our study. To obtain optimum recovery, four organic extraction solvents were evaluated including ethyl ether, ethyl acetate, ethyl ether–dichloromethane (3:2, v/v) and *n*-hexane–dichloromethane–isopropanol (20:10:1, v/v/v). It was found that *n*-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) could yield the highest recovery (>80%) for rasagiline and IS. The aqueous pH modifiers including 1 M NaOH and pH 7 phosphate buffer were also evaluated. No significant enhancement on recovery was observed, but the use of pH 7 phosphate buffer as the pH adjustment reagent could reduce the interferences to a minimum.

3.4. Method validation

3.4.1. Selectivity

The LC–MS/MS method has high selectivity because only ions derived from the analytes of interest are monitored. Fig. 2 shows the typical MRM chromatograms of a blank plasma, a spiked plasma sample with rasagiline (0.020 ng/mL) and IS (10.0 ng/mL), and a plasma sample from a healthy volunteer 0.5 h after oral administration of rasagiline mesylate tablets which equivalent to 5 mg rasagiline. In virtual of the figure, there were no significant endoge-

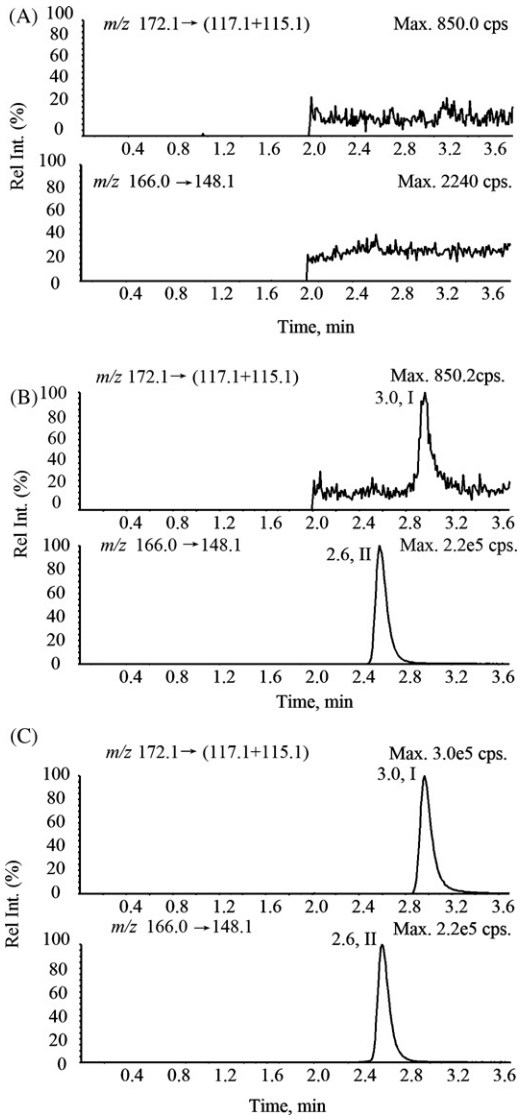

Fig. 2. Typical chromatograms of rasagiline and pseudoephedrine (IS) in human plasma by selective reaction monitoring scan mode. (A) Blank plasma sample; (B) plasma sample spiked with rasagiline at 0.020 ng/mL and pseudoephedrine (IS) at 10.0 ng/mL; (C) a volunteer plasma sample obtained at 0.5 h after an oral dose of 5 mg rasagiline. Peaks I and II refer to rasagiline and pseudoephedrine, respectively.

nous interferences observed at the retention times of the analyte and IS. Typical retention times for rasagiline and pseudoephedrine were 3.0 and 2.6 min, respectively. The corresponding capacity factors were 1.00 and 0.730, respectively.

3.4.2. Matrix effect

The absolute matrix effects for rasagiline at concentrations of 0.050 and 45.0 ng/mL were 101% and 97.0%, respectively. The relative matrix effect were 10.7% and 8.0%, respectively. The absolute and relative matrix effects for IS (10.0 ng/mL in plasma) were 97.9% and 3.9%, respectively. These results showed that ion suppression or enhancement from plasma matrix was negligible in the present condition.

