Journal of Chromatography B, 875 (2008) 515–521

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Rapid and sensitive liquid chromatography–tandem mass spectrometry: Assay development, validation and application to a human pharmacokinetic study

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ARTICLE INFO

Article history:
Received 17 July 2008
Accepted 6 October 2008
Available online 14 October 2008

Keywords:
Rasagiline
Human pharmacokinetics
Urine excretion
LC–MS/MS

ABSTRACT

Rasagiline is a highly potent, selective and irreversible second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAO-B). The present studies aimed at developing and validating a rapid and sensitive liquid chromatography–tandem mass spectrometric (LC–MS/MS) method for determination of rasagiline in human plasma and urine. LC–MS/MS analysis was carried out on a Finnigan LC-TSQ Quantum mass spectrometer using positive ion electrospray ionization (ESI^+) and selected reaction monitoring (SRM). The assay for rasagiline was linear over the range of 0.01–40 ng/mL in plasma and 0.025–40 ng/mL in urine. It took 5.5 min to analyze a sample. The average recoveries in plasma and urine samples were both >85%. The RSD of precision and bias of accuracy were less than 15% and 10%, respectively, of their nominal values based on the intra- and inter-day analysis. The developed method was proved to be suitable for use in clinical pharmacokinetic study after single oral administration of 0.5, 1 and 2 mg rasagiline mesylate tablets in healthy Chinese volunteers.

1. Introduction

Rasagiline mesylate [N-propargyl-1R(+)-aminoindan] is a highly potent, selective, irreversible, second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAO-B) [1], which has been evaluated for the treatment of Parkinson's disease. Rasagiline also possesses neuroprotective properties that are independent of its MAO inhibitory activity [2,3]. Unlike selegiline, rasagiline has no amphetamine-like metabolites and its major metabolite, 1-(R)-aminoindan, has demonstrated favourable pharmacological activity in experimental studies [4,5]. Rasagiline has shown significant beneficial effects as monotherapy in the treatment of early Parkinson's disease [6]. Monotherapy with rasagiline 1 or 2 mg once daily significantly attenuated the worsening of symptoms, compared with placebo, in patients with early Parkinson's disease in a randomised, double-blind trial ($n = 404$). Furthermore, patients treated with rasagiline for 12 months had less functional decline than patients whose treatment was delayed for 6 months ($n = 371$). In patients with moderate-to-advanced disease receiving background therapy with levodopa and additional anti-parkinsonian medications ($n = 1159$), rasagiline 0.5 or 1 mg once daily reduced the daily 'off' time by 0.49–0.94 h relative to that in placebo recipients in two randomised, double-blind trials. The efficacy of rasagiline 1 mg once daily was similar to entacapone 200 mg administered with each levodopa dose [7]. Rasagiline was generally well tolerated in clinical trials as both monotherapy and when administered with other anti-parkinsonian drugs. Adverse events with rasagiline were generally similar in frequency to those seen in placebo or entacapone recipients [8]. Preliminary data has lead to support to its adjunct use in advanced disease.

Although Thébault et al. [8] reported in 2004 the tolerability, safety, pharmacodynamics, and pharmacokinetics of rasagiline, the very detailed information about human pharmacokinetics of rasagiline was actually limited, as of this writing, not to mention the related analytical methodology applied for determining the drug's concentrations in human biomatrices. The generic forms of rasagiline were recently produced to meet the demand of the global healthcare market. To assist in regulatory filing, product quality control, and dose regimen design for clinical trials, characterization of human pharmacokinetics is generally the first and foremost clinical investigation of new drugs [9]. Since the introduction of the electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) techniques, LC–MS/MS has become an ideal and widely used method in characterization and quantitative analysis of drugs and their metabolites owing to its superior specificity, sensitivity and efficiency over others such as radioimmunoassay

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doi:10.1016/j.jchromb.2008.10.005

(RIA), gas chromatography/mass spectrometry (GC/MS) and liquid chromatography (LC) with UV [10,11]. Buffer systems made from 5 to 10 mM ammonium acetate and 0.1% acetic acid provide sufficient repeatability of the retention times and efficient ionization of basic compounds in positive ion ESI [12]. Ionization of many neutral and acidic compounds is also achieved, provided that the proton affinities are high enough. Analytical times ~5 min can easily be achieved [10]. Having said that, the aim of the present studies was to develop a rapid and sensitive LC–MS/MS method for quantitative determination of rasagiline in human biomatrices, and utilize the method, after its full validation, to characterize rasagiline's pharmacokinetic and urine excretion profile in health human volunteers.

