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ORIGINAL ARTICLE

Bio-analytical method development and validation of Rasagiline by high performance liquid chromatography tandem mass spectrometry detection and its application to pharmacokinetic study

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Abstract The most suitable bio-analytical method based on liquid–liquid extraction has been developed and validated for quantification of Rasagiline in human plasma. Rasagiline-$^{13}C_3$ mesylate was used as an internal standard for Rasagiline. Zorbax Eclipse Plus C18 (2.1 mm × 50 mm, 3.5 μm) column provided chromatographic separation of analyte followed by detection with mass spectrometry. The method involved simple isocratic chromatographic condition and mass spectrometric detection in the positive ionization mode using an API-4000 system. The total run time was 3.0 min. The proposed method has been validated with the linear range of 5–12000 pg/mL for Rasagiline. The intra-run and inter-run precision values were within 1.3%–2.9% and 1.6%–2.2% respectively for Rasagiline. The overall recovery for Rasagiline and Rasagiline-$^{13}C_3$ mesylate analog was 96.9% and 96.7% respectively. This validated method was successfully applied to the bioequivalence and pharmacokinetic study of human volunteers under fasting condition.

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1. Introduction

Rasagiline ((1R)-N-prop-2-ynyl-2,3-dihydro-1H-inden-1-amine) is used as a monotherapy in early Parkinson's disease or as an adjunct therapy in more advanced cases [1–3]. The empirical formula is $C_{12}H_{13}N$ with its molecular weight 171.24 (Fig. 1). Rasagiline is rapidly absorbed, reaching peak plasma concentration (C_{max}) in approximately 1 h. The absolute bioavailability of Rasagiline is about 36%. Food does not affect the t_{max} of Rasagiline, although C_{max} and exposure (AUC) decreased by

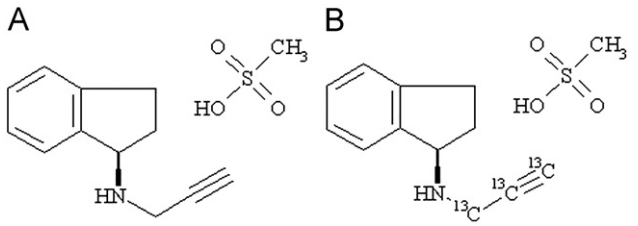

Figure 1 Chemical structures of Rasagiline (A) and Rasagiline-$^{13}C_3$ mesylate (B).

approximately 60% and 20%, respectively, when the drug is taken with a high fat meal. Rasagiline's pharmacokinetics is linear with doses over the range of 1–10 mg. Its mean steady-state half-life is 3 h but there is no correlation of pharmacokinetics with its pharmacological effect. Plasma protein binding ranges from 88%–94% with a mean extent of binding of 61%–63% to human albumin over the concentration range of 1–100 ng/mL. Rasagiline is almost metabolized in the liver and undergoes urinary excretion. Half-life of Rasagiline is about 38–45 min [4].

Literature survey reveals that only a few methods were reported for quantification of Rasagiline in human plasma and pharmaceutical analysis [5–9]. These include HPLC [5,6], crystallographic analysis [7], and LC-MS/MS [8,9]. Only two methods were reported for quantification of Rasagiline in human plasma with LC-MS/MS [8,9].

Song et al. [8] developed a method in a concentration range 0.01–40 ng/mL in human plasma and 0.025–40 ng/mL in urine with runtime 5.5 min. Papavarin was used as an internal standard and the pharmacokinetic study was conducted in 30 human volunteers. The main drawbacks of this method are longer runtime and unsuitable internal standard.

The drawbacks of Song et al. are overcome by Ma et al. [9] with shorter runtime of 3.5 min for each sample in a concentration range of 0.02–50 ng/mL, Pseudoephedrine was used as an internal standard and the pharmacokinetic study was conducted in 12 human volunteers. The main drawbacks of Ma et al. [9] method are sensitivity which is not achieved when compared with Song et al. [8] and suitable internal standard like deuterated or analogs of Rasagiline is not used.

The purpose of the present investigation is to explore rapid run analysis time (3 min), more sensitive method (5 pg/mL), with the small amount of plasma sample (100 μL plasma) utilization during sample processing, simple extraction and analyte comparison with isotope labeled internal standard (Rasagiline-$^{13}C_3$ mesylate).

2. Experimental

Rasagiline and Rasagiline-$^{13}C_3$ mesylate were obtained from TLC PharmaChem, Canada. LC grade methanol, methyl t-butyl ether and dichloromethane were purchased from J.T. Baker Inc. (Phillipsburg, NJ, USA). Analytical reagent grade formic acid and Na_2CO_3 were procured from Merck (Mumbai, India). Human plasma (K_2EDTA) was obtained from Doctors Pathological Lab, Hyderabad. The AZILECT® tablets, containing 1 mg Rasagiline per tablet, were obtained from Teva Pharma (USA).

