Journal of Chromatographic Science 2013;51:242–249
doi:10.1093/chromsci/bms134 Advance Access publication September 17, 2012

Article

Development and Validation of a Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Process Related Impurities and Degradation Products of Rasagiline Mesylate in Pharmaceutical Formulation

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Received 24 January 2012; revised 13 June 2012

A sensitive, stability-indicating gradient reverse phase high-performance liquid chromatography–ultraviolet method has been developed for the quantitative determination of process-related impurities and forced degradation products of rasagiline mesylate in pharmaceutical formulation. Efficient chromatographic separation was achieved on an ACE C8, 150 × 4.6 mm, 3 μm column with mobile phase containing a gradient mixture of solvents A and B. The flow rate of the mobile phase was 0.8 mL/min with column temperature of 30°C and detection wavelength at 210 nm. Rasagiline was subjected to the stress conditions of oxidative, acid, base, hydrolytic, thermal and photolytic degradation. Rasagiline was found to degrade significantly in acid and thermal stress conditions. The degradation products were well resolved from rasagiline and its impurities. The peak purity test results confirmed that the rasagiline peak was homogenous and pure in all stress samples and the mass balance was found to be more than 97%, thus proving the stability-indicating power of the method. The developed method was validated according to the guidelines of the International Conference on Harmonization with respect to specificity, linearity, limits of detection and quantification, accuracy, precision and robustness.

Introduction

Rasagiline is a highly potent, selective, irreversible, second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAO-B) and has been used for the treatment of idiopathic Parkinson's disease (PD). Its chemical designation is 1H-inden-1-amine,2,3-dihydro-N-2-propynyl-(1R)-methane sulfonate (Figure 1). The recommended dosage for initial monotherapy is 1 mg once daily. When rasagiline is used as adjunctive therapy with levodopa, the recommended initial dose is 0.5 mg/day and may be increased to 1 mg/day if the desired clinical effect is not achieved (1–3).

A literature survey revealed few liquid chromatography (LC) assay methods that have been reported for the determination of rasagiline in bulk dug and pharmaceutical preparation (4, 5). An assay method by high-performance liquid chromatography (HPLC) (6) describes the separation of degradation impurities from rasagiline formed during forced degradation studies, but it was out of scope because it did not separate and determine the impurities. The estimation of rasagiline in human plasma by LC–tandem mass spectrometry (MS-MS) has been performed (7, 8). However, an extensive literature survey revealed that no stability-indicating method has been reported, including major pharmacopoeias such as United States Pharmacopeia (USP), European Pharmacopeia (EP) or British Pharmacopeia (BP), for the quantitative determination of impurities and degradation products of rasagiline in pharmaceutical formulation. Therefore, it was necessary to develop an accurate, specific and stability-indicating LC method for the determination of impurities and degradation products of rasagiline in pharmaceutical formulation. The present International Conference on Harmonization (ICH) drug stability guidelines (9, 10) suggest that stress studies should be conducted on the drug product to establish its inherent stability characteristics, and the analytical method should able to separate all degradation impurities formed under stress studies to prove its stability-indicating power.

The present work concerns the method development, method validation and forced degradation studies of rasagiline. The developed LC method was validated with respect to specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy and robustness. Forced degradation studies were performed on the placebo and drug products to show the stability-indicating nature of the method. These studies were performed in accordance with established ICH guidelines.

Experimental

Chemicals and reagents

Tablets of rasagiline and its impurities, namely keto impurity and allyl impurity (Figure 1), were supplied by Dr. Reddy's Laboratories (Hyderabad, India). Amino indane, 1-indanone and 1-indanol (Figure 1) were procured from Sigma-Aldrich (Germany). HPLC-grade acetonitrile, methanol and tetrahydofuran were purchased from Rankem (New Delhi, India). Analytical grade potassium di-hydrogen ortho-phosphate, hydrochloric acid, formic acid and ortho-phosphoric acid were purchased from Merck (Mumbai, India). High-purity water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA).