2. Experimental

2.1. Chemicals and materials

Rasagiline mesylate tablets (batch no.: 20050703) and reference substance (>99.0% purity) was supplied by Changzhou Siyao Pharmaceutical group Co. Ltd. (Changzhou, China). Papaverine used as internal standard (>99.5% purity) for LC–MS/MS assay was procured from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). HPLC-grade methanol and methyl *tert*-butyl ether (MTBE) were purchased from Tedia Company Inc. (Fairfield, OH, USA). Methanoic acid, concentrated ammonia, hydrochloric acid (HCl), sodium hydroxide, ether and ammonium acetate were all purchased from Nanjing Chemical Reagent Company Inc. (Nanjing, PR China). Water was prepared with double distillation.

SPE of urine was carried out using a VISIPREPTM DL system and Supelclean^{TM} LC-18 SPE Tubes (500 mg/3 mL), both from SUPELCO (USA).

2.2. Instrumentation and conditions

Thermo-Finnigan TSQ Quantum Ultra AM tandem mass spectrometer equipped with an ESI source (San Jose, CA, USA), a Finnigan surveyor LC pump and an auto-sampler were used for the LC–MS/MS analysis. Data acquisition was performed with Xcalibur 1.2 software (Thermo-Finnigan, San Jose, CA, USA). Peak integration and calibration were carried out using LC Quan software (Thermo-Finnigan).

The separation was performed on an Inertsil ODS-3C_{18} column (5 μm, 250 mm × 4.6 mm, GL Science, Tokyo, Japan) preceded by an ODS guard column (2 mm × 4 mm) by using a mixture of 75 volumes of methanol and 25 volumes of 0.2% ammonium acetate and 0.025% ammonia in water as mobile phase with isocratic elution at 1.0 mL/min. The eluent was split so that 30% was introduced into the inlet of the mass spectrometer, and a divert valve was used to divert the eluent to waste from 0 to 3.0 min. The column temperature was maintained at 30 °C. The temperature of the sample cooler in the auto-sampler was set at 5 °C. All analyses were carried out in positive ion ESI$^+$ with the spray voltage set at 5 kV. The heated capillary temperature was set 350 °C. Nitrogen sheath and auxiliary gas were set at 300 and 35 kPa, respectively. The argon gas collision induced dissociation was used with a pressure of 0.2 Pa and the energy selected to be 20 eV. The total run time for an LC–MS/MS analysis was 5.5 min.

2.3. Calibration standard and quality control preparation in human plasma and urine samples

A 50 μg/mL stock solution of rasagiline (all the concentration was expressed as rasagiline mesylate unless otherwise indicated) was prepared in methanol. This solution was diluted to make a series of standard working solutions in the range from 0.1 to 400 ng/mL. All the working solutions were freshly prepared and stored shaded from light at 5 °C.

Calibration standard and QC samples in plasma and urine were prepared by diluting corresponding working solutions with drug-free human plasma and urine, respectively. The final concentrations of calibration standard in plasma and urine were 0.01, 0.025, 0.10, 0.20, 1.0, 2.0, 5.0, 8.0, 10, 20, 40 ng/mL and 0.025,0.05, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10, 20, 40 ng/mL, respectively. The final concentrations of QC in plasma and urine were 0.05, 1.0, 5.0 and 20.0 ng/mL and 0.10, 1.0, and 10 ng/mL, respectively. All the plasma and urine samples were stored at −20 °C.

2.4. Sample preparation

2.4.1. Sample preparation for plasma

To an aliquot 0.5 mL plasma sample in glass tube, 50 μL of IS working solution (50 ng/mL) and 200 μL of 25% ammonia solution were added then vortex-mixed briefly and extracted with 5 mL of a mixture of MTBE-ether (1:1, v/v) by vortex mixing for 3 min. After phase separation by centrifugation at 1500 × *g* force for 10 min, 4 mL of the organic layer was transferred into another glass tube and evaporated to dryness at 35 °C under a gentle stream of nitrogen. The residue was reconstituted with 150 μL of the mobile phase by vortex mixing for 1 min. The supernatant obtained after centrifugation at 16,000 × *g* force for 10 min was transferred to an auto-sampler vial and 40 μL of which was injected into the LC–MS/MS system.

