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Development and Validation of Forced Degradation Studies of Rasagiline Mesylate Using RP-HPLC and Characterization of Degradants by LC-MS/MS

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Abstract: *Aim of the present work was to develop simple, shorter and effective HPLC method with UV detection (210nm) and subsequent validation for forced degradation studies of Rasagiline mesylate using RP-HPLC and characterization of degradants by LC-MS/MS. The method uses isocratic the mobile phase mixture of Buffer and acetonitrile taken in the ratio in the ratio of 45:55(v/v) on Hypersil BDS, C18, 250 x 4.6 mm, 5µ m column. The RSD for five injections was observed to 0.7 percentage and linearity range of 25 –150 percentage of label claim established with 0.999 correlation. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.*

Key Words: *Rasagiline mesylate , Method development, LC-MS, Hypersil BDS.*

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I. Introduction

Rasagiline mesylate is a chemical inhibitor of the enzyme monoamine oxidase type-B which has a major role in the inactivation of biogenic and diet-derived amines in the central nervous system. Rasagiline is a propargylamine-based drug indicated for the treatment of idiopathic Parkinson's disease[1-7]. It is designated chemically as: 1H-Inden-1-amine, 2, 3-dihydro-N-2-propynyl-, (1R)-, methanesulfonate. The empirical formula of rasagiline mesylate is (C12H13N) CH4SO3 and its molecular weight is 267.34. Rasagiline is freely soluble in water and ethanol and sparingly soluble in isopropyl alcohol. It is a chiral compound with one asymmetric carbon atom in a five member ring with an absolute with R-configuration which is produced as single enantiomer (Clinical Therapeutics Jack J. Chen, David M. Swope and Khashayar Dashtipour) Figure-1 Chemical structure of Rasagiline mesylate. It is not official in any pharmacopoeia and till now, few spectroscopic procedures have been reported for the determination of rasagiline and its metabolites [6-9]. Literature survey reveals that, few gas chromatography mass spectrometry and high performance liquid chromatography methods have been reported for the estimation of rasagiline mesylate. A validated LCMS/MS method for the determination of rasagiline in plasma is also reported[11]. The present work describes a simple, isocratic method for the determination of lopinavir and characterization of degradation products by LC-MS/MS in tablets as for ICH guidelines.[12-18].

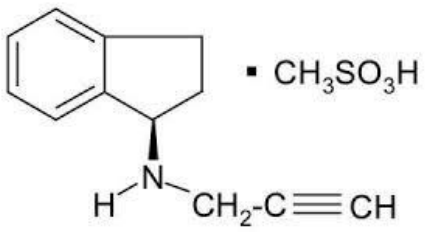


Figure: 1 Chemical structure of Rasagiline mesylate

II. Materials And Methods

Chemicals

Qualified standards and samples of Rasagiline mesylate were obtained from local laboratories and were used without any further purification. The chemicals like Potassium dihydrogen Orthophosphate , triethylamine and Ortho phosphoric acid were purchased from Merck, Mumbai. Millipore water generated from TK water system. The analytical column used was Hypersil BDS, C18, 250 x 4.6 mm, 5µ.

Instruments

A Waters prominence HPLC system equipped with a quaternary UFLC LC-20AD pump, a DGU-20A_5 degasser, a SPD-M20A diode array detector, a SIL-20AC auto sampler, a CTO-20AC column oven and CBM-20A communications bus module was used for method development and validation studies.

Standard preparation

Accurately Weighed and transferred 10mg of Rasagiline mesylate working standards into a 10 ml clean dry volumetric flask, add 7ml of water as diluent, sonicated for 30minutes and make up to the final volume with diluents. 1 mL of above stock solution pipeted out in to a 10ml volumetric flask and then make up to the final volume with water.

Preparation of sample:

5 tablets were weighed and calculate the average weight of each tablet. Then the weight equivalent to 5 tablets were transferred into a 100mL volumetric flask, 30mL of water added and sonicated for 30 minutes, further the volume made up with water and filtered. From the filtered solution 2ml pipette out into a 10ml volumetric flask and made up to 10ml with water.

