Research Article

Development and Validation of Reverse Phase High Performance Liquid Chromatographic Method for Quantitative Estimation of Rasagiline Tablet Dosage Form

Priya Patil[1]*, Pratibha Daroi[1], Vijay Kumar M[2], Smita George[2], R Mohan Singh[2], Vaidun Bhaskar[1]

[1]Department of Quality Assurance, Gahlot Institute of Pharmacy, Plot no. 59, Sector-14, Koparkhairne, Navi Mumbai, Maharashtra, India.

[2]Department of Analytical Research and Development, Central Drug Testing Laboratory, Zonal FDA Bhavan, GMSD Compound, Belasis Road, Mumbai Central, Mumbai, Maharashtra, India.

***Corresponding author's E-mail:** Patilpriya6789@gmail.com

Received: 12-06-2022; **Revised:** 19-08-2022; **Accepted:** 26-08-2022; **Published on:** 15-09-2022.

ABSTRACT

In the present study, a reverse phase high performance liquid chromatographic method was developed and validated for the determination of Rasagiline mesylate in tablet dosage form with the use of selegiline as an internal standard. Chromatographic separation was carried out on Inert Sustain C18 (4.6 mm × 100 mm i.e., 3 µm particle size) column using a mobile phase consisting of water: acetonitrile: H3PO4 (80:20:0.1 % v/v). The flow rate was maintained at 1 ml and UV detection was measured at 211 nm. The calibration curve was linear over the range 2-18 µgml % R.S.D. The results of accuracy and precision studies were within the limits. The method was simple, rapid, and easy to apply, making it suitable for routine analysis of Rasagiline mesylate in tablet dosage form.

Keywords: Rasagiline, selegiline, validation, RP-HPLC, Tablet dosage form.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v76i01.003

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v76i01.003

INTRODUCTION

Parkinson's disease (PD), or simply Parkinson's, is a long-term degenerative disorder of the central nervous system that mainly affects the motor system[1]. Globally, it is estimated 6.3 million people have the disease. Many anti-parkinsonism drugs are available in market as a monotherapy or as an adjunct therapy[2]. Rasagiline is an effective drug in market used for treatment of Parkinsonism. It is a highly potent, selective and irreversible second generation mono amino oxidase inhibitor[3]. Parkinson's disease is characterized by the death of cells that produce dopamine, a neurotransmitter. An enzyme called monoamine oxidase (MAO) breaks down neurotransmitters. MAO has two forms, MAO-A and MAO-B. MAO-B breaks down dopamine.

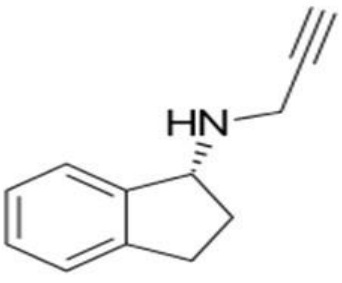

Figure 1: Structure of Rasagiline

Rasagiline prevents the breakdown of dopamine by irreversibly binding to MAO-B. Dopamine is therefore more available, somewhat compensating for the diminished quantities made in the brains of people with Parkinson's[4]. The chemical name of Rasagiline is (1R)-N-prop-2-ynyl-2,3-dihydro-1H-inden-1-amine.

Literature survey revealed that the drug Rasagiline is not official in IP, BP, USP and other pharmacopeia. However, few HPLC and GC-MS methods was reported in different journals. Other method used was a gas chromatography–mass spectrometry procedure in electron impact mode[5]. Analytical method for the quantification of Rasagiline mesylate by a reverse phase liquid chromatography method in biodegradable PLGA microspheres[6], in tablet dosage forms[7] and stability indicating method for determination of Rasagiline mesylate[8] were also reported. It was found that none of the RP-HPLC method is reported for the determination of Rasagiline using an internal standard in tablet dosage form. Hence trials were made to developed accurate, robust, HPLC method using Internal Standard.

The objective is to give an overview of the mechanism of Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) of Rasagiline and explain the basis of the retention mechanism and achieve high-speed separation without any loss of reproducibility[9].

MATERIALS AND METHODS

Chemical and reagents

Rasagiline mesylate and Selegiline was provided by Central Drug Testing Laboratory. Acetonitrile (HPLC grade) from Merk life science, o-phosphoric acid AR Grade from Merck, Ultra-purified HPLC grade distilled water was obtained from the Milli-Q®. Tablets of 0.5 mg Rasagiline (Intas

Pharmaceuticals Ltd.) were procured from local pharmacy[10].