2.4.2. Sample preparation for urine

SPE was used for urine sample pretreatment. The C_{18} extraction cartridges (3 mL) were activated subsequently once with 5 mL of methanol, 5 mL of 0.25% ammonia solution. The column was kept in a liquid state before samples were loaded. An aliquot of 1 mL urine sample mixed with 50 μL of IS working solution (50 ng/mL) was slowly applied and passed through the activated cartridge under vacuum. The column was then washed with 5 mL of 0.25% ammonia solution to elute interferences from the cartridge and dried completely after the washing. The elution of rasagiline was achieved by rinse with three portions of 1 mL each of a mixture of methanol–hydrochloric acid (99:1, v/v), and the latter eluents were collected together and evaporated to dryness under a gentle stream of nitrogen at 45 °C. The residue was reconstituted with 150 μL of the mobile phase by vortex mixing for 1 min. The supernatant obtained after centrifugation at 16,000 × *g* force for 10 min was transferred to an auto-sampler vial and 40 μL of which was injected into the LC–MS/MS system.

2.5. Assay characteristics for method validation

The method was validated for specificity, matrix effect, precision, accuracy, linearity, sensitivity, extraction recovery, and stability according to the US Food and Drug Administration (FDA) [13] and Chinese State Food and Drug Administration (SFDA) guidelines [14] for the validation of bioanalytical method.

2.5.1. Specificity

The specificity of this method was investigated by analyzing six individual human blank plasma and urine samples. Each blank sample was tested for interferences using the proposed extraction procedure and LC–MS/MS conditions.

2.5.2. Matrix effect

Matrix effect is due to co-elution of some endogenous components present in biological samples. These components may not

give a signal in SRM of target analyte, but can certainly decrease or increase the response of the analyte dramatically to affect the sensitivity, accuracy and precision of the method. Thus assessment of matrix effect constitutes an important and integral part of validation for quantitative LC–MS/MS method for supporting pharmacokinetic studies [15].

To evaluate the matrix effect, chromatographic peak areas of rasagiline from the spike-after preparation samples were compared to the neat standards at the QC concentrations.

2.5.3. Calibration curve, linearity, lower limit of quantitation

Biological samples were quantified using the ratio of peak area of rasagiline to that of IS. The calibration curves were established through a linear least-squares regression with a weighing factor of $1/C^2$, where C is the concentration of the calibration standards. Coefficients of correlations (r) were required to be 0.99 or better. Concentration in the QC and unknown biological samples were quantified from the regression equation. LLOQ was defined as the lowest concentration at which the precision, expressed as relative standard deviation (RSD), is less than 20% and the accuracy is within ±20%, and it is established using five independent samples.

2.5.4. The extraction recovery

The extraction recovery of the rasagiline was determined by comparing the responses of the analytes extracted from quintuplicate QC samples with the response of the analytes spike-after preparation standard samples at equivalent concentrations [16].

2.5.5. Accuracy and precision

The precision and accuracy of the method were assessed by intra- and inter-day validation. The intra- and inter-day accuracy and precision were determined by determining the concentrations of rasagiline in plasma and urine in five replicates of QC samples for three separate batches.

Precision was expressed as the %RSD. Accuracy was expressed in terms of %BIAS, as the percent deviation of the mean determined concentration against the spiked concentration. A %RSD and %BIAS less than or equal to a limit of 15% in measuring range above LLOQ and less than 20% at the level of LLOQ were demanded.

2.5.6. Stability

Stability experiments were performed to evaluate stabilities of the analytes in plasma and urine samples under different storage and processing conditions.

Freeze–thaw stability: it was assessed by exposing samples at moderate concentration (5.0 ng/mL of rasagiline in plasma, and 1.0 ng/mL in urine) to three freeze–thaw cycles, each cycle consisted of removing the samples from the freezer, thawing them unassisted to room temperature, kept at room temperature for 2 h and re-freezing at −20 °C.

Long-term stability: it was evaluated after storage of duplicate samples of moderate concentration level at −20 °C for 10, 20 and 30 days, respectively, a span of time long enough to enclose the entire procedure for the plasma and urine samples from collection to determination.

Bias of results should be within ±15% of their nominal values.

2.6. Method application

The established method was applied in a pharmacokinetic study performed in healthy Chinese subjects. Thirty healthy volunteers (15 male and 15 female, mean age, 34.9 ± 2.9 years; mean weight, 64.6 ± 2.2 kg; mean height, 171.3 ± 3.3 cm) were enrolled in this study in Xijing Hospital, the Fourth Military Medical University, China. The volunteers were free of cardiac, hepatic, renal, pulmonary, neurologic, gastrointestinal and hematologic disease, as assessed by physical examination, electrocardiography and the laboratory tests including hematology, biochemistry, electrolytes and urinalysis. All the subjects were instructed to abstain from taking any medication for 2 weeks before and during the whole study period. The study protocol was approved by the local Ethical Review Committee in accordance with the principles of the Declaration of Helsinki, and the recommendations of the State Food and Drug Administration of China. Written informed consent was obtained from all subjects. Data analysis was carried out using the DAS pharmacokinetic program (Drug And Statistics Version 2.0, Chinese Pharmacology Society).

