SIMPLE AND RAPID LC-MS/MS METHOD FOR THE DETERMINATION OF RASAGILINE IN HUMAN PLASMA

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ABSTRACT

An analytical method based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed and validated for the determination of rasagiline in human plasma. Rasagiline 13C3 was used as an internal standard (IS). The samples were extracted with simple solid phase extraction (SPE) and chromatographed on C_{18} column using a combination of 5mM ammonium acetate – acetonitrile (20:80, v/v) as a mobile phase. The method was validated in the range of 0.015–18.233 ng/mL as per the recent US FDA guidelines. A total of five precision and accuracy batches were analyzed during validation. The analyte was stable in aqueous as well as in plasma samples under different conditions tested and results met the acceptance criteria. The chromatographic run time was set at 2.5 min, which makes the proposed method is high through put.

KEYWORDS: Rasagiline; Solid–phase extraction (SPE); LC–MS/MS; Method validation.

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative, dopamine deficiency disorder. The symptoms include tremor at rest, bradykinesia, muscular rigidity and postural instability.[1] Rasagiline is a selective irreversible monoamine oxidase [MAO] B inhibitor, used to treat symptoms of Parkinson's disease. The drug can be used as monotherapy as adjunct therapy with levodopa. The drug decreases the oxidative deamination of dopamine, both endogenous dopamine as well as dopamine produced from exogenous levodopa there by dopamine levels are increased.[2,4]

Many analytical methods based on LC-MS/MS were reported for the determination of rasagiline in human plasma. Most of the LC-MS methods reported so far were utilizes liquid-liquid extraction (LLE) technique for the sample processing. LLE involves extraction, evaporation, drying and reconstitution steps, which is labor intensive. Ma *et al.,* 2008[5] reported a method for rasagiline with an LLOQ of 0.02 ng/mL. Pseudoephedrine was used as an internal standard. This method is having run time of >3.6 min, which is not suitable for routine bioanalytical application. Rasagiline and its metabolites were determined in plasma by LC-MS/MS method.[6] Clopidogrel was used as an internal standard. Similarly, Konda *et al.,* 2012[7] reported a method for rasagiline in human plasma. LLE with *tert-*

butyl methyl ether and dichloromethane (3:1) were used for extraction. The total run time was 3 min. Solid phase extraction (SPE) with drying and evaporation steps were used in an another LC-MS method.[8] Trimetazidine was used as internal standard and run time was 3 min. In this method pramipexole and ropinirole was simultaneously analyzed along with rasagiline. Another author Song *et al.,* 2008[9] was reported a method for the determination of rasagiline in plasma and in urine samples. This method took about 5.5 min to analyze a sample, which is very high. The analytical method should satisfy the scientists in terms of simplicity, sensitivity, runtime, time consumption, sample volume and efficient extraction procedure.[10, 11]

In view of above, the authors proposed a simple and rapid LC-MS/MS method for the determination of rasagiline in of human plasma using isotope labeled compound rasagiline 13C3 as internal standard. The extraction was carried out with simple and one step SPE technique for sample preparation.

EXPERIMENTAL
Standards and reagents
The standard sample rasagiline mesylate (99.7%) and rasagiline 13C3 mesylate (99.68% pure) were obtained from Vivan Life Sciences Limited (Mumbai, India). Ammonium acetate (analytical grade) were purchased

from Merck Ltd (Mumbai, India). HPLC grade acetonitrile and methanol were purchased from J.T. Baker (Phillipsburg, USA). HPLC grade water was obtained from Rankem (Mumbai, India). Control K2 EDATA human plasma was obtained from Deccan's Pathological Lab's (Hyderabad, India).

LC–MS/MS instrument and conditions
An HPLC system (Shimadzu, Kyoto, Japan) coupled with API-4000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with Turboionspray ™ (ESI) interface was used for the study. A 20µL aliquot of the sample was injected in to Zorbax SB C18 (50 mm x 4.6 mm, 3.5 µm) column using an isocratic mobile phase consisting of a mixture of 5mM ammonium acetate –acetonitrile (20:80, v/v). The flow rate was 0.7 mL/min with ambient temperature. The ESI was operated in positive mode with source temperature of 500°C and ion spray voltage of 5500 V. Detection of the ions was carried out in the multiple–reaction monitoring mode (MRM) with the transition pairs of *m/z* 172.1 precursor ion to the *m/z* 117.1 for rasagiline and *m/z* 175.1 precursor ion to the *m/z* 117.1 product ion for the IS. The source parameters viz. the nebulizer gas (GS1), auxiliary gas (GS2), curtain gas and collision gas were set at 40, 40, 30, and 10 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy (CE), entrance potential (EP) and collision cell exit potential (CXP) were 32, 20, 10, 10 V for rasagiline and for the IS. The chromatographic data was processed by Analyst Software™ (version 1.4.2).

