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

Dr. K. Bhavyasri*1, Dr. D. Rambabu2
*1-Rbvrr Women’s College of Pharmacy, Hyderabad.
2- Gland Pharma Pvt Ltd
Corresponding Author: Dr. K. Bhavyasri

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 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, 5 μm 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.

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.

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, 5μm.

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, 5μm.

DOI: 10.9790/3008-1402027277
Development and Validation of Forced Degradation Studies of Rasagiline Mesylate Using RP...

Instruments
A Waters prominence HPLC system equipped with a quaternary UFLC LC-20AD pump, a DGU-20A 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 30 minutes and make up to the final volume with diluents. 1 mL of above stock solution pipetted 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°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.

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>
<thead>
<tr>
<th>S.No.</th>
<th>Sample</th>
<th>Retention time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>2.930</td>
</tr>
<tr>
<td>4</td>
<td>Sample</td>
<td>2.926</td>
</tr>
</tbody>
</table>

Fig: 2 Chromatogram of Rasagiline mesylate blank

Fig: 3 Chromatogram of Rasagiline mesylate placebo
Development and Validation of Forced Degradation Studies of Rasagiline Mesylate Using RP..

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>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration in ppm</th>
<th>Area response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>309496</td>
</tr>
<tr>
<td>2.</td>
<td>50</td>
<td>610482</td>
</tr>
<tr>
<td>3.</td>
<td>75</td>
<td>876791</td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>1200152</td>
</tr>
<tr>
<td>5.</td>
<td>125</td>
<td>1491447</td>
</tr>
<tr>
<td>6.</td>
<td>150</td>
<td>1804838</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>S.No.:</th>
<th>Level in %</th>
<th>% Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>50</td>
<td>99.57</td>
</tr>
<tr>
<td>2.</td>
<td>100</td>
<td>99.44</td>
</tr>
<tr>
<td>3.</td>
<td>150</td>
<td>100.06</td>
</tr>
</tbody>
</table>

**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>
<thead>
<tr>
<th>S.No.</th>
<th>Area of Rasagiline mesylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.01</td>
</tr>
<tr>
<td>2</td>
<td>99.75</td>
</tr>
<tr>
<td>3</td>
<td>100.08</td>
</tr>
<tr>
<td>4</td>
<td>100.40</td>
</tr>
<tr>
<td>5</td>
<td>99.87</td>
</tr>
<tr>
<td>6</td>
<td>100.87</td>
</tr>
<tr>
<td>Average</td>
<td>100.12</td>
</tr>
<tr>
<td>SD</td>
<td>0.7176</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.72</td>
</tr>
</tbody>
</table>

**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.
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 (H₂O₂) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 40 µg/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, 1 ml of 2 N hydrochloric acid was added and refluxed for 30 mins at 60°C. The resultant solution was diluted to obtain 40 µg/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 30 mins at 60°C. The resultant solution was diluted to obtain 40 µg/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°C for 6 hr to study dry heat degradation. For HPLC study, the resultant solution was diluted to 40 µg/ml solution and 10 µl 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 100 µg/ml solution to UV light by keeping the beaker in UV chamber for 7 days or 200 Watt hours/m² in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 40 µg/ml and 10 µl 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 MS² analysis of the precursor ion (m/z 704) of the drug given below with molecular structure and molecular weight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.675</td>
<td>2.048</td>
</tr>
</tbody>
</table>

Table 6: LOD and LOQ establishment data
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

Acknowledgement

The author thankful to RBVRR Women’s college of pharmacy, Hyderabad for encouragement.

References


[13]. Validation of analytical procedure / Methodology as per ICH Hormnised Tripartite guidelines 1996, Q2A having reached step 4 of the ICH process at the ICH steering committee meeting on 27 October 1994, 1-8.

[14]. SOP validation studies, Indian Pharma guidance Academy, Nagpur, 1996, P- 1-3.