3.4.3. Linearity of calibration curve and lower limit of quantification

A calibration curve was established on each validation day. The calibration curve was linear over the rasagiline concentration ranges of 0.020–50.0 ng/mL in human plasma with coefficient of

Table 1
Precision and accuracy of the LC–MS/MS method to determine rasagiline in human plasma (in three consecutive days, six replicates for each day)

Concentration (ng/mL)		R.S.D. (%)		RE (%)
Added	Found	Intra-run	Inter-run	
0.0500	0.0530 ± 0.0034	5.8	9.0	6.4
2.00	2.03 ± 0.13	5.9	9.7	1.7
45.0	45.0 ± 3.52	7.2	11.2	0.1

correlation (r) >0.99. The mean (±S.D.) regression equation from replicate calibration curves from three different days was:

$$y = (0.0744 \pm 0.000839)x + (0.000577 \pm 0.000170) \qquad r = 0.9960$$

where y represents the peak-area ratio of analyte to IS and x represents the plasma concentration of rasagiline.

The lower limit of quantification of rasagiline was 0.250 ng/mL in human plasma with gas chromatography–mass spectrometry analysis, using the method described in literature [7]. In order to increase the sensitivity of rasagiline determination, we performed LC–MS/MS analysis to obtain higher sensitivity with simple technique. The LLOQ of rasagiline was improved to 0.020 ng/mL in human plasma. The intra- and inter-assay precisions were 11.9% and 9.3%, respectively, with relative error −0.5%. Under the present LLOQ, the rasagiline concentration could be determined in plasma samples up to 4.0 h after oral administration of rasagiline mesylate tablets which equivalent to 1 mg rasagiline base.

3.4.4. Precision and accuracy

The method showed good precision and accuracy. Table 1 summarizes the intra- and inter-assay precision and accuracy for rasagiline from QC samples. The intra- and inter-assay precisions were measured to be below 7.3% and 11.2%, respectively, with relative errors from 0.1% to 6.4%. The above results demonstrated that the values were within the acceptable range and the method was accurate and precise.

3.4.5. Extraction recovery

Mean extraction recoveries of rasagiline at 0.050, 2.00 and 45.0 ng/mL were 95.6 ± 7.0%, 97.9 ± 2.9% and 95.3 ± 8.3%, respectively (n = 6). Mean extraction recovery of the internal standard (10.0 ng/mL in plasma) was 91.3 ± 4.1% (n = 6).

3.4.6. Stability

The stability tests of the analytes were designed to cover anticipated conditions of handling of the clinical samples. The results of stability experiments (Table 2) showed that rasagiline was stable during sample storage (in plasma at 25 °C for 2 h, in plasma at −20 °C for 9 days), the processing (three freeze–thaw cycles) and post-treatment (in the reconstituted extract at 25 °C for 24 h).

Table 2
Summary of stability of rasagiline under various storage conditions (n = 3)

Storage conditions	Concentrations (ng/mL)		R.S.D. (%)	RE (%)
	Added	Found		
Three freeze/thaw cycles	0.0512	0.0532 ± 0.0025	4.7	3.9
	44.7	43.9 ± 1.2	2.8	−1.8
Freezing for 9 days (−20 °C)	0.0512	0.0459 ± 0.0015	2.8	−10.4
	44.7	39.2 ± 1.6	4.2	−12.3
Autosampler for 24 h (25 °C)	0.0512	0.0504 ± 0.0030	5.7	−1.6
	44.7	42.7 ± 5.9	12.4	−4.5
Short-term (2 h at 25 °C)	0.0512	0.0510 ± 0.0056	10.4	−0.4
	44.7	39.9 ± 1.2	3.0	−10.7

