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Article

LC Method for Determination of Rasagiline Mesylate in Different Plasma Matrices and its Application to Oral Pharmacokinetic Study in Rabbits

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A simple, rapid and sensitive reverse-phase liquid chromatographic method is developed and validated for estimation of rasagiline mesylate in different plasma matrices (rat, rabbit and human plasma). The method employs an isocratic elution technique with a Kromasil C18 column and has an optimized mobile phase composition of 10 mM ammonium acetate buffer–acetonitrile (40:60 v/v). The plasma samples displayed linear detector responses in the concentration range of 0.5–20 μg/mL in all plasma matrices when monitored at 265 nm using an ultraviolet detector. Because the simple protein precipitation method yields over 95% recovery of rasagiline mesylate from all plasma matrices, no internal standard was used in this method. A detailed validation study of the method proves the accuracy, precision and selectivity (in estimating rasagiline mesylate) in all plasma matrices. The drug is also stable under various processing and storage conditions in all plasma matrices, as evident from the study. The present method was applied to determine the drug-protein binding ratio in all the plasma matrices. The use of this method in determining pharmacokinetic parameters of rasagiline mesylate by non-compartmental analysis after oral dosing in rabbits is also discussed.

Introduction

Rasagiline mesylate (RM) is chemically designated as *N*-Propargyl-1(R)-aminoindan mesylate. Its structural formula is $(C_{12}H_{13}N)\bullet CH_4SO_3$ and its molecular weight is 267.34. RM is currently approved as initial monotherapy or adjunct therapy to levodopa for treatment of signs and symptoms of idiopathic Parkinson's disease in the United States and Europe (1, 2).

The oral dose of RM is 1 mg per day. RM is rapidly absorbed from the gastrointestinal tract (GIT) and has an absolute oral bioavailability of approximately 36% in humans. It undergoes extensive hepatic first pass metabolism. RM is primarily biotransformed by *N*-dealkylation and hydroxylation. Glucuronic acid and sulfate conjugation are also reported to be involved in the metabolism of RM. After oral administration, it reaches peak plasma concentration in an hour. The volume of distribution of RM in humans is 87 L. The elimination half-life of RM is approximately 0.6–2 h in humans. RM is reported to follow linear pharmacokinetics in the dose range of 1–10 mg when administered through the oral route (3).

High-performance liquid chromatography (HPLC) and ultraviolet (UV)-visible spectroscopic methods have been reported for the estimation of RM in bulk and in formulation samples. Two stability-indicating reverse-phase liquid chromatographic methods have been reported for the determination of RM in pharmaceutical dosage forms with a linearity range of 4.8–150.5 μg/mL and 10–60 μg/mL (4, 5). However, limit of detection (LOD) and limit of quantification (LOQ) values were not reported for either of these methods.

A simple UV-spectrophotometric method for the determination of RM has been reported in the literature (6) with a linearity range of 5–150 μg/mL and LOQ of 5 μg/mL. Application of this method in the analysis of RM from tablet formulation was also shown.

Another UV-spectroscopic method involving the derivatization of RM into a colored compound for quantification of RM in bulk samples has also been reported (7). An HPLC method for the estimation of RM in a novel biodegradable polylactic-*co*-glycolic acid-based microsphere formulation was reported in which the LOD and LOQ values were found to be 0.07 and 0.23 μg/mL, respectively (8). Bioanalytical methods based on liquid chromatography–mass spectrometry (LC–MS) and LC–MS-MS have been reported for the quantification of RM either alone (9, 10) or in combination with other drugs (11).

Although several methods are available for the determination and quantification of RM in bulk samples and formulation, fewer methods are available for the estimation of RM in biological samples, and none are based on liquid chromatography with UV detection.

Novel formulation approaches like buccal patches (12), nasal mucoadhesive *in situ* gelling systems and oral nanocarrier systems are being developed to improve the bioavailability of RM. The pharmacokinetic evaluation of such delivery systems in suitable animal models necessitates the development of a simple, rapid and sensitive method for the estimation of RM in relevant plasma matrices.