Sample preparation
All the stock solutions were prepared in methanol at a concentration of 1 mg/mL and working solutions were prepared in methanol and water (50:50, v/v; diluent). Calibration curve in plasma was prepared at concentration levels of 0.015, 0.030, 0.077, 0.153, 0.767, 3.648, 7.298, 10.940, 14.586 and 18.233 ng/mL. Also, the quality control (QC) samples were prepared at concentrations of 0.015 (lower limit of quantitation, LLOQ), 0.047 (low quality control, LQC), 2.000 (medium quality control, MQC1), 9.092 (MQC2) and 12.627 ng/mL (high quality control, HQC). All the sample were prepared in a single batch and stored at −70±10°C in a freezer.

To a 100 µL of thawed human plasma sample, 10 µL of the IS dilution (50 ng/mL of rasagiline 13C3) was added. To each sample 100 µL of ammonium acetate (50mM) buffer was added after vortex mixing for 10 s. The sample mixture was loaded onto an Oasis HLB (30 mg/1cc) that was pre–conditioned with 1.0 mL of methanol followed by 1.0 mL of water. The extraction cartridge was washed with 1.0 mL of 2.0 mL of water (1 mL each time) and eluted with 0.5 mL of mobile phase and injected into the LC-MS/MS system.

Method validation parameters
The present method was validated for carryover test, selectivity, matrix effect, sensitivity, linearity, precision and accuracy, recovery, dilution integrity and stability as per US FDA guidelines.[12]

RESULTS AND DISCUSSION
Mass spectrometry
Mass spectrometric conditions were optimized with 100 ng/mL solution of analyte and the IS. The solution was infused in to the ESI source at rate of 5µL/min and intensity was observed. The greater intensity was obtained in positive mode than the negative mode for the analyte and the IS. The source and compound parameters were suitable altered to obtain the high and reproducible response. Protonated form of analyte and IS, $[M+H]^+$ ion was the parent ion in the Q_1 spectrum and was used as the precursor ion to obtain Q_3 product ion spectra. The most sensitive mass transition was observed from m/z 172.1 to 117.1 for rasagiline and from m/z 175.1 to 117.1 for the IS. The dwell time for each transition was 200 ms. Here we did not present the fragmentation pattern of rasagiline and the IS as the earlier authors are described well. The MRM technique provided intrinsic selectivity and sensitivity, hence chosen for the study.[13, 14]

Method development
Mobile phase composition was tried with a variety of buffers (ammonium formate and ammonium acetate) and volatile acids (formic acid and acetic acid) in combination with organic solvents like methanol and acetonitrile. Peak shape was not good and the response was not reproducible with ammonium acetate, ammonium formate in combination with methanol and acetonitrile. Similar results were obtained with volatile buffers. A promising results were obtained with a combination of 5mM ammonium acetate –acetonitrile (20:80, v/v) as a mobile phase with a flow rate of 0.7 mL/min. Off all the columns tested, Zorbax SB C18 (50 mm × 4.6 mm, 3.5 µm) column gave good peak shape and response even at LLOQ level for the analyte. The chromatographic run time was 2.5 min, having retention time of 1.3 min for analyte and the IS.

The earlier other have employed LLE and SPE for plasma sample preparation. As a purpose to develop an effective sample preparation SPE was tested. A variety of SPE cartridges like Oasis HLB, Strata X polymeric sorbent and Orpheus C_{18} cartridges were tested. Good and reproducible recovery was obtained with Oasis HLB (30mg/1cc) cartridges at LQC, MQC2 and HQC levels for the analyte. Addition of ammonium acetate helped in attaining high recoveries for analyte and the IS. A suitable internal standard will give a measure of control for extraction, HPLC injection and ionization variability. A deuterated internal standard will have the same extraction recovery, ionization response in ESI mass spectrometry and the same chromatographic retention time. Also, these compounds will co-elute with the compound to be quantified. Hence, in the present work

rasagiline stable labeled isotope rasagiline 13C3 was used as internal standard and found to be best for the present purpose.

Carryover effect, selectivity and chromatography
The results obtained for carryover test shows no significant carryover effect in the blank samples after injection of highest concentration of analyte (ULOQ). Six plasma lots (4 were normal and one lipemic and one haemolyzed) obtained from different individual sources were screened for the interference. There was no significant interference in the blank plasma was observed at the retention time of analyte and the IS as shown in the Figure 1A. Also, no interference was observed from the IS channel at the retention time of analyte (Figure 1B). A representative chromatogram resulting from the analysis of subject blank plasma sample is shown in Figure 2.

