WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 5, 405-414. Research Article ISSN 2277– 7105

STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION FOR RASAGILINE IN ITS PHARMACEUTICAL DOSAGE FORM

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Article Received on
04 Jan. 2018,

Revised on 24 Jan. 2018,
Accepted on 14 Feb. 2018

DOI: 10.20959/wjpr20185-11242

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ABSTRACT

A new simple, rapid and sensitive stability indicating RP-HPLC method has been developed for the determination of Rasagaline in its pharmaceutical dosage form. The method employs Zorbax Extend C_{18}, 150x4.6mm, 5µ(Agilent) column for the chromatographic separation and phosphate buffer: ACN (40:60, v/v) was used as a mobile phase. Separation was completed within 10 min with a flow rate of 1 ml/min and detection was at 264 nm. The retention time of Rasagaline was found to be 4.65 min. The proposed method was found to have the linearity in the concentration range of range of 10-30 µg/ml. Linearty regression coefficient was not less than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 99.02 – 102.30% of Rasagaline mesaylate. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH Guidelines. The method was found to have suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: Rasagaline, RP-HPLC Estimation, Analytical Method Validation.

1. INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder manifested by a combination of motor and non-motor symptoms. It involves multiple neurotransmitter systems, including dopaminergic, cholinergic, noradrenergic, serotoninergic, GABAergic and glutamatergic systems, which demonstrates the complexity of this disease. The clinical criteria of PD

include a triad of resting tremor, rigidity and bradykinesia. Postural instability usually occurs later in the course of the disease. A second-generation MAO-B inhibitor, rasagiline, was developed in 1987 and was approved by the European Medicines Agency (EMEA) and the Israeli Ministry of Health in 2005 and by the US FDA and Health Canada in 2006. Chemically it is (1H-Inden-1-amine-2,3-dihydro-N-2-propynyl-(1R)-methanesulfonate (fig. 1).

Various analytical methods including UV Spectroscopy, HPLC– MS/MS methods, RP-HPLC, HPTLC are available for the estimation of Rasagaline mesyalate in pharmaceutical dosage forms. We tried to develop a simple stability indicating assay RP-HPLC method for the estimation of Rasagaline mesalyate. The proposed method was optimized and validated as per the International Conference of Harmonization (ICH) guidelines.

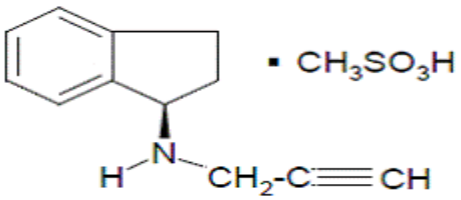

Figure 1: Structure of Rasagaline mesaylate.

2. MATERIAL AND METHOD

Instruments and Apparatus

HPLC (LC-2010 HT) equipped with UV-visible detector and photodiode array detector with vial tracks for automatic sampling, Shimadzu; Double beam UV spectrophotometer, Shimadzu, Sonicator, Branson ultrasonic corporation; Analytical weight balance, Mettler Toledo, Schwerzenbach, Switzerland; Water bath, Metalab scientific industries Ltd; Oven, Lab line, India; pH meter, Lab line, India; 0.45 micron nylon filters; Glasswares and Syringe.

Chemical, Material & Reagents

Standard Rasagaline was procured from Emcure Pharmaceuticals Ltd., Adalaj, Gandhinagar; marketed formulation named Relgin manufactured by INTAS Laboratories Pvt. Ltd. with label claim 1mg Rasagiline was procured from local market. Methanol & water (Milli-Q) of HPLC grade; Potassium dihydrogen orthophosphate, Triethylamine, Orthophosphoric acid, Hydrochloric Acid, Hydrogen Peroxide, Sodium hydroxide of AR grade (Merck) were used during the whole experimental work.

Preparation of standard stock solution of Rasagiline (100 µg/ml)

Weighed accurately 25mg of Rasagalline WS and transferred in to 25 ml volumetric flask (V.F.), about 10ml of methanol was added and sonicated to dissolve. Cooled to RT, and Volume was made up with methanol up to the mark and mixed. From this 10ml of solution was transferred to 100ml V.F and volume was made up with methanol (100 µg/ml).

Sample preparation of Rasagiline in tablets (20 µg/ml)

About 10 tablets(Label claim = 1mg) were accurately weighed and transferred into a 100 ml volumetric flask, about 50 ml of methanol was added, the solution was sonicated for 15 min with intermittent shaking. The sample was allowed to cooled to RT and diluted up to the mark with methanol and mixed well (100 µg/ml). Sample was centrifuged at 3000 rpm for 15 min. 2ml of this solution was pipette out in 10 ml V.F. and volume was made up with mobile phase upto the mark which gave final concentration of 20 µg/ml. It was filtered through 0.45µ nylon filter.

Method Validation

The method was validated according to the International Conference on Harmonization guidelines for validation of analytical procedures Q2 (R1) (ICH, 1996).

Specificity

Specificity of the method was determined by checking the interference from blank and by performing force degradation in acid, base, peroxide and thermal condition.