The study was conducted with a single-center, open-label design. Thirty volunteers were randomised into three groups. They were administered a single oral dose of 0.5, 1 or 2 mg rasagiline mesylate tablets with 250 mL of water after an overnight fast, respectively. Standardized meals were served 3 and 10 h after dosing. Venous blood samples each about 3.5 mL were drawn in heparinized tubes before dosing (0 h) and at 5, 10, 20, 30, 40, 50 min, 1, 1.5, 2, 3, 4, 6 and 8 h after oral administration. Plasma samples were separated with centrifugation at 1000 g force immediately. Urine samples were collected in following time segments, 0–2, 2–4, 4–8, 8–12 and 12–24 h post-dose. All samples were stored at −20 °C until analysis [17,18].

3. Results and discussion

3.1. Chromatographic and MS/MS conditions

Method development began with the optimization of chromatographic conditions including mobile phase composition and column type. The feasibility of various mixtures of solvents such as methanol with different buffers, such as ammonium acetate, ammonia solution and methanoic acid with variable pH in the range of 2.5–8.0 were tested. Mobile phase comprising of 85 volumes of methanol and 15 volumes of 0.2% ammonium acetate and 0.025% ammonia in water was found to be suitable for the LC sep-

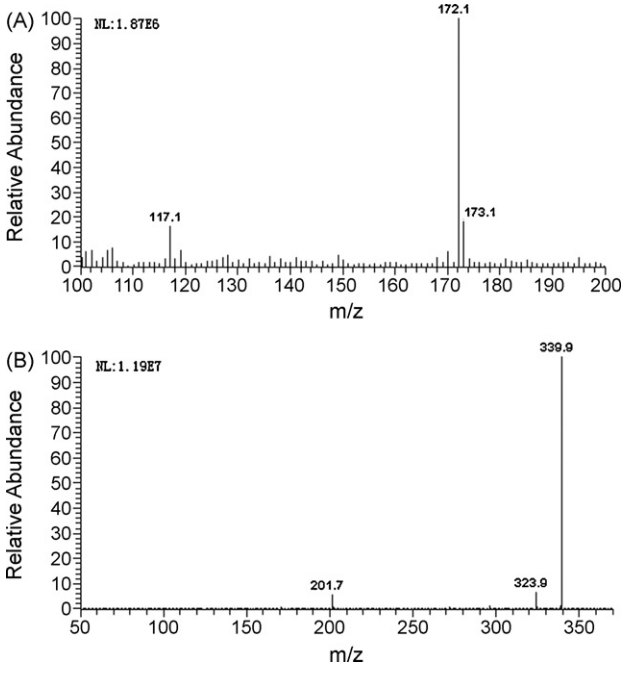

Fig. 1. Full-scan mass spectra of rasagiline (A) and IS (B).

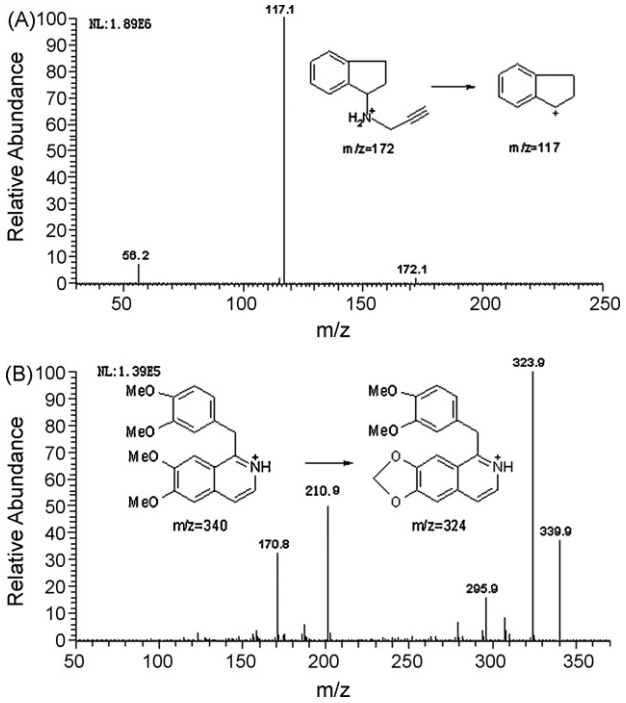

Fig. 2. Structures and product ion spectra of rasagiline (A) and IS (B).

aration with ESI mass detection of the analytes. The versatility, suitability and robustness of the method were checked with several C_{18} columns from various manufacturers, such as Lichrospher C_{18} (Merck KGaA), Zorbax Extend-C_{18} (Agilent Technologies), Shimpack VP-ODS (Shimdazu), etc. And an Inertsil ODS-3 column (5 μm, 250 mm × 4.6 mm, GL Science, Tokyo, Japan) was selected for the analysis.