Wistar rats and New Zealand white rabbits are frequently used in pharmacokinetic evaluation of novel delivery systems of a drug to study the efficacy of the delivery system at a preclinical level (13, 14). Moreover, Wistar rats and New Zealand white rabbits are also well-known and widely used animal models to study Parkinson's disease (15, 16). Hence, the development and validation of a simple reverse-phase HPLC method for the analysis of RM in rat and rabbit plasma matrices is possible. Such a method can have practical applications in preclinical, pharmacokinetic and toxicokinetic studies of RM in rat and rabbit. The applicability of the proposed method for the estimation of RM in human plasma was also confirmed by validating the method in the human plasma matrix. The proposed method can be used in the quantification of RM in human plasma samples obtained in multi-dose pharmacokinetic studies of conventional or novel drug delivery systems.

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In this study, a reverse-phase HPLC method using UV detection was developed and validated for the estimation of RM in rat, rabbit and human plasma matrices. The method was validated according to standard guidelines (17, 18) and suitable statistical tests were performed to test the significance of the results. The developed method was used in determining plasma protein binding of RM in all chosen plasma matrices. The method was also applied for studying the pharmacokinetic parameters after oral dosing of RM in rabbits.

Experimental

Chemicals and reagents

RM was supplied by Apotex Research (Bangalore, India). HPLC-grade ammonium acetate, glacial acetic acid and sodium citrate were procured from Merck (Mumbai, India). HPLC-grade acetonitrile and methanol were procured from Sigma Aldrich (Mumbai, India). A water purification system (Millipore; Milford, MA) was used to obtain high quality water.

Ultrafiltration was carried out using Microcon centrifugal filters (Amicon Ultra-0.5, NMWL 10 KDa, Millipore). Blood collection tubes (BD Microtainer; Franklin Lakes, NJ) pre-coated with K2EDTA were used for collecting blood from human volunteers.

Instruments

A Shimadzu liquid chromatographic system with binary pumps (Model LC-20AD, Prominence Liquid Chromatograph, Shimadzu, Kyoto, Japan), auto injector (Model SIL-20A HT, Prominence Auto Sampler) and photo diode array (PDA) UV detector (Model SPD-M20A, Prominence UV Detector) was used. LC Solutions software (version 1.25) was used for data collection and integration.

A vortex mixer (Model VX-200, Labnet International; Edison, NJ), sonicator (Model SONICA 2200 MH, Soltec, Italy), refrigerated centrifuge (Model C-24 BL, Remi, India) and deep freezer (Model BFS-345-S, Celfrost Innovations; India) were used for preparation and processing of samples in method development and validation. Membrane filters of 0.22 µm (Millipore) were used for filtration of the aqueous phase of the mobile phase system.

Chromatographic conditions

An endcapped C18 column (Kromasil C18, 250 mm long and 4.6 mm internal diameter, particle size 5 µm; Brewster, NY) equipped with a guard column of the same packing material was used in the study. The mobile phase consisted of 10 mM ammonium acetate buffer and acetonitrile in the ratio of 40:60. The buffer was filtered through a 0.22-µm filtration membrane. The HPLC was stabilized for 1 h at 1 mL/min flow rate through baseline monitoring before the actual analysis. RM was monitored at a wavelength of 265 nm. An injection volume of 20 µL and run time of 10 min was optimized for the final method.

Collection of blood and separation of plasma

Prior permission was obtained from the Institutional Animal Ethics Committee for all experiments involving animals (Approval No.: IAEC-02/01-11). Informed consent was obtained from all human subjects after explaining the aims and risks of the study before collection of blood samples.

Blood (0.3 mL) was collected from retro-orbital plexus of Wistar rats using a rat bleeding capillary. Marginal ear vein puncture was performed to collect blood from New Zealand white rabbits. In the case of rats and rabbits, blood was collected into microfuge tubes containing sodium citrate solution (3.8% w/v). Blood (0.5 mL) from human volunteers was collected by venous puncture directly into K2EDTA pre-coated blood collection tubes. Plasma samples in all the cases were obtained by centrifuging the blood samples in a cooling centrifuge at 3,400 rpm for 10 min at 4°C. The supernatant clear plasma was carefully collected and frozen at −20°C until further use.