2.1. Instrumentation

An HPLC system (1200 series model, Agilent Technologies, Waldbronn, Germany) connected with mass spectrometer API 4000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada) was used. Data processing was performed with Analyst 1.4.1 software package (SCIEX).

2.2. Detection

The mass spectrometer was operated in the multiple reaction monitoring (MRM) modes. Sample introduction and ionization were electrospray ionization in the positive ion mode. Sources dependent parameters optimized were as follows: nebulizer gas flow, 30 psi; curtain gas flow, 25 psi; ion spray voltage, 2000 V; temperature (TEM), 375 °C. The compound dependent parameters such as the declustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), cell exit potential (CXP) were optimized during tuning as 40, 35, 10, 12, 8 eV for Rasagiline and Rasagiline-$^{13}C_3$ mesylate, respectively. The collision activated dissociation (CAD) gas was set at 4 psi using nitrogen gas. Quadrupole 1 and quadrupole 3 were both maintained at a unit resolution and dwell time was set at 300 ms for Rasagiline and Rasagiline-$^{13}C_3$ mesylate. The mass transitions were selected as m/z 172.1 → 117.1 for Rasagiline and m/z 175.1 → 117.1 for Rasagiline-$^{13}C_3$ mesylate. The data acquisition was ascertained by Analyst 1.5.1 software.

2.3. Chromatography

Zorbax Eclipse Plus C18 (2.1 mm × 50 mm, 3.5 μm) was selected as the analytical column. Column temperature was set at 45 °C. Mobile phase composition was 0.1% formic acid:methanol (80:20, v/v). Source flow rate was 300 μL/min without split with injection volume of 10 μL. Rasagiline and Rasagiline-$^{13}C_3$ mesylate were eluted at 1.2 ± 0.2 min, with a total run time of 3.0 min for each sample.

2.4. Calibration curve and quality control samples

Two separate stock solutions of Rasagiline were prepared for bulk spiking of calibration curve and quality control samples for the method validation exercise as well as the subject sample analysis. The stock solutions of Rasagiline and Rasagiline-$^{13}C_3$ mesylate were prepared in methanol at free base concentration of 50 μg/mL. Primary dilutions and working standard solutions were prepared from stock solutions using water:methanol (50:50, v/v) solvent mixture. These working standard solutions were used to prepare the calibration curve and quality control samples. Blank human plasma was screened prior to spiking to ensure it was free of endogenous interference at retention times of Rasagiline and internal standard Rasagiline-$^{13}C_3$ mesylate. Ten point standard curve and four quality control samples were prepared by spiking the blank plasma with an appropriate amount of Rasagiline. Calibration samples were made at concentrations of 5.0, 10.0, 100.0, 600.0, 1200.0, 2400.0, 4800.0, 7200.0, 9600.0 and 12000.0 pg/mL and quality control samples were made at concentrations of 5.0, 15.0, 4500.0 and 9000.0 pg/mL for Rasagiline.

2.5. Sample preparation

For sample preparation, 100 μL of plasma sample or Rasagiline spiked standard or quality control plasma sample was added to 5 mL ria vial tubes. 50 μL of internal standard and 200 μL of 1 M Na_2CO_3 solution were added and vortexed

briefly. Then liquid–liquid extraction with 3 mL of extraction solvent (Methyl tertiary butyl ether (MTBE):Dichloromethane (DCM) (3:1, v/v)) was added to each tube and vortexed for 10 min. After centrifugation at 4000 rpm for approximately 10 min at 20 °C, the supernatant was transferred to respective ria vial tubes and evaporated to dryness under nitrogen at 25 °C. Finally, the residue was redissolved in 200 μL of reconstitution solution (MeOH:0.1% formic acid(1:4)). Further, samples were centrifuged at 4000 rpm for approximately 2 min at 20 °C and the supernatant was transferred to auto sampler vials with caps and 10 μL of sample was injected into the LC-MS/MS system.

2.6. Selectivity

Selectivity was performed by analyzing the human blank plasma samples from six different sources (donors) with an additional hemolyzed group and lipedimic group to test for interference at the retention time of analytes.

2.7. Matrix effect

Matrix effect for Rasagiline and internal standard was evaluated by comparing the peak area ratio in the post-extracted plasma sample from 6 different drug-free blank plasma samples and neat reconstitution samples. Experiments were performed at MQC levels in triplicate with six different plasma lots with the acceptable precision (% CV) of $\leq$15%.

2.8. Precision and accuracy

It was determined by replicate analysis of quality control samples ($n=6$) at a lower limit of quantification (LLOQ), low quality control(LQC), medium quality control (MQC), high quality control (HQC) levels. The % CV should be less than 15%, and accuracy should be within 15% except LLOQ where it should be within 20%.