Chromatographic conditions

Samples were analyzed on a Waters Alliance 2695 separation module (Waters Corporation, Milford, MA) equipped with a 2489 ultraviolet (UV)-visible detector and 2998 photodiode

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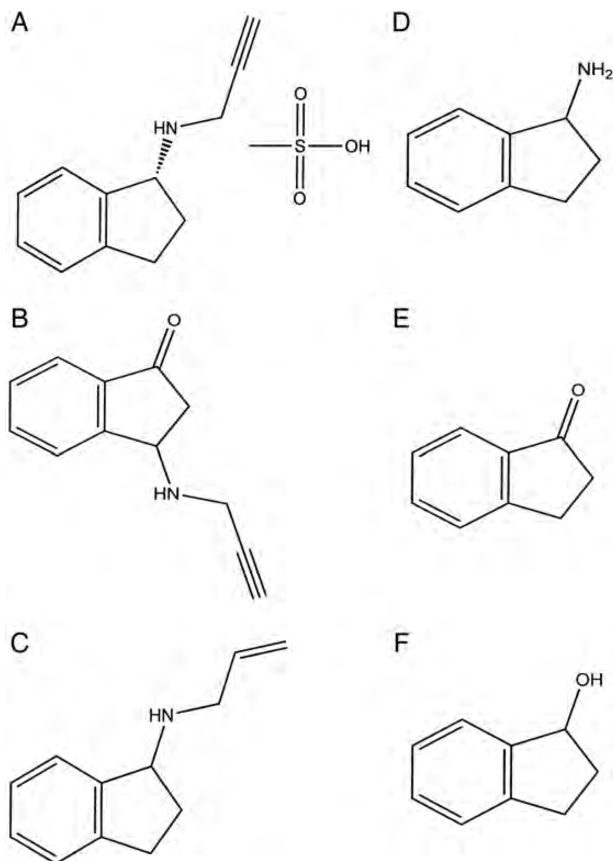

Figure 1. Structures of (A) Rasagiline mesylate, (B) Keto impurity, (C) Allyl impurity, (D) Amino indane, (E) 1-Indanone and (F) 1-Indanol.

array (PDA) detector for specificity and forced degradation studies. The method was developed using an ACE C8 (150 × 4.6 mm, 3 μm) column with mobile phase containing a gradient mixture of solvent A (mixture of 0.01 M KH_2PO_4, pH 3.0 and methanol in the ratio of 95:5 v/v) and B (mixture of 0.01 M KH_2PO_4, pH 3.0, methanol and tetrahydrofuran in the ratio of 100:850:50 v/v/v). The gradient program [Time (min)/%B] was set as 0/0, 10/0, 20/3, 25/5, 50/30, 55/30, 71/50, 80/75, 81/0 and 90/0. The flow rate of the mobile phase was 0.8 mL/min. The column temperature was maintained at 30°C and the detection wavelength was 210 nm. The sample injection volume was 30 μL.

Liquid chromatography–mass spectrometry conditions

An LC–MS-MS system (Agilent 1100 series liquid chromatograph coupled with Applied Biosystem 4000 Q Trap triple quadrupole mass spectrophotometer with Analyst 1.4 software, MDS SCIEX, Foster City, CA) was used for confirmation of the atomic mass number of degradation products formed during forced degradation studies. An ACE C8, 150 × 4.6 mm, 5 μm column was used as the stationary phase. Solvent A was 0.05% formic acid; acetonitrile and methanol in the ratio of 20:80 v/v was used as solvent B at a flow rate of 1.0 mL/min. The gradient program [Time (min)/% solvent B] was set as 0/0, 7/0, 15/3, 20/5, 40/30, 45/30, 53/50, 57/75, 61/0 and 70/0. The analysis was performed in positive electrospray/positive ionization mode. The source voltage was 5,000 V and source temperature was 450°C. Gas 1 and gas 2 were optimized to 30 and 35 psi, respectively. The curtain gas flow was 20 psi.

Preparation of system suitability solution

Milli-Q water, pH adjusted to 3.0 with HCl and methanol in the ratio of 80:20 v/v, was used as diluent. A system suitability solution of 1-indanone (4 μg/mL), 1-indanol (4 μg/mL) and rasagiline mesylate (0.64 mg/mL) was prepared by dissolving an appropriate amount of the drug in the diluent.

Preparation of standard solution

Stock solution of rasagiline mesylate was prepared in diluent with a concentration of 0.25 mg/mL. Working standard solution was prepared from diluting 5 mL of the preceding stock solution to 200 mL with diluent to a final concentration of 6.25 μg/mL.