Calibration curve and quality control standards

Primary stock of RM (1 mg/mL) was prepared by dissolving 10 mg of RM in 10 mL of deionized, high purity water. Secondary stock solutions of RM, analytical standards for studying the absolute recovery from plasma standards (0.5, 1, 2, 4, 6, 8, 10 and 20 µg/mL) and analytical quality control samples for studying the absolute recovery of plasma quality control samples (1.5, 7 and 17 µg/mL) were prepared by making appropriate dilutions in deionized high purity water.

Plasma calibration standard samples were prepared by spiking 10 µL of appropriate standard solutions of RM in 90 µL of drug-free rat, rabbit and human plasma to obtain final concentrations of 0.5, 1, 2, 4, 6, 8, 10 and 20 µg/mL for the calibration curve; the same procedure was followed for plasma quality control (QC) samples, which were considered as lower quality control (LQC = 1.5 µg/mL), medium quality control (MQC = 7 µg/mL) and higher quality control (HQC = 17 µg/mL) samples. Blank plasma sample was prepared by spiking 10 µL of deionized high purity water into 90 µL of drug-free plasma.

Extraction technique

The protein precipitation method was followed to extract RM from all plasma matrices. One hundred microliters of drug-spiked plasma samples were pipetted into an RIA vial and 150 µL of acetonitrile (protein precipitating agent) was added, followed by vortex-mixing for 2 min. This was followed by centrifugation of samples at 10,000 rpm for 20 min at 4°C. From the centrifuged samples, supernatant was transferred to a sample loading vial and injected into the HPLC system.

Method development

In the process of HPLC method development for RM, mobile phase composition and flow rate were optimized by experimenting with different aqueous phase and non-aqueous phase combinations at different flow rates. Buffers with different strengths and in varying compositions with acetonitrile and/or methanol were investigated.

The mobile phase composition and flow rate were finally selected based on the criteria of peak properties (retention time, tailing factor, plate height, number of plates and peak purity index), sensitivity (height and area), ease of preparation and applicability to analyze RM in chosen plasma matrices.

Method validation

The developed method was validated according to standard guidelines and suitable statistical tests were performed to test the significance of the results. Selectivity was established in all the plasma matrices by comparing six different lots of drug-free plasma samples and RM-spiked plasma samples. Calibration curves were constructed from blank sample and eight non-zero samples ranging from 0.5 to 20 µg/mL. The linearity of the method was assessed by plotting peak area against the nominal concentrations of RM. Calibration curves were fitted using unweighted linear regression analysis. Precision and accuracy were determined across the three QC samples in all the matrices. Intra-day precision and accuracy were assessed by replicate analysis ($n = 3$), twice in a day at each of the QC levels. Inter-day precision and accuracy were determined by replicate analysis of the same QC samples on three different days ($n = 18$). The percent relative standard deviation (%RSD) was calculated from the predicted concentration obtained by regression equation.

Sensitivity was assessed by determination of lowest limit of quantification (LLOQ), the minimum quantifiable concentration with %RSD less than 20. The LOD and LOQ values were also determined using the standard deviation of the response and the slope of the calibration curve. Recovery of the drug was determined at all concentration levels (including three QC levels) in triplicate by comparing the peak area obtained from plasma (extracted) samples with analytical standard (unextracted) samples at the same nominal concentration.

Stability of RM in all plasma matrices was evaluated under three different stress conditions: three freeze and thaw cycles, long-term storage (up to 28 days at −20°C) and post-extraction storage (in auto-injector up to 24 h). Stability was determined by triplicate analysis of LQC and HQC samples in each of the previously described conditions. The percentage deviation from the mean concentrations observed at zero time was calculated. The stability of RM stock solution was also established by storing it at room temperature for a period of 24 h and comparing the response for a 17 µg/mL solution prepared from this stock solution (after 24 h storage period) against the same concentration prepared using a fresh stock solution.

Dilution integrity (DI) studies were performed to prove the application of the proposed method in quantifying samples that lie well above the linearity range. For this study, three series of DI standards of RM were prepared to yield concentrations of 100, 200 and 400 µg/mL separately in all plasma matrices. These concentrations were 5, 10 and 20 times higher than the last point of the linearity range (20 µg/mL). The DI standards were then diluted 5, 10 and 20 times, respectively, with their respective blank plasma, vortex-mixed for 5 min and then analyzed after being processed in the same way as described previously.