2.9. Recovery

The extraction efficiencies of Rasagiline and Rasagiline-$^{13}C_3$ mesylate were determined by analysis of six replicates at each quality control concentration level for Rasagiline and at one concentration for Rasagiline-$^{13}C_3$ mesylate. The percentage recovery was evaluated by comparing the peak areas of extracted standards to the peak areas of nonextracted standards (spiked into mobile phase).

2.10. Stability

Stock solution stability was performed by comparing the area response of analyte and internal standard in the stability sample, with the area response of sample prepared from fresh stock solution. Stability studies in plasma were performed at the LQC and HQC concentration levels using six replicates at each level. Analyte was considered stable if the change is less than 15% as per US FDA guidelines [10]. The stability of spiked human plasma samples stored at room temperature (bench top stability) was evaluated for 24 h. The stability of spiked human plasma samples stored at 2–8 °C in autosampler (autosampler stability) was evaluated for 55 h. The autosampler sample stability was evaluated by comparing the extracted plasma samples that were injected immediately (time 0 h), with the samples that were reinjected after storing in the autosampler at 2–8 °C for 26 h. The reinjection reproducibility was evaluated by comparing the extracted plasma samples that were injected immediately (time 0 h), with the samples that were re-injected after storing in the autosampler at 2–8 °C for 26 h. The freeze–thaw stability was conducted by comparing the stability samples that had been frozen at −30 °C and thawed three times, with freshly spiked quality control samples. Six aliquots each of LQC and HQC concentration levels were used for the freeze–thaw stability evaluation. For long-term stability evaluation the concentrations obtained after 78 days were compared with initial concentrations.

2.11. Application of method

The validated method has been successfully used to analyze Rasagiline concentrations in 22 human volunteers under fasting conditions after administration of a single tablet containing 1 mg (1 × 1 mg) Rasagiline as an oral dose. The study design was a randomized, two-period, two-sequence, two-treatment single dose, open label, bioequivalence study using AZILECT® manufactured by Teve Pharma, USA as the reference formulation. The test formulation was conducted for APL Research Pvt. Ltd., India. The study was conducted according to current GCP guidelines and after obtaining signed consent of the volunteers. Before conducting the study it was also approved by an authorized ethics committee. There were a total of 19 blood collection time points including the predose sample, per period. The blood samples were collected at time intervals (0, 0.083, 0.167, 0.25, 0.333, 0.417, 0.5, 0.667, 0.833, 1, 1.25, 1.5, 2, 2.5, 3, 3.75, 4.5, 5.5 and 6.5 h) in separate vacutainers containing K_2EDTA as an anticoagulant. The plasma from these samples was separated by centrifugation at 4000 rpm within the range of 10 °C. The plasma samples thus obtained were stored at −30 °C until analysis. The pharmacokinetic parameters were computed using Win-Nonlin® software version 5.2 and 90% confidence interval was computed using SAS® software version 9.2.

3. Results and discussion

3.1. Method development

During method development, different options were evaluated to optimize mass spectrometry detection parameters, chromatography and sample extraction.

3.1.1. Mass spectrometry detection parameters optimization

Electrospray ionization (ESI) provided a maximum response over atmospheric pressure chemical ionization (APCI) mode, and was chosen for this method. The instrument was optimized to obtain sensitivity and signal stability during infusion of the analyte in the continuous flow of mobile phase to electrospray ion source operated at both polarities at a flow rate of 10 μL/min. Rasagiline gave more response in positive ion mode as compare to the negative ion mode. The predominant peaks in the primary ESI spectra of Rasagiline and Rasagiline-$^{13}C_3$ mesylate correspond to the MH+ ions at

m/*z* 172.1 and 175.1 respectively (Fig. 2A and C). Product ions of Rasagiline and Rasagiline-$^{13}C_3$ mesylate scanned in quadrupole 3 after a collision with nitrogen in quadrupole 2 had an *m*/*z* of 117.1 and 117.2 respectively (Fig. 2B and D).