Mass spectrometric conditions were optimized so as to achieve the maximum stable response of the parents and the major product ions of the analytes. Selective reaction monitoring (SRM) afforded by tandem mass spectrometry has great advantage in reducing interference and enhancing sensitivity over the selected ion monitoring (SIM). SRM was set for the detection of rasagiline and IS in this study. The full-scan mass spectra of rasagiline and IS are shown in Fig. 1A and B, respectively, and their fragmentation schemes of product ions from the parent ions $[M+H]^+$ are shown in Fig. 2A and B, respectively.

The SRM transitions selected for the detection of rasagiline and IS are m/z 172.1 → 117.1 and m/z 339.9 → 323.9, respectively. Parameters such as spray voltage, capillary temperature, and nitrogen sheath gas and the collision gas pressure and energy were tuned to maximize the specificity and sensitivity for the detection.

3.2. Sample preparation

For plasma, several extraction solvents were examined, such as ethyl acetate, ether and solvents mixture of MTBE–ether (1:1, v/v). The solvents mixture of MTBE–ether (1:1, v/v) was chosen due to its high extraction recovery and less matrix effects. 200 μL of 25% ammonia solution was added to alkalize the plasma sample, to free the drug from the plasma protein bonding, and to reduce the interferences from endogenous substances.

For urine, liquid–liquid extraction was attempted by using MTBE–ether (1:1, v/v) mixture as the extraction solvent with different alkaline reagents, such as 1 mol/L sodium hydroxide, ammonia–ammonium chloride buffer (pH 10) and ammonia solution of different concentrations. However, due to the low molecule weight of rasagiline and less matrix interferences in urine comparing to plasma, the evaporation process have greater influence on urine. Therefore, the extraction recovery was found to be of great diversity. SPE (solid phase extraction) is often preferred over liquid/liquid extraction because of the practical advantages of no emulsions, better recoveries, cleaner extracts achievable, the ability to remove many interferences and matrix components selectively, and so on. And what has become one of the most important advantages for SPE is that it is much more capable of being automated, which is important in reducing overall cycle times for clinical studies. Thus, a C_{18} cartridge SPE was tested for sample pretreatment.

In order to establish the optimum conditions for the SPE procedures, the washing solvents such as water and 0.25% ammonia solution were evaluated. The results indicated that proteins and interfering endogenous compounds were removed by 0.25% ammonia solution without any loss of the analytes. The eluting solution adopted a mixture of methanol–hydrochloric acid (99:1, v/v) ensured the complete elution of the extracted free base of rasagiline and converted the free base into the much stable and no-evaporative salt. All these procedures mentioned above ensured higher extraction recovery, better reproducibility and less interference.

3.3. Specificity and matrix effects

Specificity was assessed by comparing the chromatograms of drug-free human plasma and urine with the corresponding spiked plasma and urine, for the test of endogenous interferences. Observed retention time were 4.5 and 4.4 min for rasagiline and IS, respectively, with a total run time of 5.5 min. A representative chromatogram of the plasma and urine blank are shown in Figs. 3A and 4A. No additional peaks due to endogenous substances were observed that would interfere with the detection of the compounds of interest. Figs. 3B and 4B show the chromatograms of calibration standard containing 10 ng/mL of rasagiline, and 50 ng/mL of the IS in plasma and urine, respectively. Typical chromatograms of plasma and urine sample are shown in Figs. 3C and 4C, which were collected 2 and 8 h after oral administration of a single dose of 1 mg rasagiline mesylate tablets for plasma and urine, respectively. The corresponding concentrations of rasagiline in plasma and urine were found to be 0.03 and 0.11 ng/mL, respectively.

Matrix interferences caused by plasma and urine endogenous materials were evaluated by comparing the peak areas of the post-spiked standards with those of the neat standards at the QC concentrations, the ratio was within the acceptable limits (85–115%). No significant ion suppression or enhancement was observed at the expected retention time of the targeted ions.