Preparation of sample solution

Tablet powder equivalent to 15.6 mg rasagiline mesylate was dissolved in 20 mL diluent with sonication for 20 min and diluted to 25 mL with diluent. This solution was filtered through a 0.45 μm nylon membrane filter.

Preparation of placebo solution

Approximately 1,985 mg of placebo powder was weighed and transferred to a 25 mL volumetric flask. Twenty milligrams of diluents were added, dissolved with sonication for 20 min and diluted to 25 mL with diluent. This solution was filtered through a 0.45 μm nylon membrane filter.

Forced degradation studies

Forced degradation studies were performed at a 625 μg/mL concentration of rasagiline mesylate in tablets to provide an indication of the stability-indicating property and specificity of the proposed method. A peak purity test was conducted for the rasagiline peak by using a PDA detector on stress samples. All solutions used in forced degradation studies were prepared by dissolving the drug product in a small volume of stressing agents. After degradation, these solutions were diluted with diluent to yield a stated rasagiline mesylate concentration of approximately 625 μg/mL. Conditions employed for performing the stress studies are described in the following (9–11).

Acid degradation

Tablet powder equivalent to 15.6 mg rasagiline mesylate was accurately weighed and dissolved in 5 mL of diluent, 5 mL 5 N HCl was added and the mixture was kept at 70°C for 10 min. The solution was brought to ambient temperature, neutralized by the addition of 5 mL 5 N NaOH and diluted to 25 mL with diluent.

To prepare the blank, 5 mL of 5 N HCl and 5 mL of 5 N NaOH were diluted to 25 mL with diluent.

Base degradation

Tablet powder equivalent to 15.6 mg rasagiline mesylate was accurately weighed and dissolved in 5 mL of diluent, 5 mL 5 N NaOH was added and the mixture was kept at 70°C for 5 min. The solution was brought to ambient temperature, neutralized by the addition of 5 mL 5 N HCl and diluted to 25 mL with diluent.

To prepare the blank, 5 mL of 5 N NaOH and 5 mL of 5 N HCl were diluted to 25 mL with diluent.

Hydrolytic degradation

Tablet powder equivalent to 15.6 mg rasagiline mesylate was accurately weighed and dissolved in 5 mL of diluent, 10 mL of water was added and the mixture was kept at 70°C for 10 min. The solution was brought to ambient temperature and diluted to 25 mL with diluent.

To prepare the blank, 10 mL of water was diluted to 25 mL with diluent.

Oxidation degradation

Tablet powder equivalent to 15.6 mg rasagiline mesylate was accurately weighed and dissolved in 5 mL of diluent, 5 mL of 3% hydrogen peroxide was added and the mixture was kept at 70°C for 10 min. The solution was brought to ambient temperature and diluted to 25 mL with diluent.

To prepare the blank, 3% hydrogen peroxide was diluted to 25 mL with diluent.

Thermal degradation

Tablet powder equivalent to 15.6 mg rasagiline mesylate was stored at 105°C for 9 h, dissolved and diluted to 25 mL with diluent.

Photolytic degradation

The susceptibility of the drug product to the light was studied (9). Tablet powder for photostability testing was placed in a photostability chamber and exposed to a white florescent lamp with an overall illumination of 1.2 million lux hours and near UV radiation with an overall illumination of 200 watt/m^2/h at 25°C. Following removal from the photostability chamber, the sample was prepared for analysis as previously described.

Stability sample analysis (accelerated and long-term conditions)

The drug product was placed in a stability chamber and exposed to accelerated conditions (40°C temperature and 75% relative humidity for a period of three months) and long-term conditions (25°C temperature and 60% relative humidity for a period of 24 months). Following removal from the stability chamber, samples were prepared for analysis as previously described.

Method Validation

The proposed method was validated according to ICH guidelines (11). The following validation characteristics were addressed: specificity, accuracy, precision, LOD, LOQ, linearity, range and robustness.

System suitability

System suitability was determined before sample analysis from duplicate injections of the standard solution containing 6.25 μg/mL of rasagiline mesylate. The acceptance criteria included area ratio between 0.9 and 1.1 for rasagiline peak, USP tailing factor less than 2.0 and USP plate count not less than 3,000 for rasagiline peak from the standard solution and system suitability solution, minimum resolution of 2.0 between 1-indanol and 1-indanone peaks.