3.1.2. Chromatography optimization

Initially, a mobile phase consisting of ammonium acetate and acetonitrile in varying combinations was tried, but a low response was observed. The mobile phase containing acetic acid:acetonitrile (20:80, v/v) and acetic acid:methanol (20:80, v/v) gave the better response, but poor peak shape was observed. A mobile phase of 0.1% formic acid in water in combination with methanol and acetonitrile with varying combinations was tried. The best signal along with a marked improvement in the peak shape was observed for Rasagiline and Rasagiline-$^{13}C_3$ mesylate using a mobile phase containing 0.1% formic acid in water in combination with methanol (20:80, v/v). Short length columns, such as Symmetry Shield RP18 (50 mm × 2.1 mm, 3.5 μm), Inertsil ODS-2V (50 mm × 4.6 mm, 5 μm), Hypurity C18 (50 mm × 4.6 mm, 5 μm) and Hypurity Advance (50 mm × 4.0 mm, 5 μm), YMC basic (50 mm × 2 mm, 5 μm), Zorbax Eclipse Plus C18 (2.1 mm × 50 mm, 3.5 μm), were tried during the method development. The best signal and good peak shape was obtained using the Zorbax Eclipse Plus C18 (2.1 mm × 50 mm, 3.5 μm) column. It gave satisfactory peak shapes for both Rasagiline and Rasagiline-$^{13}C_3$ mesylate. Flow rate of 0.3 mL/min without splitter was used and reduced the run time to 3.0 min. Both the drug and internal standard were eluted in shorter time at 2.0 min. For an LC-MS/MS analysis, utilization of stable isotope-labeled or suitable analog drugs as an internal standard proves helpful when a significant matrix effect is possible. In our case, Rasagiline-$^{13}C_3$ mesylate was found to be best for the present purpose. The column oven temperature was kept at a constant temperature of about 45 °C. Injection volume of 10 μL sample is adjusted for better ionization and chromatography.

3.1.3. Extraction optimization

Prior to load the sample for LC injection, the co-extracted proteins should be removed from the prepared solution. For this purpose, initially we tested with different extraction procedures like Protein precipitation(PPT), Liquid–liquid extraction(LLE) and Solid phase extraction(SPE). We found ion suppression effect in protein precipitation method for the drug and internal standard. Further, we tried with SPE and LLE. Out of all, we observed LLE is suitable for extraction of the drug and internal standard. We tried with several organic solvents (ethyl acetate, chloroform, n-hexane, dichloromethane and methyl tertiary butyl ether) individually as well with