3.4. Linearity and LLOQ

The validated assay was linear in the range from 0.01 to 40 ng/mL and 0.025 to 40 ng/mL for rasagiline in human plasma and urine, respectively. The typical calibration curves of rasagiline in human plasma and urine were $Y = 0.04472C + 0.00178$ and $Y = 0.097434C + 0.00253$, respectively, with both coefficients of correlation (r) about 0.999. The lowest concentration of determination with RSD < 20% was taken as the LLOQ and was found to be 0.01 ng/mL for plasma and 0.025 ng/mL for urine. In all the cases, the calculated concentrations in the calibration curves were within ±15% bias from the nominal value except at the LLOQ, which was set at ±20%, which are in agreement with criteria guidelines.

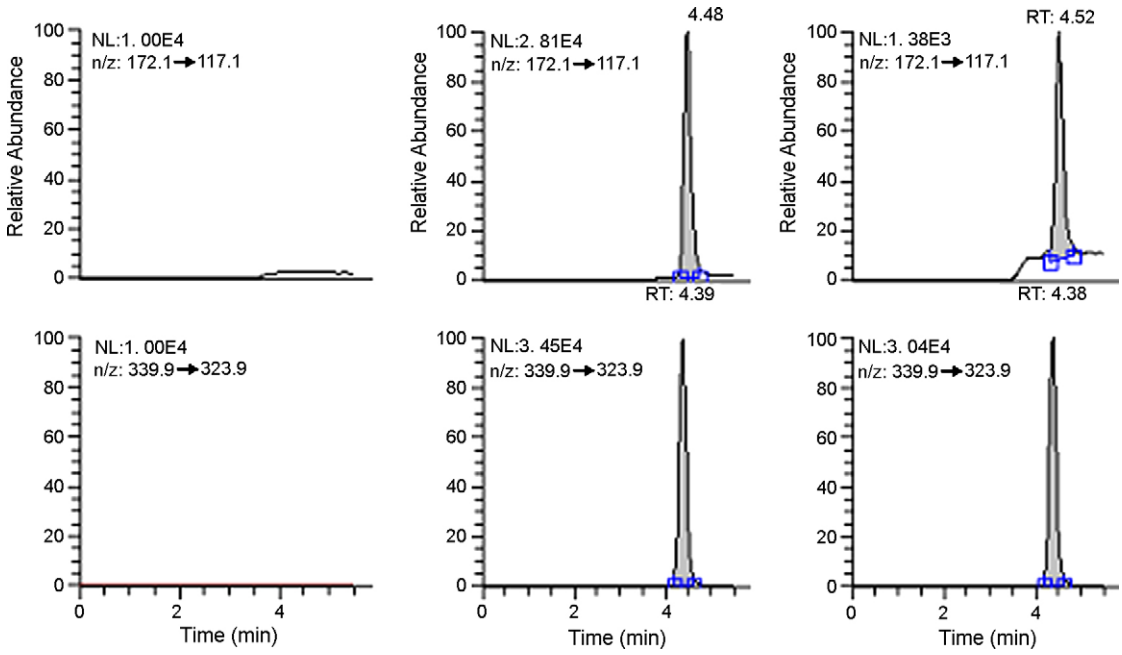

Fig. 3. Representative chromatograms for rasagiline (SRM transition *m*/*z* 172.1 to *m*/*z* 117.1) and IS (SRM transition *m*/*z* 339.9 to *m*/*z* 323.9) from (A) a drug-free blank plasma, (B) a calibration standard plasma sample containing 10 ng/mL rasagiline and (C) a plasma sample from a healthy volunteer 2 h after oral administration of 1 mg rasagiline mesylate tablets (concentration found was 0.03 ng/mL).

3.5. Extraction recovery

The observed value of extraction recovery of the method from plasma and urine and %RSD ($n = 5$) are shown in Tables 1 and 2, respectively. Recoveries were more than 90% at different QC concentrations for both plasma and urine with acceptable variability.

Table 1
Results of extraction recovery of rasagiline in plasma ($n = 5$).