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and excipients. Placebo interference was evaluated by analyzing the placebo prepared by the test method. No peak due to placebo was detected at the retention time of rasagiline and its impurities. The specificity of the developed LC method for rasagiline was conducted in the presence of its impurities and degradation products.

Precision

The precision of method was verified by repeatability and intermediate precision. Repeatability was checked by injecting six individual preparations of rasagiline mesylate tablets spiked with its five impurities at 1.0% level (1.0% of impurities with respect to 625 μg/mL rasagiline mesylate). The percent relative standard deviation (RSD) of the area for each impurity was calculated. The intermediate precision of the method was also evaluated using different analysts and different instruments and performing the analysis on different days.

Limits of detection and quantification

The LOD and LOQ for rasagiline impurities were determined at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. A precision study was also conducted at the LOQ level by injecting six individual preparations of rasagiline impurities and calculating the RSD of the area.

Linearity

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% of specification level of each impurity (i.e., LOQ, 0.25, 0.50, 1.0, 1.5 and 2.0%). Calibration curves were plotted between the responses of peak versus analyte concentrations. The coefficient correlation, slope and *y*-intercept of the calibration curve are reported.

Accuracy

The accuracy of the method for keto and allyl impurities, amino indane,, 1-indanone and 1-indanol was evaluated in triplicate using four concentration levels from LOQ to 150% (i.e., LOQ, 2.0, 4.0 and 6.0 μg/mL). The percentage recovery for each impurity was calculated at each level.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered,and system suitability parameters for rasagiline standard and the resolution between 1-indanone and 1-indanol were recorded. The variables evaluated in the study were pH of the mobile phase buffer ($\pm$0.2), column temperature ($\pm$5°C), flow rate ($\pm$0.2 mL/min) and percent organic in the mobile phase ($\pm$10%).

Solution stability

The solution stability of rasagiline and its impurities was determined by leaving the test solution and standard solutions in tightly capped volumetric flasks at room temperature for 48 h and measuring the amount of five impurities at every 24 h against freshly prepared standard solution.

Results and Discussion

Method development

Three process impurities; namely keto impurity, 1-indanol, allyl impurity and two degradation impurities, 1-indanone and amino indane, are present in rasagiline mesylate tablets. All process impurities were provided by the synthetic chemistry division of Dr. Reddy's Laboratories Ltd. The atomic mass number of all impurities was confirmed by LC–MS-MS. The primary aim of the chromatographic method development was to achieve the separation of critical, closely eluting pairs of compounds, rasagiline and its allyl impurity, and 1-indanone and 1-indanol, and to elute rasagiline as symmetrical peak. All impurities of rasagiline were subjected to separation by reverse-phase LC on a Hypersil BDS C18, 150 × 4.6 mm, 5 μm column with pH 3.5, 0.01M KH_2PO_4 buffer–methanol in 95:5 ratio as a solvent A and pH 3.5, 0.01M KH_2PO_4 buffer–methanol in 10:90 ratio as solvent B. Two compounds, 1-indanone and 1-indanol, were strongly retained and merged together, and the allyl impurity was not well resolved from rasagiline.

To separate closely eluting compounds, the pH of the mobile phase buffer was reduced to 3.0 and an ACE C8, 150 × 4.6 mm, 3 μm column was selected for separation. With the addition of 5% tetrahydrofuran to solvent B, 1-indanone and 1-indanol impurities were eluted with the desired separation and retention times. After several experiments for gradient profile, the conditions were further optimized as described previously.

Forced degradation behavior

HPLC studies of samples obtained during forced degradation studies of rasagiline under different conditions suggested the degradation behavior described in the following.

Acid degradation

The drug was found to be moderately unstable in 5 N HCl at 70°C for 10 min. The major impurities in the study were found to be amino indane (0.75%) and 1-indanone (0.82%), with 0.61% of a maximum unknown degradant at a relative retention time (RRT) of approximately 0.80, with total impurities of approximately 3.75% (Table I, Figure 2A).

Base degradation

The drug was found to be slightly unstable in 5 N NaOH at 70°C for 5 min. The major degradants in the study was found to be amino indane (0.63%) and 1-indanone (0.45%), with a maximum unknown degradant (0.21%) at an RRT of approximately 2.07, with total impurities of approximately 2.01% (Table I).