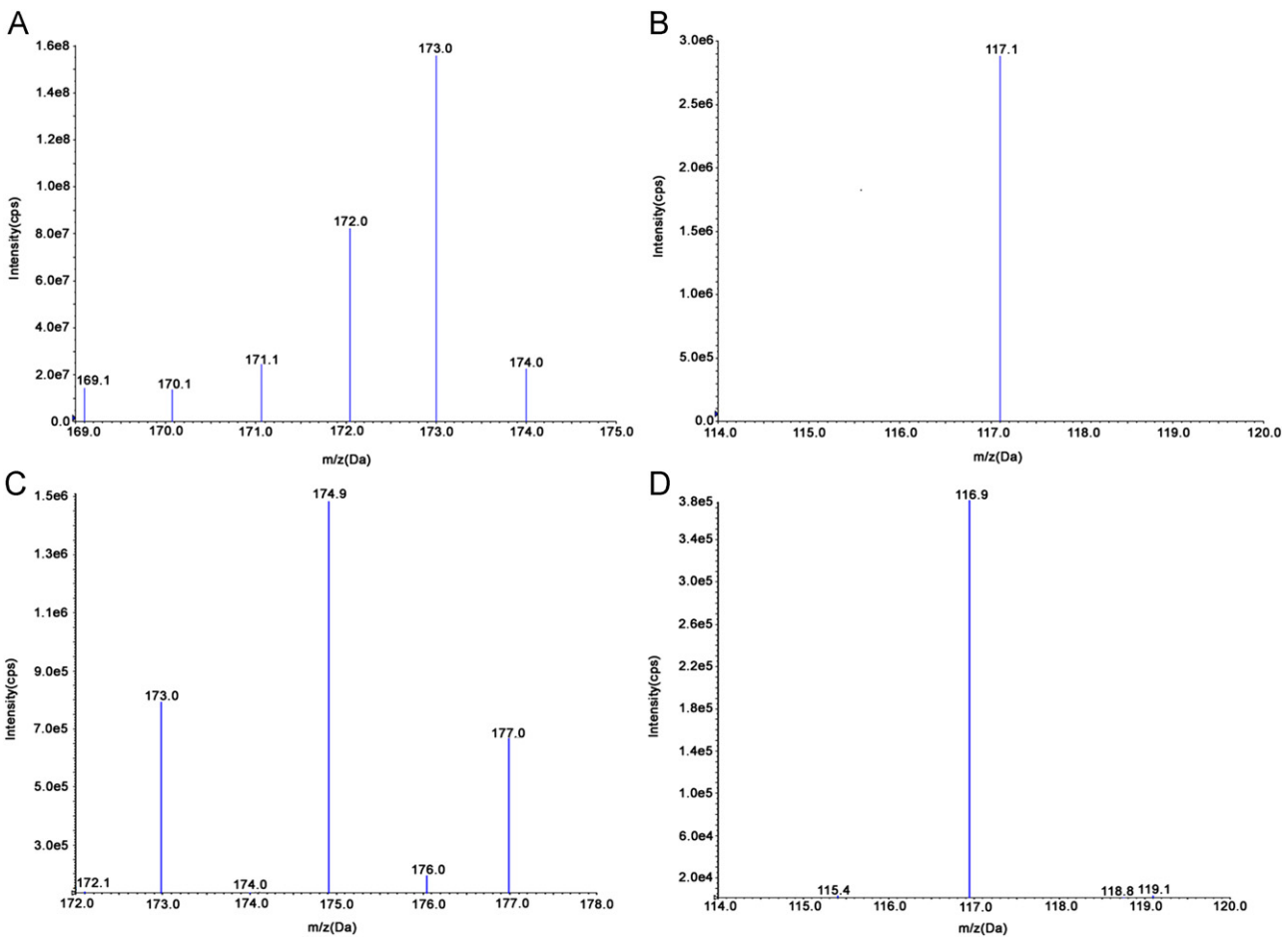

Figure 2 Mass spectra (A) Rasagiline Parent ion, (B) Rasagiline Product ion, (C) Rasagiline-$^{13}C_3$ mesylate Parent ion, and (D) Rasagiline-$^{13}C_3$ mesylate Product ion.

combinations in LLE to extract analyte from the plasma sample. In our case methyl tertiary butyl ether:dichloromethane (75:25) combination served as good extraction solvent. Auto sampler wash is optimized as 80% methanol. Several compounds were investigated to find a suitable internal standard, and finally Rasagiline-$^{13}C_3$ mesylate was found to be the most appropriate internal standard for the present purpose. There was no significant effect of IS on analyte recovery, sensitivity or ion suppression. High recovery and selectivity was observed in the liquid–liquid extraction method. These optimized detection parameters, chromatographic conditions and extraction procedure resulted in reduced analysis time with accurate and precise detection of Rasagiline in human plasma.

3.2. *Method validation*

A thorough and complete method validation of Rasagiline in human plasma was done following US FDA guidelines [10]. The method was validated for selectivity, sensitivity, matrix effect, linearity, precision and accuracy, recovery, reinjection reproducibility and stability.

3.2.1. *Selectivity and sensitivity*

Representative chromatograms obtained from blank plasma and plasma spiked with a lower limit of quantification (LOQ) sample are shown in Figs. 3 and 4 for Rasagiline and Rasagiline-$^{13}C_3$ mesylate. The mean % interference observed at the retention time of analytes between six different lots of human plasma, including hemolyzed and lipedemic plasma containing K_2EDTA as an anti-coagulant was 0.00% and 0.00% for Rasagiline and Rasagiline-$^{13}C_3$ mesylate respectively, which was within acceptance criteria. Six replicates of extracted samples at the LLOQ level in one of the plasma sample having least interference at the retention time of Rasagiline were prepared and analyzed. The % CV of the area ratios of these six replicates of samples was 1.1% for Rasagiline, confirming that interference does not affect the quantification at the LLOQ level. The LLOQ for Rasagiline was 5 pg/mL. All the values obtained below 5 pg/mL for Rasagiline were excluded from statistical analysis as they were below the LLOQ values validated for Rasagiline.

3.2.2. *Matrix effect*

The % CV of ion suppression/enhancement in the signal was found to be 1.0% at MQC level for Rasagiline, indicating that the matrix effect on the ionization of analyte is within the acceptable range under these conditions.

3.2.3. *Linearity*

The peak area ratios of calibration standards were proportional to the concentration of Rasagiline in each assay over the nominal concentration range of 5–12000 pg/mL. The calibration curves appeared linear and were well described by least-squares linear regression lines. As compared to the 1/x