Plasma protein binding studies

Plasma protein binding (%) of RM in all matrices was determined by an ultracentrifugation technique using centrifugal filters. Rat, rabbit and human plasma samples spiked with RM at two different concentrations of 17 and 34 µg/mL were incubated for 4 h at 37°C (19). After the incubation period, 500-µL aliquots of plasma samples were transferred to a sample reservoir of centrifugal filters and centrifuged at 4,500 rpm for 10 min at 4°C. The filtrate obtained in the lower vial was analyzed by the developed method. Plasma protein binding (%) of RM was determined using the following equation:

$$\text{Plasma protein binding (\%)} = [(C_t - C_u)/C_t] \times 100$$

where C_t is the total drug concentration in plasma sample and C_u is the concentration of drug in filtrate collected after ultracentrifugation.

Pharmacokinetic application in rabbit oral dosing study

RM formulation for oral administration was prepared by dissolving the drug in deionized high-purity water just before the commencement of the study. Formulation was administered through oral route as a single oral dose in female New Zealand white rabbits ($n = 3$), weighing 2.2 to 2.5 kg, at a dose of 10 mg/kg. Blood samples were drawn from marginal ear veins at 5, 15, 30, 45, 60, 120, 180 and 240 min post-dosing in microfuge tubes pretreated with sodium citrate solution (3.8% w/v). A baseline blank plasma sample was drawn from each animal before drug administration. All samples were processed according to the procedure described earlier and analyzed using the validated HPLC method.

Various pharmacokinetic parameters were calculated from measured RM plasma concentrations versus time profiles after oral administration using the non-compartmental model in Phoenix WinNonlin software (Version 6.0, Pharsight Corporation; Cary, NC).

Results and Discussion

Method development

A mobile phase consisting of aqueous phase (10 mM ammonium acetate) and acetonitrile in the ratio of 40:60 at a flow rate of 1 mL/min was selected as the optimal condition for the developed method. With optimized mobile phase condition, the retention time of RM was found to be 5.72 ± 0.17 min with a maximum tailing factor of 1.09 ± 0.03 across all the plasma matrices. Chromatographic peak parameters obtained for the method across all plasma matrices and across three QC levels are presented in Table I.

Method validation

Selectivity

A simple and efficient one-step precipitation technique was found to be suitable for the estimation of RM in all the plasma matrices. No interference was observed from endogenous protein impurities at the retention time of RM, which is evident from overlaid chromatograms of the blank plasma

sample and plasma calibration standard of each plasma matrix, as shown in Figure 1. Thus, the proposed method was found to be specific and selective for the estimation of RM in all three plasma matrices.

Linearity

The calibration curve was linear in the selected concentration range of 0.5 to 20 μg/mL in all plasma matrices. In the three plasma matrices at all concentration levels, %RSD values did not exceed 5.0. From the unweighted linear regression analysis, slope, intercept and standard error of estimate were found and the results are summarized in Table II. Low values of standard error of the estimate indicate the high precision of the developed method in all plasma matrices. In all cases, the F_{cal} values were found to be lower than the F_{crit} values at $P < 0.05$, further indicating the precision of the method.

Accuracy

Across all plasma matrices, QC samples (LQC, MQC and HQC) showed an accuracy ranging from −1.32 to 2.84% with maximum %RSD of 3.35, establishing the accuracy of method for RM estimation (Table III).

Table I
Chromatographic Peak Parameters for Rasagiline Mesylate in Various Plasma Matrices*

Plasma matrix	QC sample level	Retention time (t_R) (min) (mean ± SD)[†]	Tailing factor (mean ± SD)[†]	Plate height (H) × $10^{-2‡}$ (mm)	Number of plates (N)[‡]	Peak purity index[‡]
Rat	LQC	5.71 ± 0.18	0.98 ± 0.02	2.61	9,562.38	0.9999
	MQC	5.64 ± 0.14	1.01 ± 0.06	2.60	9,606.66	0.9998
	HQC	5.78 ± 0.13	1.11 ± 0.08	2.62	9,513.05	0.9999
Rabbit	LQC	5.76 ± 0.11	1.00 ± 0.04	2.60	9,613.46	0.9997
	MQC	5.77 ± 0.13	1.05 ± 0.03	2.63	9,481.02	0.9999
	HQC	5.64 ± 0.19	1.11 ± 0.01	2.60	9,611.72	0.9999
Human	LQC	5.78 ± 0.21	1.03 ± 0.02	2.56	9,747.72	0.9998
	MQC	5.72 ± 0.24	1.09 ± 0.02	2.58	9,679.07	0.9999
	HQC	5.69 ± 0.19	1.07 ± 0.03	2.60	9,599.30	0.9998