Matrix effect and sensitivity
Matrix effect, expressed as IS normalized matrix factor (MF) and was calculated using the below formula:
IS normalized matrix Factor = Peak response area ratio in presence of matrix ions
Mean peak response area ratio in absence of matrix ions

Matrix effect assessed by at LQC and HQC levels comparing the mean area response of post–extraction spiked samples with mean area of aqueous samples (neat samples). These samples were prepared in mobile phase and injected. The IS normalized matrix factor was 0.995 for LQC and 0.993 for HQC. The results indicate no significant matrix effect was found in all the plasma lots tested.

The lowest limit of reliable quantification (LOQ) for the present method was set at 0.015 ng/mL. At this concentration, the precision and accuracy of analyte was found to be 6.59 and 104.44%, respectively.

Linearity, precision and accuracy
A total of five successful calibration curves run during the validation in the concentration range of 0.015–518.233 ng/mL for rasagiline with a mean correlation coefficient of ≥ 0.99. After comparing the two weighting models ($1/x$ and $1/x^2$), a regression equation with a weighting factor of $1/x^2$ of the drug to the IS concentration was found to produce the best fit for the concentration–detector response relationship.

The results of intra–day and inter–day analysis are summarized in Table 1. The intra–day and inter day precision deviation values were all within 15% of the relative standard deviation (RSD) at low, middle and high quality control level, whereas within 20% at LLOQ QCs level. The intra–day and inter–day accuracy deviation values were all within $100 \pm 15\%$ of the actual values at low, middle and high quality control level, whereas within $100 \pm 20\%$ at LLOQ QCs level. The results revealed good precision and accuracy.

Recovery
The recovery of rasagiline at LQC, MQC2 and HQC levels were found to be 95.62%, 94.62% and 95.24%, respectively with mean recovery of $95.16 \pm 0.50\%$. Similarly, the recovery of IS was 90.13% with the precision range of 2.16–3.02%.

Dilution integrity
Sample dilution was performed to extent the ULOQ suitable of higher dose of rasagiline. The ULOQ can be extended up to 30.940 ng/mL by 1/2 and 1/4 dilutions with screened human blank plasma. The precision (%CV) an accuracy for two-fold dilution was 0.57% to 92.81%, respectively. Similarly, the precision (%CV) an accuracy for four-fold dilution was 0.45% to 94.76%, respectively.

Stability studies
Analyte stability at various conditions was evaluated. In the different stability experiments carried out viz. bench top stability (9 h), autosampler stability (38 h), wet extract stability at 2–8°C (40 h), repeated freeze–thaw cycles (3 cycles), reinjection stability (35 h) and long term stability at −70°C for 50 days the mean % nominal values of the analyte were found to be within ±15% of the predicted concentrations for the analyte at their LQC and HQC levels (Table 2). Therefore, the results were found to be within the acceptable limits during the entire validation.

Table 1: Precision and accuracy data for rasagiline.

Quality control	Run	Concentration found Mean±SD (ng/mL)	Precision (%)	Accuracy (%)
Intra–day variations (*n*=12 at each concentration)				
LLOQ		0.017 ± 0.001	6.80	111.67
LQC		0.045 ± 0.002	4.55	96.63
MQC1		2.207 ± 0.18	8.00	110.35
MQC2		9.693 ± 0.60	6.14	106.61
HQC		13.212 ± 1.33	10.03	104.64
Inter–day variations (*n*=30 at each concentration)				
LLOQ		0.017 ± 0.001	6.12	111.33
LQC		0.048 ± 0.004	8.57	101.91
MQC1		2.182 ± 0.12	5.60	109.10
MQC2		9.597 ± 0.41	4.26	105.56
HQC		12.901 ± 1.00	7.72	102.17
Nominal concentrations of LLOQ, LQC, MQC1, MQC2 and HQC are 0.015, 0.047, 2.000, 9.092 and 12.627 ng/mL, respectively.				

Table 2: Stability data for rasagiline in plasma (*n*=6).