Spiked *C* (ng/mL)	Mean found *C* (ng/mL)	Recovery (%)	RSD (%)
0.05	0.046	92.5	10.7
1.0	0.92	91.7	3.2
5.0	4.5	90.5	4.8
20.0	20.3	101.6	5.1

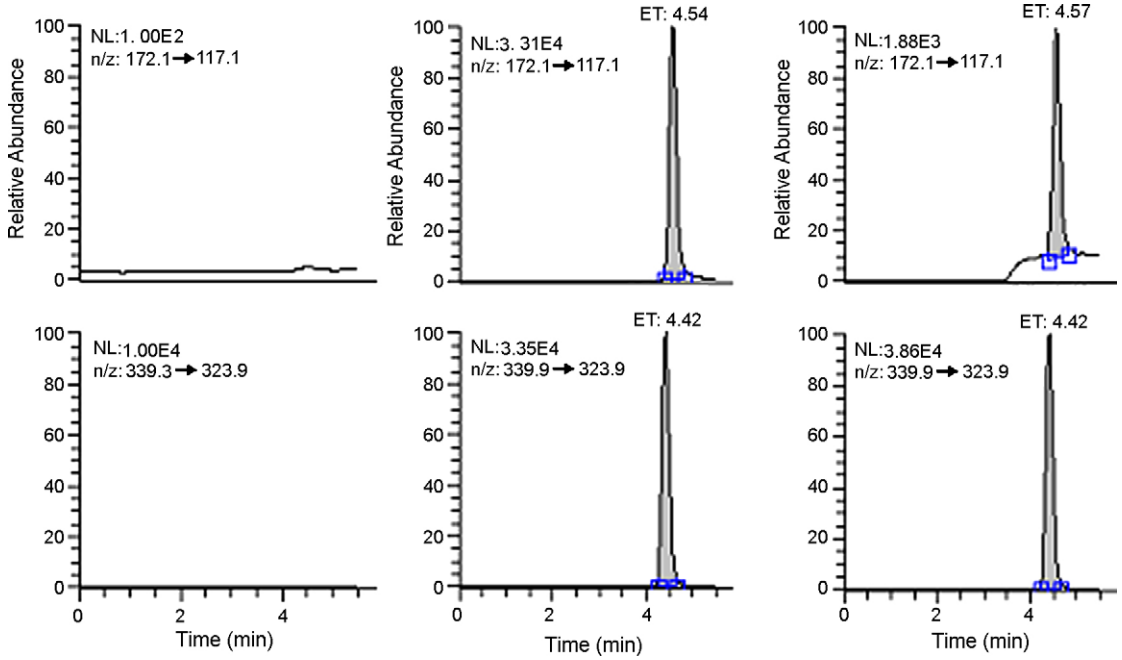

Fig. 4. Representative chromatograms for rasagiline (SRM transition *m*/*z* 172.1 to *m*/*z* 117.1) and IS (SRM transition *m*/*z* 339.9 to *m*/*z* 323.9) from (A) a blank urine, (B) a calibration standard urine sample containing 10 ng/mL of rasagiline and (C) a urine sample from a healthy volunteer 2 h after oral administration of 1 mg rasagiline rasagiline mesylate tablets (the concentration found was 0.11 ng/mL).

Table 2
Results of extraction recovery of rasagiline in urine ($n = 5$).

Spiked C (ng/mL)	Mean found C (ng/mL)	Recovery (%)	RSD (%)
0.10	0.11	108.7	6.2
1.0	1.0	100.6	1.8
10.0	9.8	97.8	6.0

Table 3
Intra- and inter-day precision and accuracy for rasagiline in plasma ($n = 5$).

	Spiked C (ng/mL)			
	0.05	1.0	5.0	20.0
Intra-day run				
Mean found C (ng/mL)	0.046	0.92	4.5	20.3
Accuracy (%BIAS)	−7.5	−8.3	−9.5	1.6
Precision (%RSD)	2.5	2.6	2.1	2.3
Inter-day run				
Mean found C (ng/mL)	0.047	0.98	4.9	19.8
Accuracy (%BIAS)	−4.7	−2.4	−2.8	−1.1
Precision (%RSD)	11.3	14.1	14.2	6.8

Table 4
Intra- and inter-day precision and accuracy for rasagiline in urine ($n = 5$).

	Spiked C (ng/mL)		
	0.10	1.0	10.0
Intra-day run			
Mean found C (ng/mL)	0.11	1.0	9.8
Accuracy (%BIAS)	8.7	0.6	−2.2
Precision (%RSD)	2.9	0.6	1.4
Inter-day run			
Mean found C (ng/mL)	0.10	1.0	10.1
Accuracy (%BIAS)	2.4	3.4	0.6
Precision (%RSD)	14.2	14.1	12.8

3.6. Precision and accuracy

Five quality control samples at each concentration level (0.05, 1.0, 5.0, and 20 ng/mL for plasma and 0.10, 1.0, and 10 ng/mL for urine) were processed and calculated each run of five for three runs to provide precision and accuracy of this method. The intra- and inter-day precision and accuracy data are summarized in Table 3 for plasma and Table 4 for urine. The data obtained for rasagiline were within the acceptable criteria limits to meet the guidelines for bioanalytical methods requirements.