*Note: in all cases, LQC = 1.5 μg/mL, MQC = 7 μg/mL and HQC = 17 μg/mL.
[†]Values of six independent determinations ($n = 6$).
[‡]Mean value of six independent determinations ($n = 6$).

Precision

In the repeatability study, the %RSD ranged from 1.14 to 4.11 across all QC samples in all plasma matrices (Table IV). The %RSD value for intra-day variation was not more than 4.11, and that for inter-day variation was less than 4.86 across all plasma matrices (Table IV). Acceptable %RSD values indicate the repeatability and intermediate precision of the method in all selected plasma matrices.

Sensitivity

The mean percentage accuracy of six independent samples of 0.5 μg/mL, calculated against the linear regression equation, was found to be 2.02 (%RSD = 4.45) for rat plasma, 1.34 (%RSD = 3.23) for rabbit plasma and 1.67 (%RSD = 4.35) for human plasma. The LOD and LOQ values are given in Table II. In all plasma matrices, the LOQ was found to be below 0.5 μg/mL; therefore, 0.5 μg/mL was considered to be the LLOQ for the proposed method. The reported LC–MS and LC–MS-MS methods for the determination of RM in biological matrices are expensive and involve many processing steps, although though their sensitivity is higher than the proposed method. Moreover, the reported methods require at least 0.5 mL of plasma for processing in the analysis of RM (9–10). Hence, these methods are less suitable in preclinical studies, in which the volume of blood that can be collected from rats or rabbits at each time point is limited. Because the proposed method is validated in different plasma matrices with plasma volume of 0.1 mL, it can easily be used in preclinical and clinical pharmacokinetic studies involving novel/controlled release formulations of RM in which the number of sampling points and the duration of the study are higher.

Recovery

The absolute recovery of RM from the drug-spiked plasma in various matrices across QC samples when compared with aqueous equivalent samples was within 96.09 to 102.72%, with %RSD less than 2.86 at each of the concentration levels. A high mean percent recovery value forestalls the use of an internal standard in the method, and with low %RSD values, the extraction efficiency of the solvent selected for precipitation is also established. The data are presented in Table III.

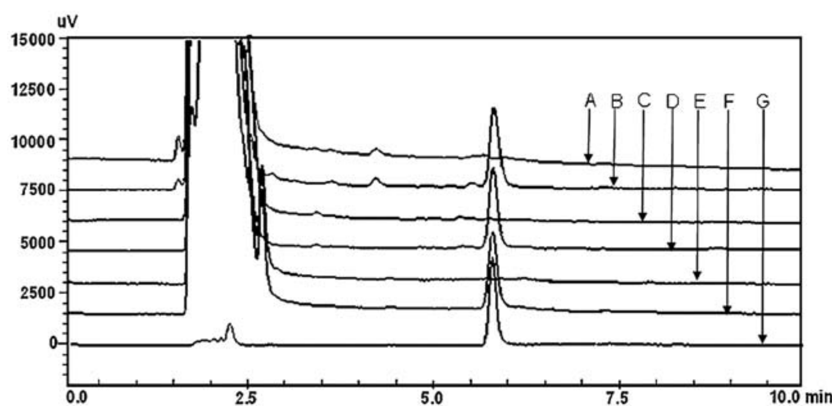

Figure 1. Drug-free blank rat plasma (A); overlaid chromatogram of rasagiline mesylate HQC (17 μg/mL) spiked in rat plasma (B); drug-free blank rabbit plasma (C); RM spiked in rabbit plasma (D); drug-free blank human plasma (E); RM spiked in human plasma (F); aqueous equivalent of HQC sample (G).