Instrumentation

Thermo Scientific Ultimate 3000 system equipped with chromeleon 7.4.2 software was used to perform quantitative detection. Column used was C-18 Inert Sustain. For all Spectrophotometric measurements Perkin Elmer UV/VIS spectrometer lambda 25 equipped with software Perkin Elmer UV win lab was used. Analytical weighing balance, vacuum filter pump, Millipore filtration kit, Sonicator, Water bath, sample filtration assembly and different type of glassware's were used throughout the experiment[11].

Chromatographic Conditions

The composition of the mobile phase used was water: acetonitrile: H_3PO_4 (80:20:0.1 % v/v). The mobile phase was vacuum filtered through 0.45m nylon Millipore membranes and degassed by ultrasonication for 10min before use. The mobile phase flow rate was set at 1 ml. After equilibration with the solvent to obtain a stable baseline, aliquots of samples (10µl) were injected in the column. The total run time was 10 min. The absorbance of the eluent was monitored at 211 nm. Selegiline (10µgml) was used as an internal standard[12].

Determination of wavelength of maximum Absorbance:

Rasagiline standard 10 mg weighed accurately transferred to the 100 ml volumetric flask and the volume was make up to the mark with diluent i.e., 100ppm. From stock solution further dilutions were made to get a concentration of 10ppm.Then the solution was scanned in UV visible Spectrophotometer in the range of 300.0 nm to 190.0 nm. Rasagiline showed maximum absorbance at 211 nm as shown in Fig. 2 Hence the same wavelength selected for the analysis of Rasagiline.

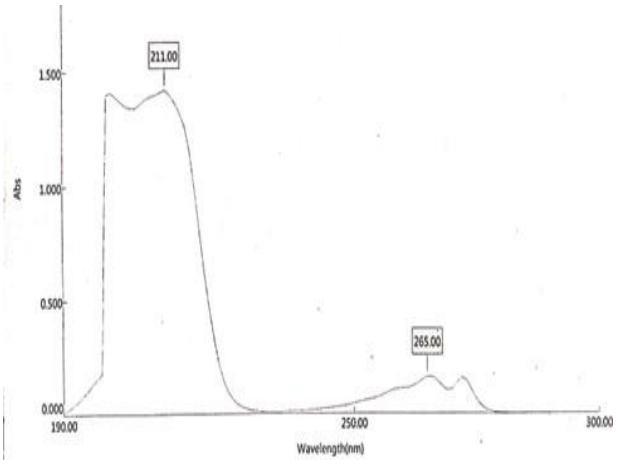

Figure 2: UV spectra of Rasagiline

Selection of Solvent (Diluent)

Based on the molecular structure and chemical nature of Rasagiline, water and acetonitrile in the ratio of (80:20 v/v) was selected as a diluent for preparation of standard and sample solutions.

Preparation of Mobile Phase

The composition of the mobile phase used was water: acetonitrile: H_3PO_4 (80:20:0.1 % v/v). The mobile phase was sonicated and filtered through 0.45µm nylon filter.

Preparation of standard solution

A standard solution of concentration 10 µg/ml of Rasagiline and 10 µg/ml selegiline was prepared using a diluent.

Analysis of marketed formulation

Sample solutions were prepared by weighing 20 tablets accurately. Average weight determined and finely powdered. Equivalent 2mg powder of Rasagiline and Selegiline is dissolved in diluent and sonicated. Further dilutions were made to set a concentration 10 µg/ml of Rasagiline and selegiline in diluent.

Method Optimization

Rasagiline is weakly basic non-polar molecule. Hence, Base deactivated column (Inert Sustain C18 4.6 mm × 100 mm i.e., 3 µm particle size) was selected for the present study. Structurally similar molecule Selegiline were selected as Internal Standard.