Stability test	QC (spiked concentration (ng/mL)	Mean ± SD (ng/mL)	Precision (%)	Accuracy/Stability (%)
Process[a]	0.047	0.048 ± 0.002	3.46	101.06
	12.627	12.698 ± 0.10	0.82	100.56
Process[b]	0.047	0.050 ± 0.002	3.87	106.74
	12.627	12.747 ± 0.13	0.88	100.95
Bench top[c]	0.047	0.050 ± 0.001	2.19	106.38
	12.627	12.725 ± 0.12	0.98	100.78
FT[d]	0.047	0.051 ± 0.002	3.82	108.16
	12.627	12.733 ± 0.093	0.73	100.84
Reinjection[e]	0.047	0.050 ± 0.003	5.37	106.38
	12.627	13.128 ± 0.10	0.74	103.97
Long–term[f]	0.047	0.050 ± 0.002	4.38	106.38
	12.627	12.720 ± 0.08	0.64	100.73

[a] after 38 h in autosampler at 10°C; [b] after 40 h at 2–8°C; [c] after 9 h at room temperature; [d] after 3 freeze and thaw cycles; [e] after 35 h of Reinjection; [f] at –70°C for 50 days
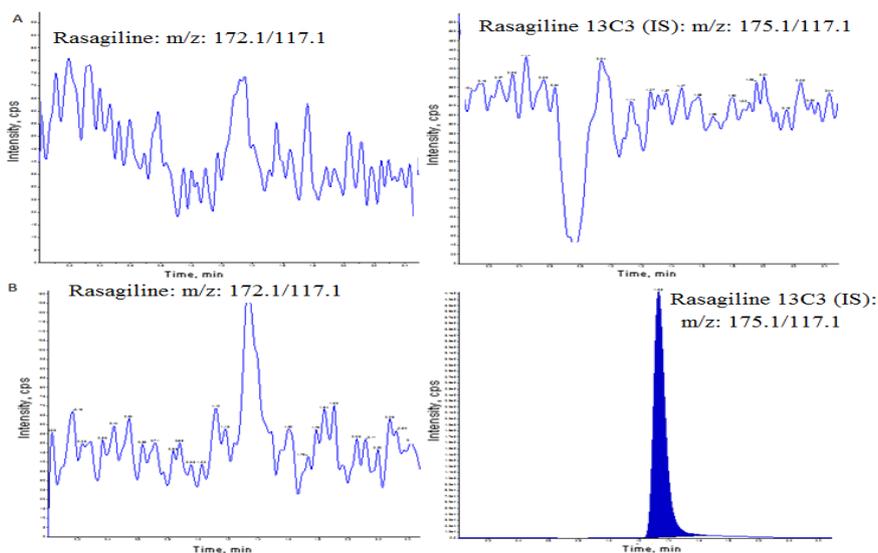

Figure 1. Typical MRM chromatograms of rasagiline (left panel) and IS (right panel) in human blank plasma (A) and human plasma spiked with IS (B).
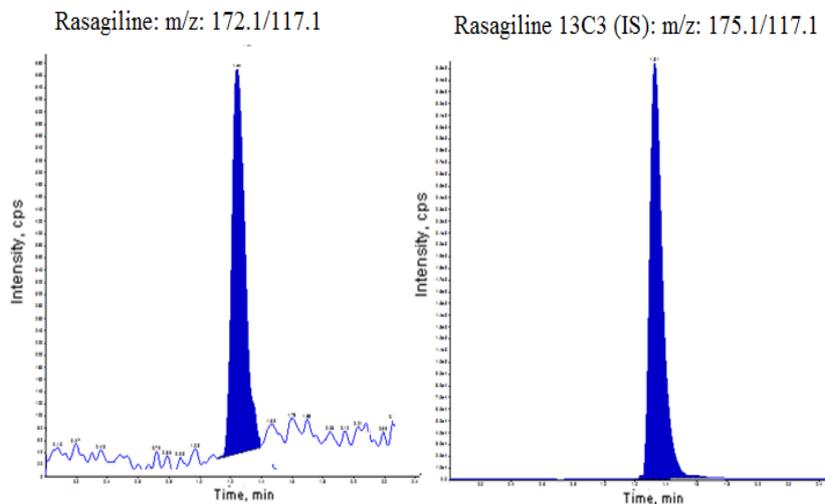

Figure 2. Typical MRM chromatograms of a LLOQ sample along with IS.

CONCLUSIONS

This paper defines a simple and rapid LC–MS/MS method for the quantification of rasagiline in human plasma. The method is owing advantages like use of stable labeled isotopes as internal standard, simple and efficient SPE procedure, use of low plasma volume (100µL) and rapid run time of 2.5min. The method was fully validated and the results are well within the acceptance limits as per the US FDA guidelines. From the validation results, we can conclude that the developed method can be useful for bioavailability and bioequivalence (BA/BE) studies and routine drug analysis of rasagiline.

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