Water degradation

The drug was found to be quite unstable in water at 70°C for 15 min. The major degradants in the study were found to be amino indane (0.66%) and 1-indanone (0.43%), with a maximum unknown degradant (0.33%) at an RRT of approximately 1.96, with total impurities of approximately 2.37% (Table I, Figure 2B).

Oxidation degradation

The drug was found to be slightly unstable under conditions of 3% hydrogen peroxide at 70°C for 10 min. The major

Table I
Summary of Forced Degradation Results*

Stress condition	Impurity (%)						Rasagiline			
	Keto impurity	Amino indane	Allyl impurity	1-Indan-one	1-Indanol	MUI	Degrad-ation (%)	Mass balance	Purity angle	Purity threshold
Acid hydrolysis	ND	0.75	ND	0.82	0.06	0.61	3.75	97.4	1.321	1.657
Base hydrolysis	ND	0.63	ND	0.45	0.03	0.21	2.01	97.6	0.818	1.689
Oxidation degradation	ND	0.34	ND	0.47	0.38	0.21	2.23	99.9	0.567	1.154
Thermal degradation	ND	1.53	ND	1.71	0.07	0.76	6.02	97.1	0.509	1.331
Water degradation	0.08	0.66	ND	0.43	0.11	2.37	2.37	99.5	0.582	1.282
Photolytic degradation	ND	0.09	ND	0.03	0.02	0.02	0.18	100.9	0.535	1.144
Stability sample (accelerated condition)	ND	0.28	ND	0.15	ND	0.14	0.76	100.5	0.508	1.143
Stability sample (long-term condition)	ND	0.25	ND	0.14	ND	0.12	0.70	99.9	0.512	1.153

*Note: ND = not detected; MUI = maximum unknown impurity.