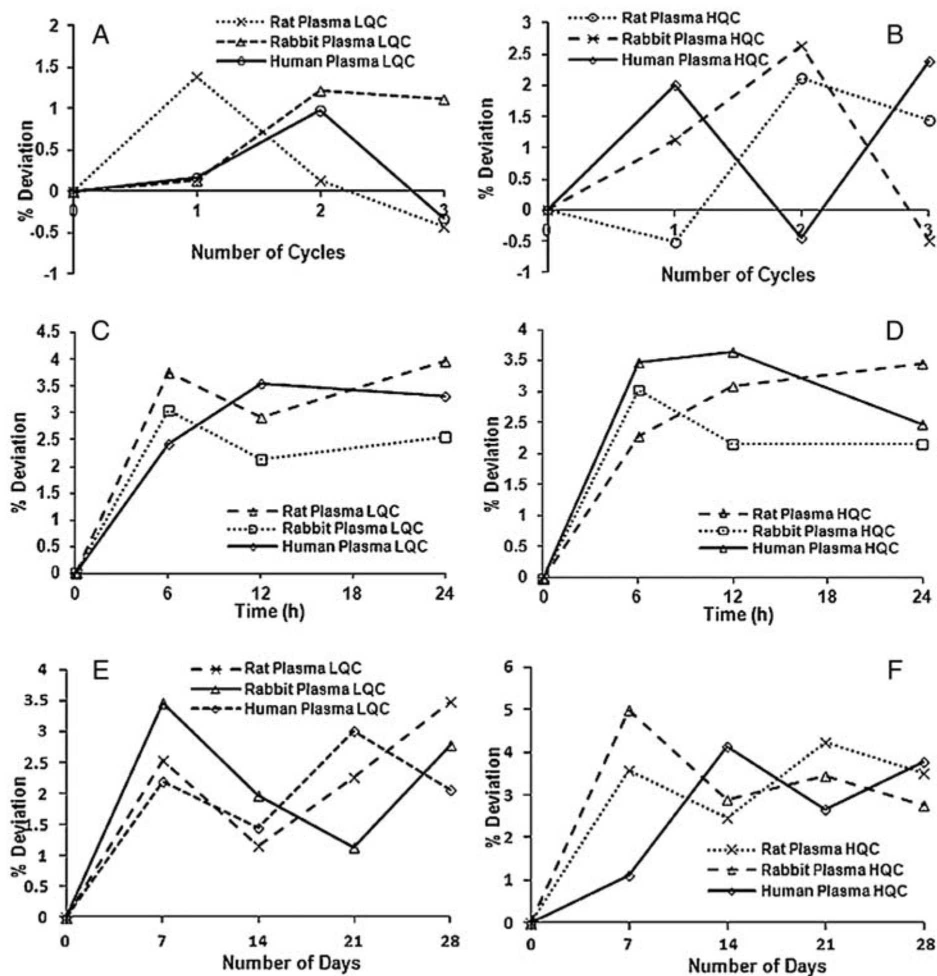

Figure 2. Stability study of rasagiline mesylate LQC = 1.5 μg/mL and HQC = 17 μg/mL in plasma samples: freeze thaw stability (A, B); post-preparative stability (C, D); long-term stability (E, F). Each point represents the mean of three independent determinations.

Table II
Data from Linear Regression Analysis of Calibration Curves, LOD, LOQ and LLOQ for Rasagiline Mesylate in Various Plasma Matrices*

Plasma matrix	Slope (mean ± SEM) ($n = 6$)	Intercept (mean ± SEM) ($n = 6$)	r^2	SEE	$F_{cal} \times 10^{-3}$†	LOD and LOQ‡ (μg/mL)	Selected LLOQ (μg/mL)
Rat	1,917.76 ± 14.78	137.17 ± 34.87	0.994	0.017	3.1	0.147 and 0.445	0.5
Rabbit	2,006.06 ± 12.51	151.81 ± 33.51	0.997	0.021	1.6	0.156 and 0.472	
Human	1,967.83 ± 13.64	147.22 ± 36.74	0.993	0.019	2.5	0.151 and 0.457	

*Note: standard error of mean (SEM), regression coefficient (r^2), standard error of estimate (SEE).
†At $P < 0.05$, F_{crit} (5.42) = 2.44. The F_{cal} value is significantly less than F_{crit}.
‡LOD = 3.3 × (standard deviation of intercepts/mean value of slopes); LOQ = 10 × (standard deviation of intercepts/mean value of slopes).