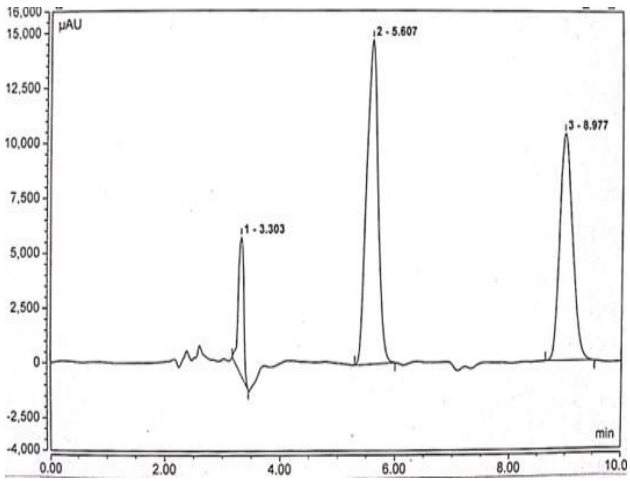

Figure 3: Chromatogram of Rasagiline standard solution

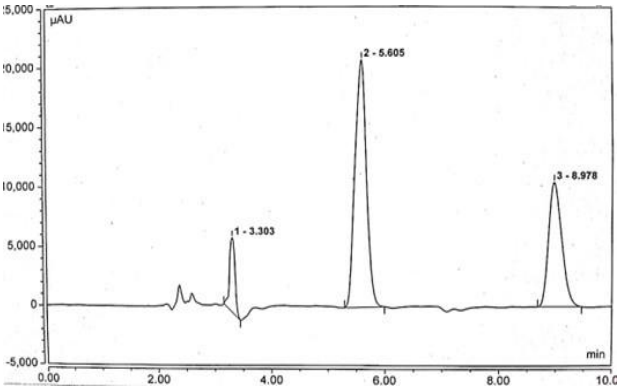

Figure 4: Chromatogram of Rasagiline sample solution

Different mobile phase systems of different composition and ratios were tried for the separation of Rasagiline mesylate and siligiline (Internal Standard). The trials include Methanol:Water (50:50% v/v), Acetonitrile : Water

(50:50% v/v) gave poor peak shape. After the addition of 0.1% H_3PO_4 it was found that peak shape was improved. Further trials were done to get acceptable resolution between drug and Internal Standard. Finally the mobile phase comprising of water: acetonitrile : H_3PO_4 (80:20:0.1 % v/v) gave good resolution of acceptable SST parameters and results were mentioned in tables.

Method Validation

Validation of developed RP-HPLC method was done for parameters such as linearity, precision, accuracy and recovery and robustness as per ICH guidelines[13].

Linearity

Appropriate aliquots from standard Rasagiline stock solutions were prepared to obtain concentrations of 2 - 18µg/ml. Linearity was determined over the range of (2-18 µg/ml) for the Rasagiline. Regression equation obtained was y = 902.61x + 301.13. The method is having good linearity (r^2 = 0.9994). The results established that the analyte response is proportional to the analyte concentration in the selected concentration range. The calibration curve for Rasagiline is done as shown in Table no. 2 and Figure no. 5.

Precision

Precision of System was ascertained by six replicate analysis of Standard solution of Rasagiline. Precision of method (Repeatability) was ascertained by six replicate analyses of homogeneous sample solution of concentration 10 µg/ml. The Intermediate precision was studied by injecting freshly prepared working standard solution of Rasagiline on two different days (Interday) and on same day but at three different time (Intraday).

System precision

The % RSD values were found to be within the limit that is less than 2 %. The results are briefed in Table No 3.

Method Precision

The mean assay percentage results are briefed in Table No. 4 and were found to be within the limit.

Intermediate Precision

The % assay, average, SD, % RSD for Day-1 and Day- 2, and HPLC-1 were found to be not more than 2 %. The results are briefed in Table No. 5.

Accuracy

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of Rasagiline (110, 120, and 130%) of standard solution was added to the pre analysed formulation. This solution was analysed as previously described. The assay was repeated over 3 injections of each concentration to obtain data. The resultant % RSD for this study was found to be < 2.0 % with a corresponding percentage recovery value. Accuracy results at various levels of concentration are summarized in Table No.6. And mean recovery was found to be 97.43% which is within the limits, hence the method is found to be accurate.

Robustness

The Robustness of the method was established by deliberate change in detection wavelength by ±2 nm, change in the temperature by ± 2°C and flow rate by ± 0.2 ml in the estimation of Rasagiline tablet dosage form. The reproducible results were obtained which proves that method is robust. Robustness was performed and by analysing, resulted values were found to be within the limit that is less than 2 %, thus the developed method was proved to be robust. The results are briefed in Table No. 8.