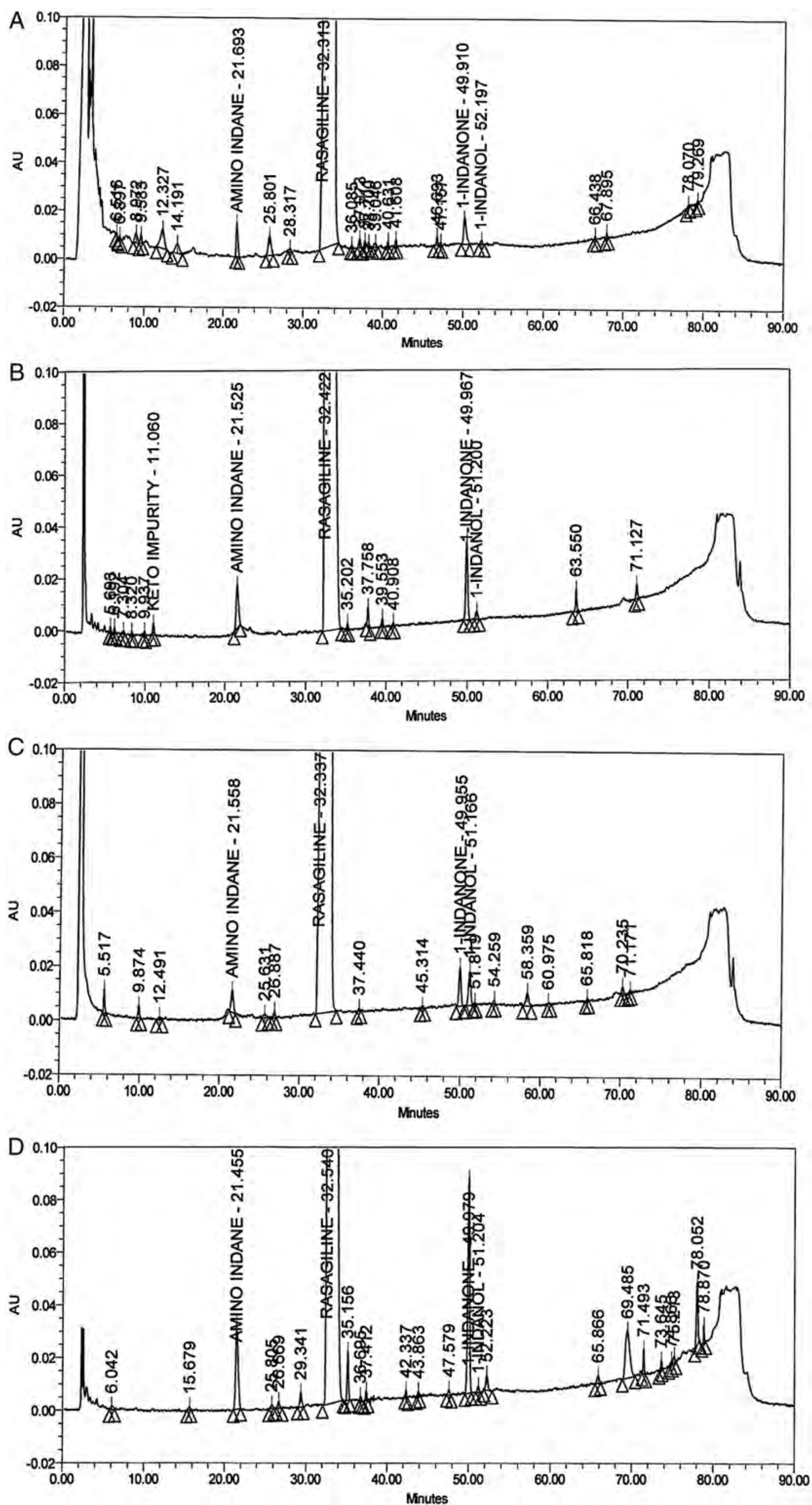

Figure 2. Typical chromatograms of (A) acid degradation sample, (B) hydrolytic degradation sample, (C) oxidative degradation sample and (D) thermal degradation sample.

impurities in the study were found to be amino indane (0.34%), 1-indanone (0.47%), 1-indanol (0.38%) and maximum unknown degradant (0.21%) at an RRT of approximately 1.80, with total impurities of approximately 2.23% (Table I, Figure 2C).

Thermal degradation

Rasagiline was found to be highly susceptible to thermal exposure. The major degradant observed in the sample, exposed to dry heat at 105°C in a hot air oven for 9 h, was found to be a maximum unknown degradant (0.76%) at an RRT of 2.14. The major known impurities in the study were found to be amino indane (1.53%) and 1-indanone (1.71%), with total impurities of approximately 6.02% (Table I, Figure 2D).

Photolytic degradation

Upon subjecting the rasagiline sample to both UV and visible light, no degradation was observed (Table I).

Stability sample analysis (accelerated and long-term conditions)

The drug was found to be stable in accelerated and long-term stability conditions. In accelerated conditions, the major degradants in the study were found to be amino indane (0.28%), 1-indanone (0.15%) and maximum unknown degradant (0.14%), with total impurities of approximately 0.76%. In long-term conditions, the major degradants in the study were found to be amino indane (0.25%), 1-indanone (0.14%) and maximum unknown degradant (0.12%), with total impurities of approximately 0.70% (Table I).

Table II
System Suitability Test Results

Parameters	Specification	Observed values	
		Precision	Intermediate precision
Resolution*	≥2.0	3.02	2.42
Area ratio	≥0.9 and ≤1.1	1.00	1.00
USP tailing	≤2.0	1.1	1.2
USP plate count	>3,000	5,539	5,821

*Resolution between 1-indanone and 1-indanol.

Method Validation

System suitability

System suitability was checked for the conformance of suitability and reproducibility of the chromatographic system for analysis. The system suitability was evaluated on the basis of peak area ratio, USP tailing factor and USP plate count for the rasagiline peak from standard solution, and for the resolution between 1-indanone and 1-indanol from the system suitability solution. All tested critical parameters met the acceptance criteria (Table II).