Stability

The stability of RM in all plasma matrices was evaluated using QC samples (LQC and HQC) under different stress conditions and the results are shown in Figure 2. In freeze thaw stability, no significant degradation of RM was observed up to three cycles over a period of three days in all matrices. Deviation from the zero time concentration was found to be less than 2.7% at the end of three freeze thaw cycles, as shown in Figures 2A and B. In the postpreparative stability study of the processed samples, RM was found to be stable for 24 h, with a maximum deviation of less than 4.0% from the zero time concentration, as shown in Figures 2C and D.

In long-term stability studies, RM was found to be stable for 28 days when stored at −20°C. The deviation in recoveries of RM after analysis at 7, 14, 21 and 28 days of sample preparation was found to be less than 5.0% (Figures 2E and F). The results of this study indicate that storage temperature of −20°C was adequate for storing the samples for at least 28 days.

Dilution integrity

The three series of DI standards showed an accuracy ranging from −0.42 to 2.16% with maximum %RSD of 4.03, indicating the acceptability of the method in analyzing samples up to 20

Table III
Accuracy, Precision and Absolute Recovery Data of Proposed Method for Rasagiline Mesylate in Various Plasma Matrices*

Plasma matrix	QC level	Predicted concentration† (μg/mL)			Mean accuracy‡ (%)	Recovery§	
		Obtained range (μg/mL)	Mean ± SD‡	%RSD		Mean ± SD (%)	%RSD
Rat	LQC	1.44–1.54	1.49 ± 0.05	3.35	2.11	98.89 ± 2.67	2.69
	MQC	6.19–7.13	6.93 ± 0.21	3.03	−1.08	101.14 ± 1.58	1.56
	HQC	16.87–17.40	17.11 ± 0.29	1.69	1.49	99.75 ± 2.86	2.86
Rabbit	LQC	1.48–1.55	1.51 ± 0.04	2.64	2.84	98.65 ± 1.43	1.45
	MQC	6.86–7.17	7.01 ± 0.16	2.28	0.79	99.69 ± 2.17	2.17
	HQC	16.87–17.36	17.18 ± 0.14	1.56	−1.11	99.47 ± 1.61	1.62
Human	LQC	1.46–1.53	1.50 ± 0.04	2.67	−1.32	98.23 ± 2.14	2.18
	MQC	6.81–7.15	6.98 ± 0.19	2.72	1.73	99.68 ± 2.05	2.65
	HQC	16.80–17.10	16.95 ± 0.25	1.47	2.59	100.12 ± 1.51	1.51

*Note: in all cases, LQC = 1.5 μg/mL, MQC = 7 μg/mL and HQC = 17 μg/mL.
†Predicted concentration of RM was calculated by linear regression equation.
‡Values of six independent determinations ($n = 6$).
‡Accuracy is given in relative error % = [100 × (predicted concentration − nominal concentration)/nominal concentration]. Mean of six independent determinations ($n = 6$).
§Percent recovery = [(Peak area of plasma standard/peak area of analytical standard of same concentration) ×100].

Table IV
Results of Intermediate Precision Study in Various Plasma Matrices*

Plasma matrix	QC level	Intra-day repeatability (%RSD) ($n = 3$)			Inter-day repeatability (%RSD) ($n = 18$)
		Day 1	Day 2	Day 3	
Rat	LQC	4.11	3.14	2.96	3.38
		3.75	2.12	3.84	
	MQC	1.48	2.51	1.65	3.12
		1.14	2.26	2.12	
	HQC	3.14	3.01	3.56	3.04
		3.09	3.18	3.36	
Rabbit	LQC	3.41	3.36	3.47	4.86
		2.33	3.15	3.86	
	MQC	2.44	3.15	3.64	3.30
		3.89	3.65	3.44	
	MQC	3.70	3.65	2.29	2.89
		3.85	3.97	3.12	
Human	LQC	3.69	3.15	3.46	3.32
		3.24	3.36	3.28	
	MQC	3.46	2.39	3.44	2.89
		3.26	3.14	3.96	
	HQC	3.26	3.34	3.89	3.14
		3.14	3.12	3.78	

*Note: in all cases, LQC = 1.5 μg/mL, MQC = 7 μg/mL and HQC = 17 μg/mL. Intra-day repeatability was assessed by replicate analysis ($n = 3$) twice a day at each QC level.