RESULT AND DISCUSSION

Simple RP HPLC method have been developed for the determination of Rasagiline in Tablet dosage form. The chromatographic conditions were optimized by taking into consideration the chemical structures of Rasagiline mesylate and Selegiline was used as Internal Standard which makes the method more precise and accurate. % RSD for the six replicate injections was found to be 2% and ensured that entire testing system and chemicals used could generate precise and accurate outcome. All the SST parameters like theoretical plates were observed greater than 2000 of Rasagiline drug. This result is briefed in Table 1.

The optimized chromatographic conditions were found satisfactory to yield well retained, sharp and symmetrical peak at 5.5 min of Rasagiline and 8.9 of Selegiline. The results of linearity studied over the concentration range 2 - 18µg/ml showed the linear detector response with correlation coefficient of 0.9994 and the regression equation of y = 902.61x + 301.13. (Table 2 and Fig 5)

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. Mean Recovery was observed to be 99.87% representing the accuracy of the method. (Table 6)

Replicate estimations (n=6) of Rasagiline in standard solution and sample solution by proposed method have yielded % RSD of 0.45% indicating substantially high precision of system and method (repeatability). (Table 3,4)

The intermediate precision study was ascertained on the basis of intra-day and inter-day data obtained by analysing Rasagiline tablet by proposed method and it is found to be very much reproducible with minimum % RSD. i.e. 0.19% and 0.99%. (Table 5)

The method was sufficiently robust for normally expected variations in chromatographic conditions such as wavelength, temperature and flow rate and mobile phase. (Table 8)

The number of Average theoretical plates was 3421and tailing factor was 0.9 for Rasagiline which indicates *efficient* performance of the column.

Table 1: System suitability studies of Rasagiline

Sr. No.	Peak Area	Retention Time
1	3344.1	5.582
2	3358.4	5.578
3	3316	5.573
4	3357.7	5.578
5	3345.5	5.562
6	3352.1	5.547
Average	3345.63	5.57
S.D.	15.69	0.01
%R.S.D.	0.46	0.23
Limits	NMT 2.0%	NMT 1.0%

*Average Mean of Six determination, SD = Standard Deviation, % RSD = Percentage relative standard deviation, NMT = Not more than, NLT = Not less than

Table 2: Linearity data of Rasagiline

Linearity level	Concentration	Area
1	2	652
2	5	1485.66
3	8	2426.71
4	10	3242.78
5	12	4155.77
6	15	5135.55
7	18	6066.78

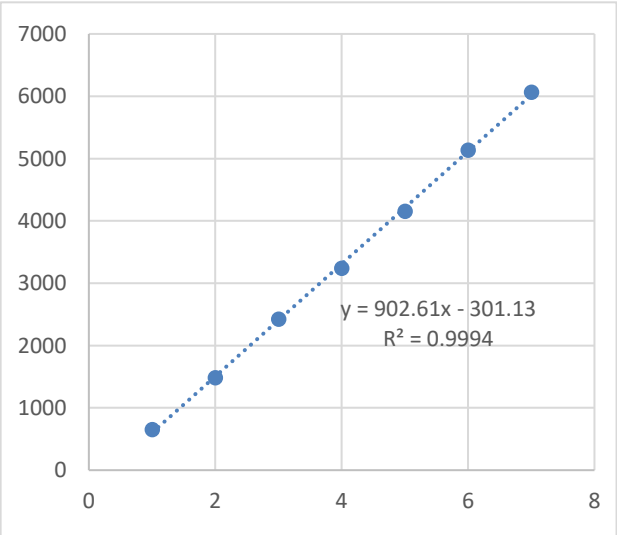

Figure 5: Linearity graph of Rasagiline

Table 3: System Precision (Standard)

Sr. No.	Area
1	3344.1
2	3358.4
3	3316
4	3357.7
5	3345.5
6	3352.1
Mean	3345.63
SD	15.69
% RSD	0.46
Limit	NMT 2.0 %

SD= Standard Deviation. %RSD= Percentage relative standard deviation. NMT= Not more than.

