METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF 
RASAGILINE MESYLATE BY HPTLC

Saidulu Soppari*1, Mangulal Kethavath2, M. D. Seema Farheen3

1Department of Pharmacology, Slc’s College of Pharmacy, Pigilipur (V), R.R (Dist)-501512, Telangana, India.
2Department of Pharmaceutics, Slc’s College of Pharmacy, Pigilipur (V), R.R (Dist)-501512, Telangana, India
3Department of Pharmaceutics,Slc’s College of Pharmacy, Pigilipur (V), R.R (Dist)-501512, Telangana, India.

ABSTRACT
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 or the treatment of idiopathic Parkinson’s disease. Rasagiline is freely soluble in water, 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 R-configuration which is produced as single enantiomer. A simple and sensitive thin-layer chromatographic method has been established for analysis of Rasagiline Mesylate in pharmaceutical dosage form. Chromatography on silica gel 60 F254 plates with 6:1: 2(v/v/v) butanol-methanol water as mobile phase furnished compact spots at RF 0.76±0.01. Densitometry analysis was performed at 255 nm. To show the specificity of the method, Rasagiline Mesylate was subjected to acid, base, neutral hydrolysis, oxidation, photolysis, and thermal decomposition, and the peaks of degradation products were well resolved from that of the pure drug. Linear regression analysis revealed a good linear relationship between peak area and amount of Rasagiline Mesylate in the range of 100–300 ng/band. The minimum amount of Rasagiline Mesylate that could be authentically detected and quantified was 11.15 and 37.11 ng/band, respectively. The method was validated, in accordance with ICH guidelines for precision, accuracy, and robustness. Since the method could effectively separate the drug from its degradation products, it can be regarded as stability indicating.

KEYWORDS: Rasagiline Mesylate, Mono Amine Oxidise type-B, Chromatography, Silica Gel, Isopropyl alcohol, butanolt-methanol water.

INTRODUCTION
The objective of this study is to develop a new method for the estimation of Rasagiline Mesylate in Bulk Drugs and it’s Pharmaceutical Dosage Forms by HPTLC and the method will be validated for various parameters [1, 5] like accuracy, precision, system suitability, linearity and robustness as per ICH guidelines. Rasagiline Mesylate is an irreversible inhibitor of monoamine oxidase chemically known as (R)-N-(prop-2-ynyl)-2-3-dihydro-1H-inden-1-amine.[1,6] used as a mono therapy in early disease[1,2,3,7]. Validation is the process of collecting documented evidence that the method performs according to its intended purpose [1, 2, 3, 6, and 7].
EXPERIMENTAL METHODS

a. Calibration Plots of Rasagiline Mesylate:

A stock solution containing 100 µg/mL of Rasagiline Mesylate was prepared by dissolving an accurately weighed 10mg portion of the drug in methanol in 100mL volumetric flask. Different volumes of stock solution(1,1.5,2,2.5,3,3.5µL)were spotted on an HPTLC plate in triplicate to obtain concentrations of100,150,200,250,300 and 350ng band of Rasagiline Mesylate, respectively[1,3,4]. The data of peak are versus drug concentration were treated by linear least-squares regression [5, 6].

B. Chromatographic conditions and equipment:-

The chemical sand reagent souse were of AR grade and procured from S.D.Fine-Chem. Chromatography was performed on 10cmx10cm aluminium foil plate speculated with 0.2-mm layer so f silicagel60F254. Butanol-methanol-water6:1:2(v/v/v) was used as the mobile phase [6,7]. Linear ascending development was performed in a twin-trough glass chamber previously saturated with mobile phase vapour for 30min at room temperature (RT, 25±2°C) and relative humidity60±5% [3,5]. Densitometry scanning, at 254nm, was performed with a Camag TLC scanner 3 in absorbance mode [7]. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190-400 nm [4, 5].

c. Forced degradation of doripenem:

A stock solution containing 10mg Rasagiline Mesylate in 100ml methanol (100µg/mL) was used for forced degradation to provide an indication of the stability-indicating ability and specificity of the proposed method [1, 3, and 4]. Studies of acid-induced decomposition were performed by exposing the solution of the drug to 5M hydrochloric acid by heating the solution under refluxat80°C for 5h [5, 6, and 7]. The resulting solution was applied to TLC plates such that 250ng per band was applied to the plates. Neutral hydrolysis was carried 25mg of the drug in 25mLHPLC grade water and the solution was refluxed for 12hr sat 50°C[1, 3, 5, 6].
RESULTS

A typical HPTLC densitogram of Rasagiline Mesylate at 254nm (Rf – 0.76 ± 0.01)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TLC densitometry</th>
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</thead>
<tbody>
<tr>
<td>Linear range</td>
<td>100-300 ng/band</td>
</tr>
<tr>
<td>Correlation coefficient (r) ±SD</td>
<td>0.9993 ± 0.02</td>
</tr>
<tr>
<td>Slope ±SD</td>
<td>2.0001 ±1.52</td>
</tr>
<tr>
<td>Intercept ±SD</td>
<td>23.15 ± 1.791</td>
</tr>
<tr>
<td>LOD (ng/spot)</td>
<td>11.15</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>37.11</td>
</tr>
</tbody>
</table>

Linear regression data for the calibration curves (n=6)

A typical HPTLC densitogram of Rasagiline Mesylate from tablet formulation at 254nm (Rf – 0.76 ± 0.01).

DISCUSSION

Validation of Method Parameters:

Linearity:

The linear regression analysis data for the calibration plots showed a good linear relationship ($r^2=0.9993±0.02$) with respect to peak area in the concentration range of 100-
The mean values of the slope and intercept were 5.40 ± 0.257 and 23.15 ± 1.791, respectively, for densitometry analysis at 254 nm.

**Robustness:**
The low values of RSD obtained after introducing small, deliberate changes in the mobile phase composition, mobile phase volume, chamber saturation time, time from application to development and time from development to scanning in the developed HPTLC method indicated the robustness of the method.

**LOD and LOQ:**
LOD and LOQ were determined by the SD method and were found to be 11.12 and 37.21 ng/band, respectively.

**Accuracy:**
Accuracy of the method was obtained by recovery after spiking with 50, 100 and 150% of additional drug. The study was carried out in triplicate by standard addition method and found to be in the range of 99.10–101.0%.

**Specificity:**
The R_f value (0.76 ± 0.01) of the sample and standard was almost identical, and spectra of the sample and the standard were super imposable. These results indicated the specificity of the method.

**CONCLUSION**
The introduction of HPTLC to pharmaceutical analysis is a major step in quality assurance. Statistical analysis proved the method is suitable for analysis of Rasagiline Mesylate as the bulk drug and in a pharmaceutical formulation without interference from excipients. This is a typical stability-indicating assay, established in accordance with the recommendations of the ICH guidelines. The method can be used to determine the purity of a drug and it is also proposed for analysis of drug and degradation products in stability samples obtained during industrial production.

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