Chromatographic conditions

The chromatographic column used was Hypersil BDS column with dimensions of 250 mm X 4.6 mm with 5µm particle size. The column temperature was maintained at 30^{o}C and detection was monitored at a wavelength of 210nm. Injection volume was 10 µl and the mobile phase flow was set at 1.2mL/min.

METHOD VALIDATION

The developed method for determination of Rasagiline mesylate was validated for system suitability along with method selectivity, specificity, linearity, range, precision, accuracy, range, ruggedness, robustness according to the ICH guidelines.

Method validation parameters

The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with Rasagiline mesylate in the range of 25 to 150% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using Rasagiline mesylate and evaluated by making six replicate injections. The Accuracy of the method by recoveries of Rasagiline mesylate sample solutions at different concentration levels ranging from 50 to 150%.The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

III. Results And Discussion

Optimization of chromatographic conditions:

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 213 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Rasagiline mesylate . Preliminary development trials were performed with various ODS and BDS columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Hypersil BDS, C18, 250 x 4.6 mm, 5µm column there a significant improvement in the peak shapes with 1.6 tailing factor.

System suitability:

The RSD from five replicate injections of standard preparation was 0.7 %, tailing factor for Rasagiline mesylate peak was 1.7 and theoretical plates obtained 6050.

Selectivity:

Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation and placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of Rasagiline mesylate . Specificity results of Rasagiline mesylate given in the below Table-2.The selectivity chromatograms shown in the Figures 2-6

Table 2: Selectivity results of Rasagiline mesylate

S.No.	Sample	Retention time
1	Blank	-
2	Placebo	-
3	Standard	2.930
4	Sample	2.926