Table 4: Method Precision (Sample)

Sr. No.	Analyst A
	Day 1 %
1	97.61
2	97.28
3	97.49
4	97.48
5	97.34
6	97.06
Mean	97.37
SD	0.19
% RSD	0.19

Table 5: Intermediate Precision/ Interday Precision

Sr. No.	Analyst A	Analyst B
	Day 1 %	Day 2 %
1	97.61	97.29
2	97.28	97.16
3	97.49	97.03
4	97.48	97.18
5	97.34	97.08
6	97.06	97.06
Mean	97.37	97.13
SD	0.19	0.09
% RSD	0.19	0.09

Table 6: Accuracy studies of Rasagiline

% Level	STD spiked (µg/ml)	Amount recovered (mg)	% Amount recovered	% Recovery	Mean % recovery
110	2	0.532	106.57	99.5	99.87
120	4	0.585	117.04	99.94	
130	6	0.636	127.24	100.18	

Table 7: Assay results of Rasagiline

Sample no.	Weight of standard (mg)	Sample Weight	Mean Area of standard at 245nm	Area of sample at 245nm	% Assay
1	10.41	282.84	3345.63	4805.2	97.61
2		282.7		4788.9	97.28
3		282.86		4799.35	97.49
4		282.45		4798.4	97.48
5		282.59		4791.55	97.34
6		282.53		4778.2	97.06
Mean				4793.6	97.37

Table 8: Robustness studies of Rasagiline

Parameter	Change in parameter (±)	% Estimation	Mean	SD	% RSD
Wavelength (± 2 nm)	213 211 209	96.79 97.37 97.38	97.18	0.33	0.34
Mobile Phase composition (± 2 nm)	82:18 80:20 78:22	96.55 97.37 96.79	97.03	0.42	0.43
Flow rate (± 0.02 ml/min)	0.98 1 1.02	96.12 97.37 96.75	96.91	0.62	0.64
Temperature $\pm 2^0$c	42 40 38	97.94 97.37 96.97	97.42	0.48	0.50

CONCLUSION

The RP-HPLC method developed for Rasagiline mesylate in tablet dosage form was found to be simple, rapid, and can generate accurate and precise results. Moreover, the duration of analysis time is less as well as lesser mobile phase consumption confirmed that the method is rapid and economical. Validation parameters that is linearity, accuracy and recovery, precision, robustness, assay is successfully validated as per ICH Q2 (R1) guidelines. According to ICH guidelines the parameters were within the range. Hence the proposed RP-HPLC technique can be used for routine analysis and quality control of Rasagiline mesylate in tablet dosage form.

REFERENCES

1. *"Understanding Parkinson's". Parkinson's Foundation. Retrieved 12 August 2020.*
2. Anthony HV, Schapira MD. Timing of treatment initiation in Parkinson's disease: A need for reappraisal Obeso. J Annals Neurol 2006; 59 (3): 559.
3. Abassi ZA, Binah O, Youdim MBH. Cardiovascular activity of rasagiline, a selective and potent inhibitor of mitochondrial monoamine oxidase B: comparison with selegiline. Bri J Pharmacol 2014; 143: 371.
4. Stocchi F, Fossati C, Torti M "Rasagiline for the treatment of Parkinson's disease: an update". Expert Opinion on Pharmacotherapy 2015; 16 (14): 2231–41.
5. Stefanova N, Poewe W, Wenning G K. Rasagiline is neuroprotective in a transgenic model of multiple system atrophy. Exper Neurol 2008; 210(2): 421.
6. Fernandez M, Barcia E, Negro S. Development and validation of a reverse phase liquid chromatography method for the quantification of rasagiline mesylate in biodegradable PLGA microspheres. J Pharm Biomed Anal. 2009; 49: 1185.
7. Lakshmi MV, Rao JVLNS, Rao AL Development and validation of RP-HPLC method for the estimation of rasagiline tablet dosage forms. Rasayan J chem 2010; 3(4): 621.
8. Kumar RN, Rao GN, Naidu PY. Stability indicating RP-LC method for determination of rasagiline mesylate in bulk and pharmaceutical dosage forms. Int J Appl Bio Pharm Tech 2010; 1(2): 247.
9. P.D Sethi, RajatSethi, Santosh V. Gandhi, Nitin Dubey, High-Performance liquid Chromatography, Sethi's Quantitative analysis of pharmaceutical formulation, volmues-5, CBS publisher & distributers pvt. Ltd.2015; 8.
10. Chatwal G, Anand S, Instrumental Method of Chemical Analysis, Himalaya publication, 2007; 5(2): 624-2.
11. Skoog, West, Holler, Crouch, Fundamental of Analytical chemistry 2013; 9: 973.
12. Mahadev B. K Shirsagar, P. Mahajan, Sanjay D. Sawant, Department of quality. Method development and validation by RP-HPLC for estimation of drug in bulk and pharmaceutical dosage form2017; 10(7): pp 843-849.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.