Specificity/stress studies

No interferences were observed due to placebo and sample diluent at the retention time of rasagiline and its impurities (Figure 3). All forced degradation samples were analyzed using a PDA detector to ensure the homogeneity and purity of the rasagiline peak. All known impurities and unknown degradation products were satisfactorily separated under all forced degradation study conditions, and the purity angle was found to be less than the purity threshold. Apart from the homogeneity of the peaks, the PDA spectrum for all related impurities and rasagiline were compared against their standard spectra. Identification for the impurities and rasagiline were performed by determining the atomic mass numbers of known impurities by LC–MS-MS and comparing their PDA spectrs, purity plots and RRTs along with those of the standard, and were found to match. The mass balance (% assay + % sum of all degradants + % sum of all

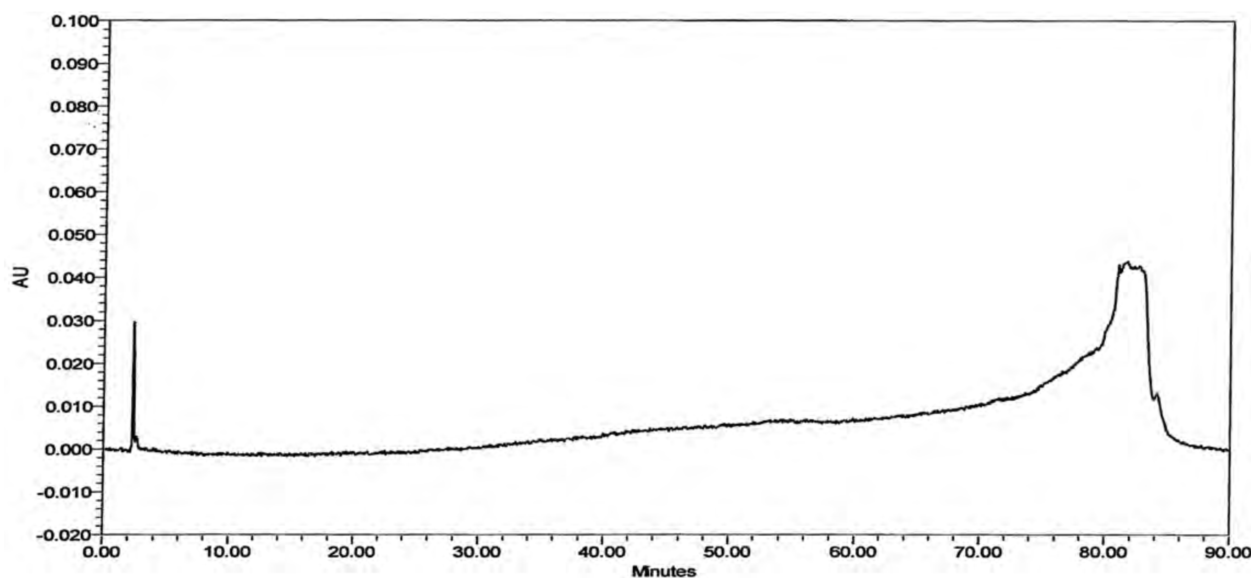

Figure 3. Typical chromatogram of rasagiline placebo.

impurities) results were calculated for all degradation samples and found to be more than 97% (Table I).

Precision

The RSD for the areas of keto impurity, amino indane, allyl impurity, 1-indanone and 1-indanol in the repeatability study was within 1.6%, and in the intermediate precision study was within 0.8%, which confirms the good precision of the method. The RSD values are presented in Table III.

LOD and LOQ

The LOD, LOQ and precision at LOQ values for all five impurities are reported in Table III.

Linearity

The linearity calibration plots for the keto impurity, amino indane, allyl impurity, 1-indanone and 1-indanol was obtained over the calibration ranges tested, i.e., LOQ to 200% of specification level of each impurity (i.e., LOQ, 0.25, 0.50, 1.0, 1.5 and 2.0%). The correlation coefficient was greater than 0.999 (Table III). These results show that an excellent correlation existed between the peak area and concentration of keto impurity, amino indane, allyl impurity, 1-indanone and 1-indanol.

Accuracy

The percentage recovery of keto impurity, amino indane, allyl impurity, 1-indanone and 1-indanol in rasagiline samples varied from 94.0 to 106.0%. The LC chromatogram of the spiked sample at 1.0% level of all five impurities in the rasagiline mesytlate sample is shown in Figure 4. The recovery values for rasagiline impurities are presented in Table IV.

Robustness

In all deliberately varied chromatographic conditions (flow rate, column temperature, pH of mobile phase buffer and composition of organic solvent), all analytes were adequately resolved and elution order remained unchanged. The resolution between the critical pair, i.e., for 1-indanone and 1-indanol, was greater than 2.0, tailing factor for the rasagiline peak from the standard solution was less than 1.3, and area ratio for the peak areas was between 0.95 and 1.05.