Table V
Plasma Protein Binding of Rasagiline Mesylate in Rat, Rabbit and Human

Plasma	Concentration of RM used (μg/mL)	Plasma protein binding (%) ($n = 3$)	(Mean ± SD)* (%)
Rat	17	81.71 ± 2.15	82.13 ± 1.78
	34	82.56 ± 1.42	
Rabbit	17	96.48 ± 3.04	95.67 ± 2.73
	34	94.86 ± 2.42	
Human	17	81.15 ± 2.16	81.07 ± 2.74
	34	80.99 ± 3.32	

*Mean and SD values obtained from two levels of RM spiked into corresponding plasma matrix ($n = 6$).

times higher than that captured in the linearity range in all plasma matrices.

Plasma protein binding studies

The developed and validated HPLC method was applied to determine the plasma protein binding of RM in all plasma matrices. The plasma protein binding (%) was found to be 82.13 ± 1.78 in Wistar rats, 95.67 ± 2.73 in New Zealand white rabbits and 81.07 ± 2.74 in humans (Table V). No significant difference was observed in plasma protein binding of RM in rat and human ($t_{cal} = 0.22$, $t_{crit} = 2.23$, $\alpha = 0.05$ and $d_f = 10$). Statistically significant difference was observed in plasma protein binding of RM in rabbit and human ($t_{cal} = 12.93$, $t_{crit} = 2.23$, $\alpha = 0.05$ and $df = 10$) and rabbit and rat ($t_{cal} = 14.09$, $t_{crit} = 2.23$, $\alpha = 0.05$ and $d_f = 10$). The obtained values are in agreement with the reported values for *in vitro* plasma protein binding of RM in rat plasma (75.2–81.3%) and in human plasma (88–94%) (1).

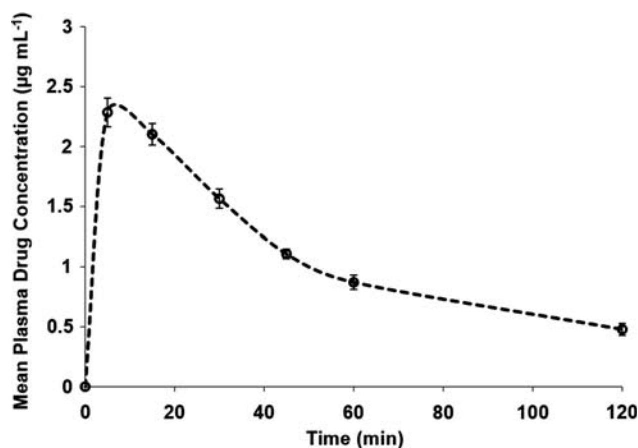

Figure 3. Mean plasma concentration versus time profile of rasagiline mesylate in rabbits after a single oral dose of the drug (10 mg/kg, $n = 3$).

Pharmacokinetic application in rabbit oral dosing study

The developed and validated HPLC method for RM estimation was applied to a pharmacokinetic study of RM administered as single oral dose in rabbits. The mean plasma concentration versus time profile of RM following oral administration is given in Figure 3.

The pharmacokinetic parameters obtained from the study using non-compartmental analysis were: area under the curve

(AUC) = 2.91 ± 0.08 h μg/mL, Lambda_z = 0.65 ± 0.02 h, mean retention time—oral (MRT_{oral}) = 1.43 ± 0.03 h, total systemic clearance (Cl_s) expressed as Cl_s/F = 143.18 ± 5.89 mL/min and volume of distribution (V_{ss}) expressed as V_{ss}/F = 13.22 ± 0.11 L.

Conclusions

A rapid, sensitive and selective reverse-phase HPLC method is described for the qualitative and quantitative analysis of RM in rat, rabbit and human plasma matrices. The developed method is accurate and precise, with near full recovery of the drug from all plasma matrices. An oral pharmacokinetic study in rabbits to determine pharmacokinetic parameters proves the applicability of this method to real-time sample analysis. Because this method is validated in different plasma matrices, it can be used in pre-clinical and clinical pharmacokinetic studies of novel formulations of RM.

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