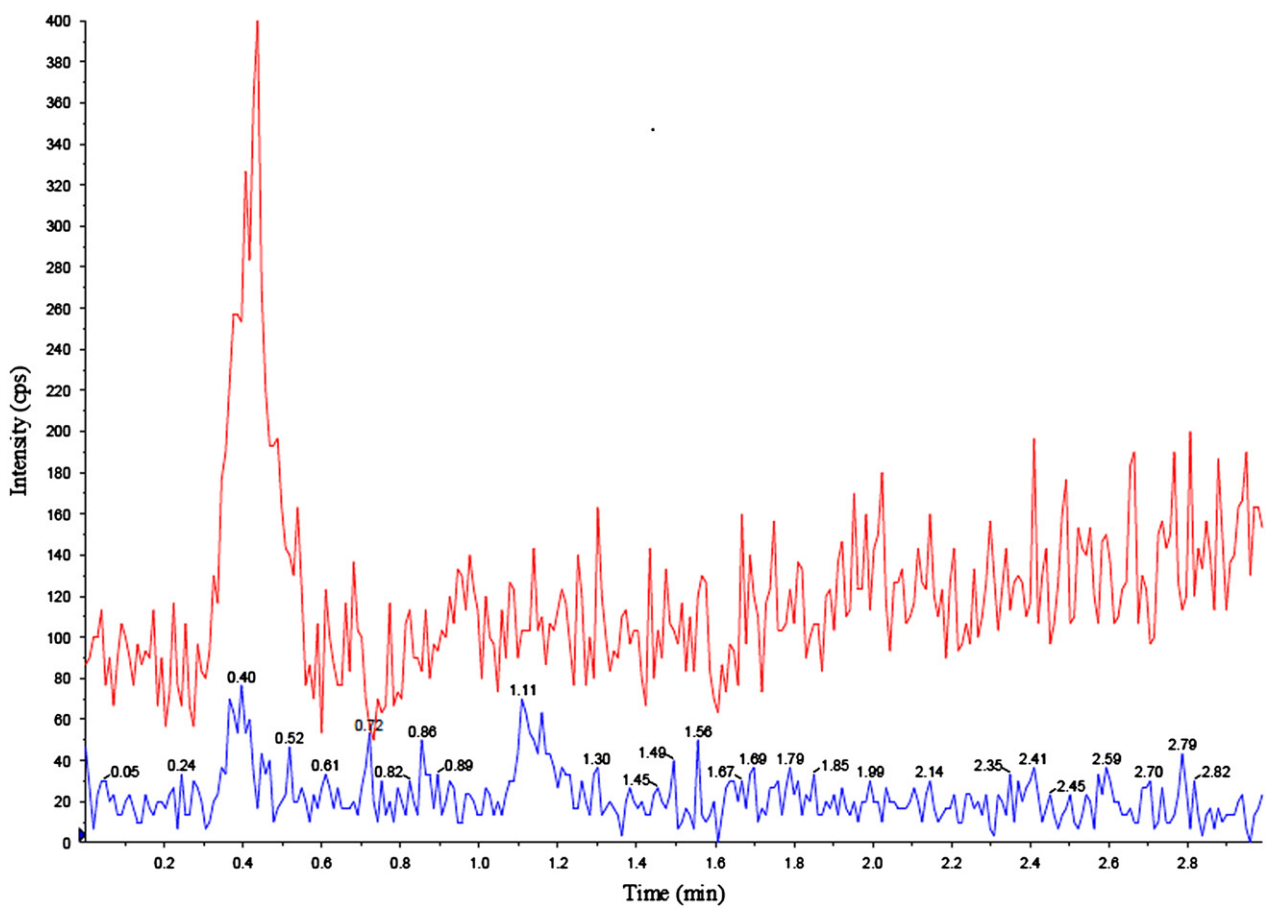

Figure 3 Blank plasma chromatograms of Rasagiline and Rasagiline-$^{13}C_3$ mesylate in human plasma.

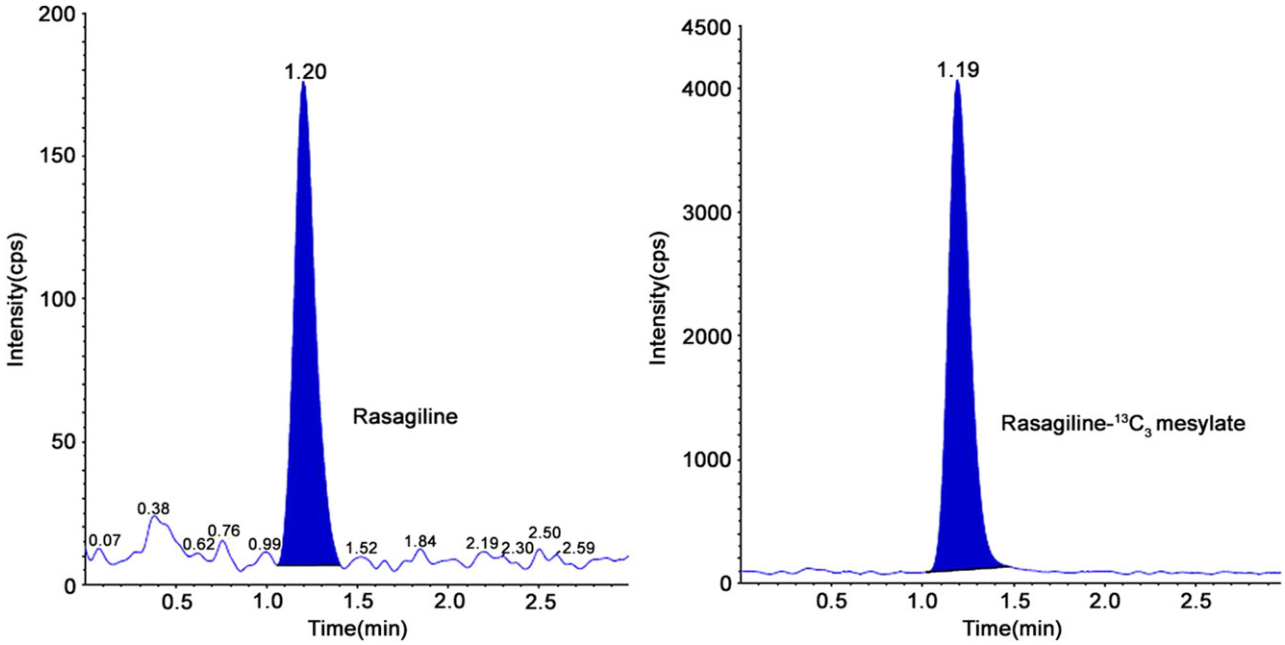

Figure 4 LLOQ chromatograms of Rasagiline and Rasagiline-$^{13}C_3$ mesylate in human plasma.