3.7. Stability

The results of long-term storage and freeze–thaw stability data indicated that samples were stable at −20 °C for at least 30 days and through three freeze–thaw cycles in both human plasma and urine. There was no significant degradation observed since the bias in concentration was within ±15% of their nominal values.

3.8. Application of the method in pharmacokinetic studies

The established LC–MS/MS method was successfully applied to the determination of rasagiline in the plasma and urine samples after oral administration of 0.5, 1 or 2 mg rasagiline mesylate tablets in healthy Chinese volunteers, respectively.

The mean plasma concentration–time profiles are shown in Fig. 5. In addition, the main pharmacokinetic parameters for rasagiline are presented in Table 5. The values of the area under plasma concentration–time course (AUC) were proportional to the dose levels of rasagiline, indicating that the blood concentrations of rasagiline increase when the oral doses increase. The cumulative urine excretion rate of rasagiline versus time after oral administration of the drug to the human subjects is shown in Table 6 following our previous data processing paradigm [17,18]. The cumulated rate of rasagiline excreted in urine was consistent with the reported data that the drug excreted from urine in prototype less than 1% [19].

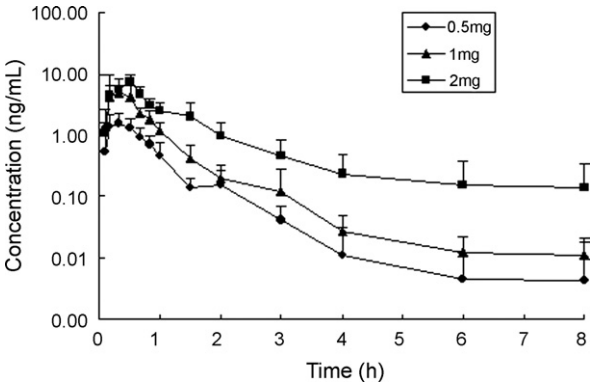

Fig. 5. The mean (±SD) plasma concentration–time profiles of rasagiline in healthy Chinese volunteers ($n = 10$) after a single oral administration of 0.5, 1 and 2 mg rasagiline mesylate tablets, respectively.

Table 5
The main pharmacokinetic parameters for rasagiline after single oral doses of 0.5, 1 and 2 mg to healthy volunteers, respectively (mean ± SD, $n = 10$).

Parameters	0.5 mg	1 mg	2 mg
C_{max} (ng/mL)	2.12 ± 0.33	5.52 ± 1.23	8.91 ± 3.52
T_{max} (h)	0.3 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
AUC_{0-8} (ng h/mL)	1.40 ± 0.30	3.62 ± 0.38	8.14 ± 2.52
$AUC_{0-\infty}$ (ng h/mL)	1.47 ± 0.31	3.79 ± 0.43	8.62 ± 3.30
$t_{1/2}$ (h)	1.35 ± 0.74	1.75 ± 0.44	1.78 ± 0.67
CL/F (L/h)	351.9 ± 81.0	271.1 ± 26.5	256.6 ± 98.4

Table 6
Cumulative urine excretion (% of dose) of rasagiline after single oral dose of 0.5, 1 and 2 mg to healthy volunteers, respectively (mean ± SD, $n = 10$).

Time after dosing (h)	Dose (mg)		
	0.5	1	2
0–2	0.09 ± 0.05	0.16 ± 0.06	0.18 ± 0.03
2–4	0.19 ± 0.03	0.22 ± 0.04	0.21 ± 0.05
4–8	0.21 ± 0.05	0.24 ± 0.04	0.22 ± 0.04
8–12	0.21 ± 0.04	0.24 ± 0.03	0.22 ± 0.06
12–24	0.21 ± 0.06	0.24 ± 0.04	0.22 ± 0.04

4. Conclusion

The LC–MS/MS methods described in this paper allow the LLOQ of rasagiline in human plasma down to 0.01 ng/mL as well as the LLOQ in urine down to 0.025 ng/mL. This method offers advantages in terms of a simple liquid–liquid extraction or a solid phase extraction without any other cleanup procedures with a short run time (5.5 min), which is important for large batches of clinical investigation samples. The method has been fully validated and the assay performance results indicate that it is precise and accurate enough for the routine determination of rasagiline in human plasma and urine. The method has been successfully applied to the determination of the plasma and urine levels of rasagiline in human for investigation of the clinical pharmacokinetics of a rasagiline mesylate tablets.

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