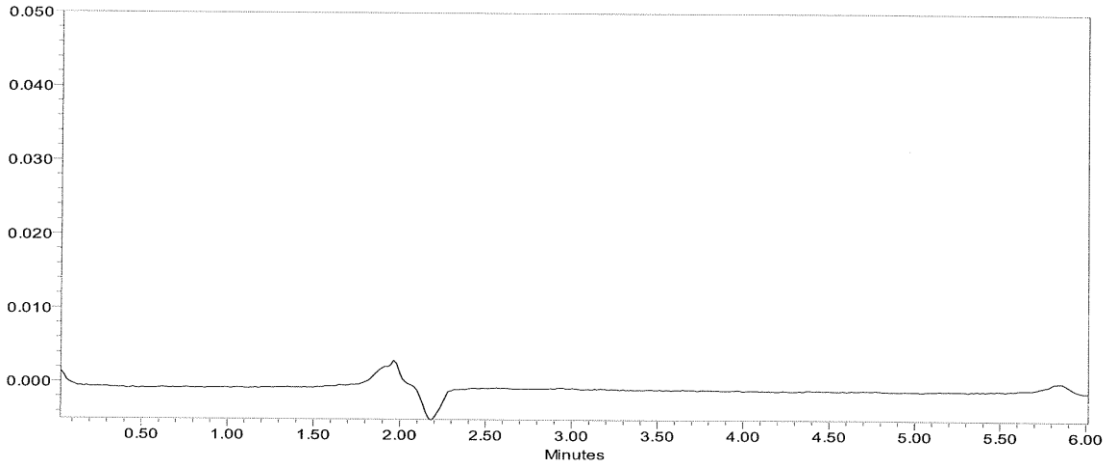


Fig: 2 Chromatogram of Rasagiline mesylate blank

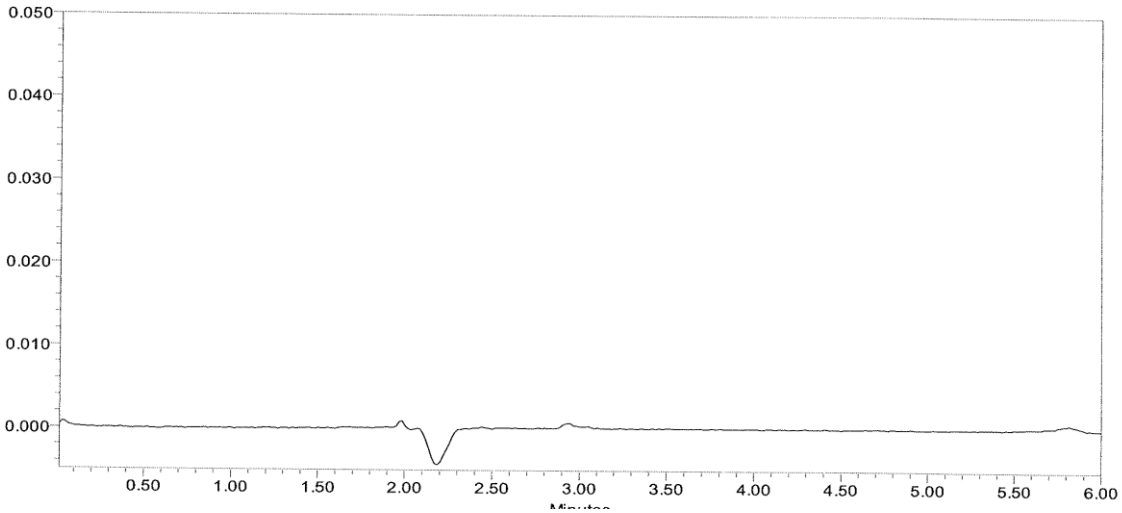


Fig: 3 Chromatogram of Rasagiline mesylate plcebo

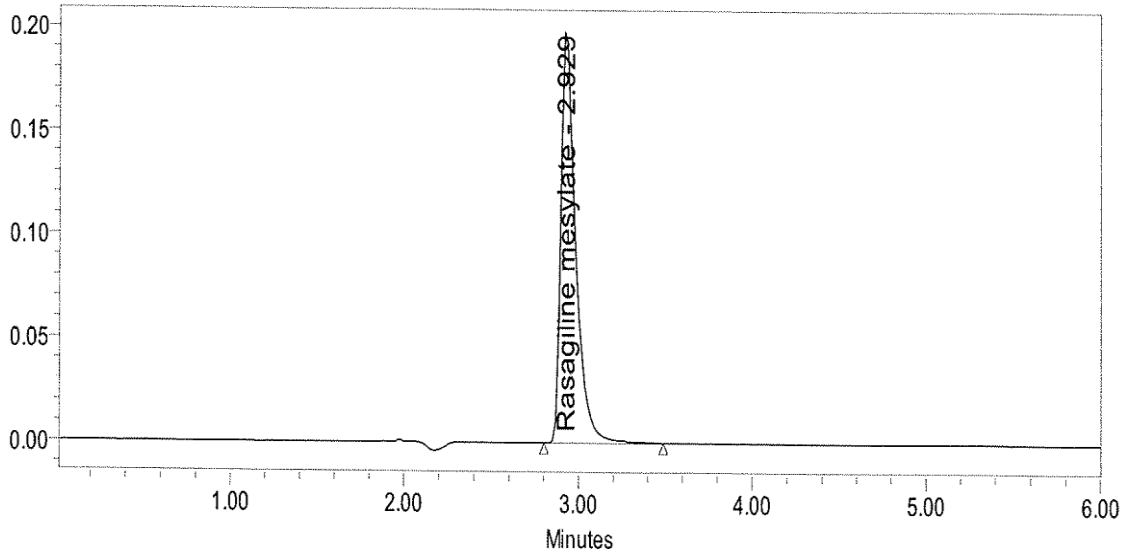


Fig: 2 Chromatogram of Rasagiline mesylate

Linearity:

To demonstrate the linearity with rasagiline mesylate standard in the range of 25 to 150% of specification limit**.** Correlation coefficient of rasagiline mesylate was 0.999.The linearity results shown in the below Table -3

Table 3: Linearity results of Rasagiline mesylate

S.No.:	Concentration in ppm	Area response
1.	25	309496
2.	50	610482
3.	75	876791
4.	100	1200152
5.	125	1491447
6.	150	1804838

Accuracy:

Accuracy study found that the mean % of recovery was more than 97.0% and less than 103.0% at each level 50 to 150% of concentration levels, hence method is accurate. The accuracy results are given Table-4.

Table4: Accuracy results

S.No.:	Level in %	% Mean Recovery
1.	50	99.57
2.	100	99.44
3.	150	100.06

Precision:

The precision of test method was validated by assaying six samples prepared on Rasagiline mesylate and calculate relative standard deviation of Assay results. The precision results are given Table-5.