Table 1 Calibration curve details.

Concentration (pg/mL)	Mean (pg/mL)	SD	CV (%)	Accuracy
5.0	4.8	0.0	0.4	96.0
10.0	9.8	0.2	1.7	98.0
100.0	100.5	2.6	2.6	100.5
600.0	595.2	16.7	2.8	99.2
1200.0	1180.6	22.4	1.9	98.4
2400.0	2496.1	69.9	2.8	104.0
4800.0	4505.4	108.1	2.4	93.9
7200.0	7268.4	247.1	3.4	101.0
9600.0	9468.2	236.7	2.5	98.6
12000.0	11864.5	178.0	1.5	98.9

SD: Standard deviation.

weighing factor, a weighing factor of 1/x2 achieved the best result and was chosen to achieve homogeneity of variance. The correlation coefficient was $\geq$0.9991 for Rasagiline. The observed mean back-calculated concentration with accuracy and precision (% CV) of five linearity's analyzed during method validation is given in Table 1. The deviations of the back calculated values from the nominal standard concentrations were less than 15%. This validated linearity range justifies the concentration observed during real sample analysis.

3.2.4. *Precision and accuracy*

The inter-run precision and accuracy were determined by pooling all individual assay results of replicate (n=6) quality control over five separate batch runs analyzed on four different days. The inter-run, intra-run precision (% CV) was $\leq$5% and inter-run, intra-run accuracy was in between 85 and 115 for Rasagiline. All these data presented in Table 2 indicate that the method is precise and accurate.

3.2.5. *Recovery*

Six aqueous replicates (samples spiked in reconstitution solution) at low, medium and high quality control concentration levels for Rasagiline were prepared for recovery determination, and the areas obtained were compared with the areas obtained for extracted samples of the same concentration levels from a precision and accuracy batch run on the same day. The mean recovery for Rasagiline was 96.9% with a precision of 2.4%, and the mean recovery for Rasagiline-$^{13}C_3$ mesylate was 96.7% with a precision of 2.1%. This indicates that the extraction efficiency for Rasagiline as well as Rasagiline-$^{13}C_3$ mesylate was consistent and reproducible.

3.2.6. *Reinjection reproducibility*

Reinjection reproducibility exercise was performed to check whether the instrument performance remains unchanged after hardware deactivation due to any instrument failure during real subject sample analysis. The change was less than 2.5% at LQC and HQC concentration levels; hence batch can be reinjected in the case of instrument failure during real subject sample analysis. Furthermore, samples were prepared to be reinjected after 27 h, which shows % change less than 2.8% at LQC and HQC concentration levels; hence batch can be reinjected after 27 h in the case of instrument failure during real subject sample analysis.

3.2.7. *Stabilities*

Stock solution stability was performed to check stability of Rasagiline and Rasagiline-$^{13}C_3$ in stock solutions prepared in methanol and stored at 2–8 °C in a refrigerator. The freshly prepared stock solutions were compared with stock solutions prepared before 28 days. The % change for Rasagiline and Rasagiline-$^{13}C_3$ mesylate was −0.01% and 0.02% respectively, which indicates that stock solutions were stable at least for 28 days. Bench top and autosampler stability for Rasagiline was investigated at LQC and HQC levels. The results revealed that

Table 2 Within-run and between-run precision and accuracy.

Nominal added concentration (pg/mL)	Within-run ($n=6$)				Between-run ($n=36$)			
	Mean (pg/mL)	SD	Precision (CV, %)	Accuracy	Mean (pg/mL)	SD	Precision (CV, %)	Accuracy
5.0	4.9	0.1	1.3	98.0	5.1	0.1	1.6	102.0
15.0	15.2	0.2	1.3	101.3	15.2	0.2	1.6	101.3
4500.0	4485.6	130.1	2.9	99.7	4465.2	98.2	2.2	99.2
9000.0	8965.3	215.2	2.4	99.6	8965.2	251.0	2.8	99.6

SD: Standard deviation, CV = Coefficient of variation.

Table 3 Stability of the samples.