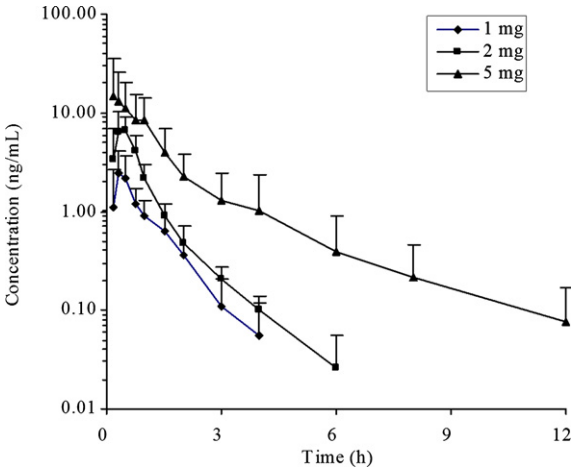

Fig. 3. Mean plasma concentration–time curve of rasagiline after single oral doses of 1, 2 and 5 mg to 12 healthy volunteers, respectively (mean ± S.D.).

Table 3
The main pharmacokinetic parameters for rasagiline after single oral doses of 1, 2 and 5 mg to healthy volunteers, respectively (mean ± S.D., n = 12)

Parameters	1 mg	2 mg	5 mg
C_{max} (ng/mL)	3.04 ± 1.43	8.20 ± 2.93	22.2 ± 16.6
T_{max} (h)	0.596 ± 0.364	0.478 ± 0.193	0.562 ± 0.368
AUC_{0-24} (ng h/mL)	2.43 ± 0.782	6.11 ± 1.16	20.7 ± 14.3
$AUC_{0-\infty}$ (ng h/mL)	2.48 ± 0.801	6.17 ± 1.16	20.9 ± 14.4
$t_{1/2}$ (h)	0.759 ± 0.323	1.18 ± 0.743	2.87 ± 1.94
CL/F (L/h)	440 ± 133	337 ± 81.2	437 ± 434

All RE values between post-storage and initial QC samples were within ±15%. In addition, stock solution of rasagiline in methanol was shown to be stable for 9 days at 4 °C (RE from −3.7 to 4.2%).

3.5. Application of the method to pharmacokinetic study in healthy volunteers

The LC–MS/MS method described above had been applied successfully to the pharmacokinetic study of rasagiline in Chinese healthy subjects. Using this analytical method, we were able to measure the concentration of rasagiline up to 4 h for all subjects after a single oral administration of rasagiline mesylate tablets which equivalent to 1 mg rasagiline base, up to 6 h for 20% of the subjects and up to 8 h for 10% of the subjects. Fig. 3 shows the profile of the mean rasagiline plasma concentration versus time. Meanwhile, the main pharmacokinetic parameters for rasagiline are presented in Table 3.

Rasagiline is mainly metabolized via hepatic CYP1A2 enzyme [16]. The report has demonstrated that the interindividual variation in CYP1A2 activity is extensive [17]. In our study, a large interindividual variability in rasagiline concentration–time profiles was observed with the C_{max} ranging from 1.21 to 5.44 ng/mL (1 mg rasagiline), 2.65 to 14.1 ng/mL (2 mg rasagiline), 1.49 to 50.0 ng/mL (5 mg rasagiline). Therefore a wide linear concentration range of the calibration curve (0.020–50.0 ng/mL) is necessary for determination of rasagiline in human plasma.

4. Conclusion

An LC–MS/MS method with high selectivity was developed and validated for the determination of rasagiline in human plasma. This method was sensitive enough to monitor the low-dosage PK studies of rasagiline in human plasma. The drug remains detectable in the

plasma up to 4 h (1 mg), 6 h (2 mg) and 12 h (5 mg). The profiling duration was almost twice longer than those reported. This method offered advantages of wide linear concentration range, short run time and simple sample preparation. It was successfully applied to characterize the pharmacokinetics of rasagiline in Chinese healthy volunteers.

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