Table: 5 Precision results

S.No	Area of Rasagiline mesylate
1	99.01
2	99.75
3	100.08
4	100.40
5	99.87
6	100.87
Average	100.12
SD	0.7176
% RSD	0.72

LOD and LOQ

The limit of detection and limit of quantification of test method was validated based on signal to noise ratio method. The LOD and LOQ data given table 6.

Table6: LOD and LOQ establishment data

Parameter	Concentration in ppm
LOD	0.675
LOQ	2.048

3.3. Degradation of rasagiline mesylate

The degradation behavior of LV under various stress conditions was investigated by LC. Typical chromatograms are shown in Figure 2

Oxidation:

To 1 ml of stock solution of rasagiline mesylate, 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 40µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation

To 1 ml of stock solution rasagiline mesylate, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 40µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution rasagiline mesylate , 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 40µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal/ Dry Heat Degradation Studies:

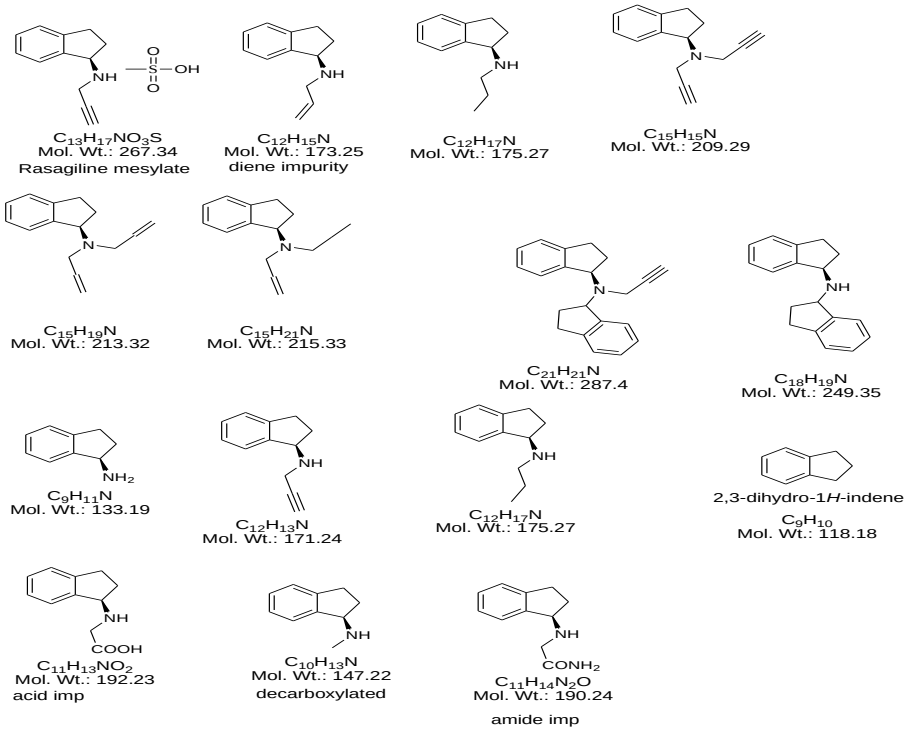
The standard drug solution was placed in oven at 105^0c for 6 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability Studies:

The photochemical stability of the drug was also studied by exposing the 100µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m^2 in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 40µg/ml and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Mass spectral fragmentation:

The analysis of the degradation products was carried by LC and LC-MS. Rasagiline mesylate was subjected to LC-MS/MS with atmospheric pressure chemical ionization (APCI) to know the fragmentation pattern of drug. The MS^2 analysis of the precursor ion (m/z 704) of the drug given below with molecular structure and molecular weight.



IV. Conclusion

A validated stability indicating assay LC-PDA method was developed to study the degradation behavior of Rasagiline mesylate under hydrolysis (acid, base and neutral), oxidation, thermal and UV conditions. LC-MS/MS characterization of degradation products was carried out and pathways of decomposition were proposed. The drug was found to be degraded extensively in all conditions except oxidation due to presence of carbamate and urea linkage, which were susceptible to hydrolysis

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