Solution stability

The variability in the estimation of all five rasagiline impurities was within $\pm 10\%$ during the solution stability. The results from solution stability experiments confirmed that the sample solution and standard solutions were stable up to 48 h.

Conclusions

A simple and efficient reversed-phase HPLC method was developed and validated for the quantitative analysis of rasagiline impurities in pharmaceutical dosage forms. The method was found to be precise, accurate, linear, robust and rugged during validation. Satisfactory results were obtained from the validation of the method. The method is stability-indicating and can

Table III
LOD, LOQ, Linearity and Precision Data

Parameter	Keto impurity	Amino indane	Allyl impurity	1−Indanone	1−Indanol
LOD (μg/mL)	0.011	0.013	0.012	0.006	0.010
LOQ (μg/mL)	0.030	0.039	0.039	0.018	0.029
Linearity (μg/mL)	0.030−8.001	0.039−8.101	0.039−8.117	0.018−8.030	0.029−8.098
Correlation coefficient	0.9998	0.9998	0.9999	0.9999	0.9997
Intercept	−6,513.78	−6,660.37	−2,518.61	−8,022.76	−8,475.09
Slope	159,348.56	144,029.83	94,079.08	342,254.59	144,783.10
Precision (%RSD)	1.6	0.5	0.5	0.9	0.9
Intermediate precision (%RSD)	0.8	0.3	0.8	0.6	0.5
Precision at LOQ (%RSD)	3.8	3.0	1.2	1.9	0.9

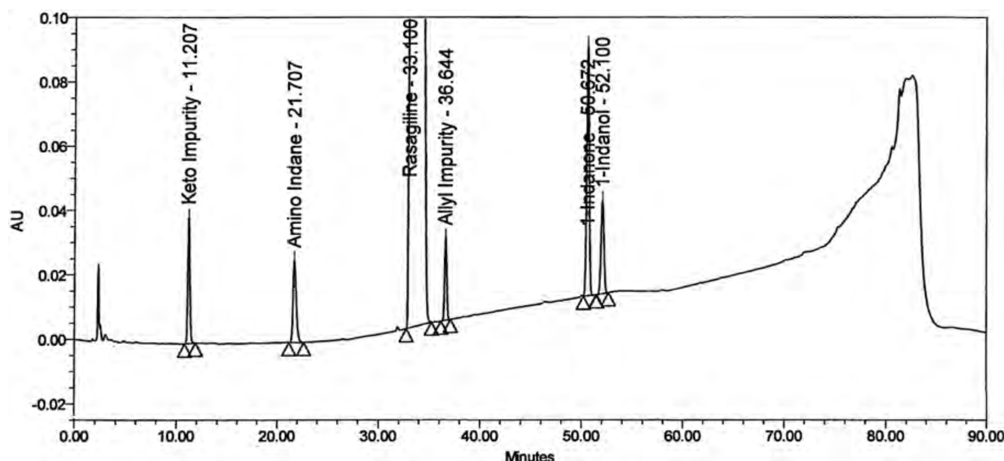

Figure 4. Typical chromatogram of rasagiline sample spiked with its five impurities.

Table IV
Evaluation of Accuracy Study

Amount spiked*	Recovery (%)†				
	Keto impurity	Amino indane	Allyl impurity	1-Indanone	1-Indanol
LOQ	106.0 ± 0.5	100.2 ± 1.3	100.8 ± 0.9	105.3 ± 1.8	96.2 ± 2.3
50%	97.6 ± 0.8	99.3 ± 1.1	102.8 ± 1.7	101.4 ± 0.6	96.1 ± 0.3
100%	94.2 ± 1.4	96.1 ± 1.6	100.4 ± 1.1	99.5 ± 0.9	95.5 ± 0.7
150%	102.7 ± 0.4	97.6 ± 1.9	101.3 ± 0.7	104.9 ± 0.3	103.1 ± 0.8

*Amount of five impurities spiked individually with respect to 1.0% specification level.
†Mean ± %RSD for three determinations.

be used for the routine analysis of production samples and to check the stability of rasagiline mesylate tablets.

Acknowledgments

The authors are thankful to the management of Dr. Reddy's Laboratories Ltd., Hyderabad for providing facilities to carry out this work.

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