Stability experiments	Spiked plasma concentration ($n=6$, pg/mL, mean ± SD)	Concentration measured ($n=6$, pg/mL, mean ± SD)	CV (%, $n=6$)
Room temperature stability (24 h)	15.0	14.9 ± 0.2	1.3
	9000.0	8799.3 ± 211.2	2.4
Autosampler stability (55 h)	15.0	15.2 ± 0.2	1.3
	9000.0	8896.4 ± 213.5	2.4
Long-term stability (78 days)	15.0	14.6 ± 0.2	1.3
	9000.0	8897.5 ± 213.5	2.4
Freeze–thaw stability (cycle 3, 48 h)	15.0	14.9 ± 0.2	1.3
	9000.0	8897.5 ± 213.5	2.4

Rasagiline was stable in plasma for at least 24 h at room temperature, and 55 h in an auto sampler at 20 °C. It was confirmed that repeated freezing and thawing (three cycles) of plasma samples spiked with Rasagiline at LQC and HQC levels did not affect their stability. The long-term stability results also indicated that Rasagiline was stable in a matrix up to 78 days at a storage temperature of −30 °C. The results obtained from all these stability studies are tabulated in Table 3.

3.3. *Application*

The validated method has been successfully used to quantify Rasagiline concentrations in 22 human volunteers, under fasting conditions after administration of 1 mg (1 × 1 mg) tablet containing Rasagiline as an oral dose. The study was carried out after obtaining signed consent from the volunteers. These volunteers were contracted in APL Research Centre, Hyderabad, India. The study protocol was approved from an IEC (independent ethics committee) as per DCGI (Drug Control General of India) guidelines. The pharmacokinetic parameters evaluated were C_{max} (maximum observed drug concentration during the study), $AUC_{0-6.5}$ (area under the plasma concentration–time curve measured 6.5 h, using the trapezoidal rule), t_{max} (time to observe maximum drug concentration), K_{el} (apparent first order terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve, using the method of the least square regression) and $t_{1/2}$ (terminal half-life as determined by the quotient $0.693/K_{el}$, Table 4).

The Test/Reference ratios for C_{max}, $AUC_{0-6.5}$, and $AUC_{0-\infty}$ were 80.22, 90.86 and 90.70 respectively, and they were within the acceptance range of 80%–125% demonstrating the bioequivalence of the two formulations of Rasagiline [11–12]. The mean concentration versus time profile of Rasagiline in human plasma from 22 subjects that are receiving 1 × 1 mg oral dose of Rasagiline tablet as test and reference is shown in Figure 5.

Table 4 Mean pharmacokinetic parameters of Rasagiline in 22 healthy volunteers after oral administration of 1 mg (1 × 1 mg) test and reference products.

Pharmacokinetic parameter	Rasagiline	
	Test	Reference
AUC_{0-t} (pg h/mL)	4005.11	4407.78
C_{max} (pg/mL)	4529.87	5647.15
$AUC_{0-\infty}$ (pg h/mL)	4022.33	4434.56
K_{el}	0.64892	0.59415
$t_{1/2}$	1.07	1.17
t_{max}(h)	0.417	0.417

$AUC_{0-\infty}$: area under the curve extrapolated to infinity; AUC_{0-t}: area under the curve up to the last sampling time; C_{max}: the maximum plasma concentration; t_{max}: the time to reach peak concentration; K_{el}: the apparent elimination rate constant. $t_{1/2}$: $0.693/K_{el}$

4. Conclusion

The proposed bio-analytical method is simple, highly sensitive, selective, rugged and reproducible. The major advantage of this method is rapid analysis time (3 min), less plasma volume (0.1 mL) usage for analysis, suitable internal standard usage. This method was successfully applied in bioequivalence study to evaluate the plasma concentrations of Rasagiline in healthy human volunteers.

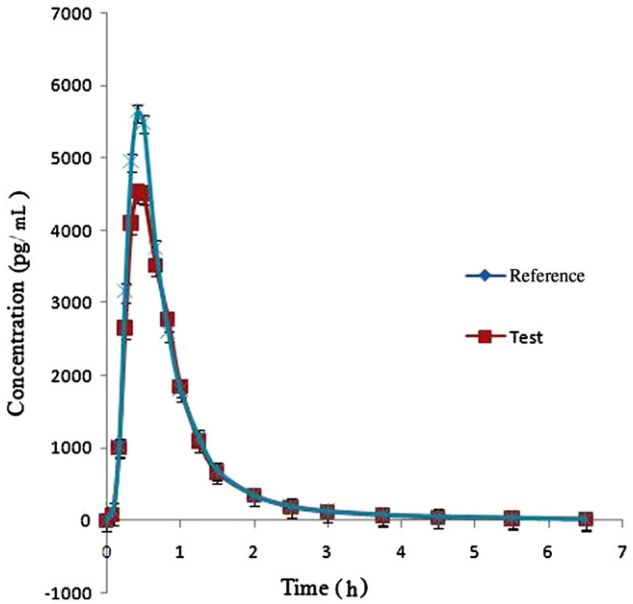

Figure 5 Mean plasma concentrations of test versus reference after 1 mg dose (1 × 1 mg tablet) in 22 healthy volunteers.

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