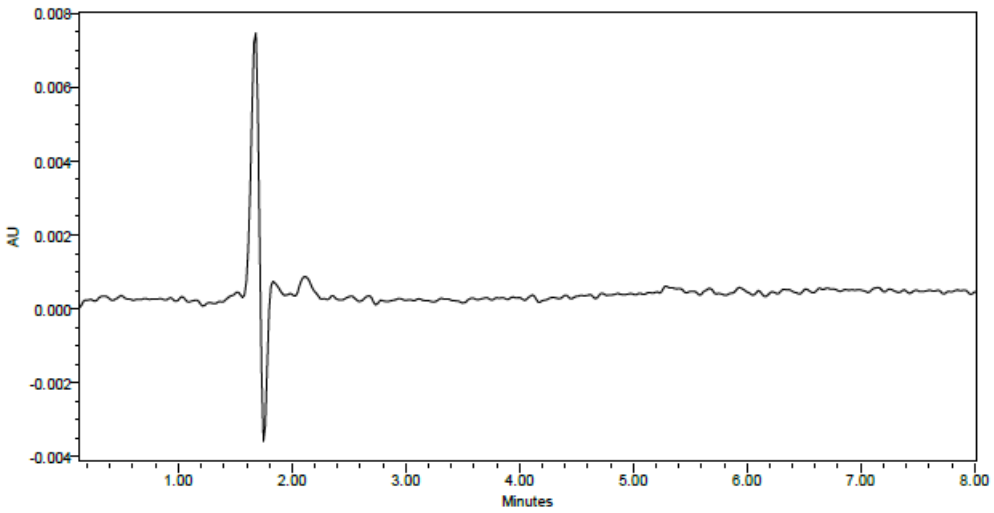

Fig. 2: Chromatogram of Blank.

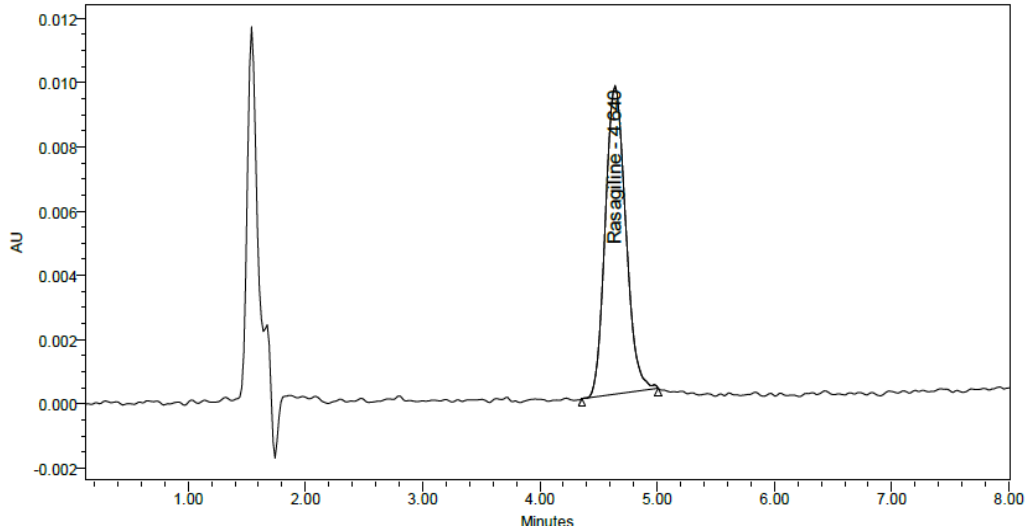

Fig. 3: Chromatogram of Rasagiline Standard at 264nm.

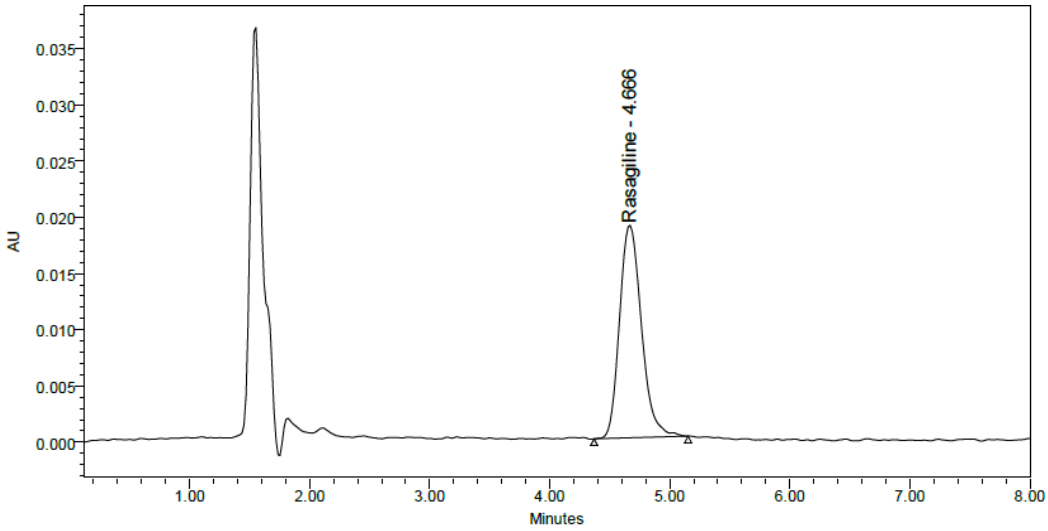

Fig. 4: Chromatogram of Rasagiline Sample at 264nm.

Table 1: Degradation profile of Rasagiline.

Stress condition	% Degradation of Rasagiline
Acidic	20.25
Alkaline	15.27
Oxidative	9.13
Thermal	6.18

Linearity and Range (n=5)

It was found that Lambert-Beer's law was followed in the concentration ranges of 10 - 50 µg/ml (10, 20, 30, 40, 50 µg/ml) for Rasagiline. The straight line equation and correlation coefficient for Rasagiline is shown in below tables.

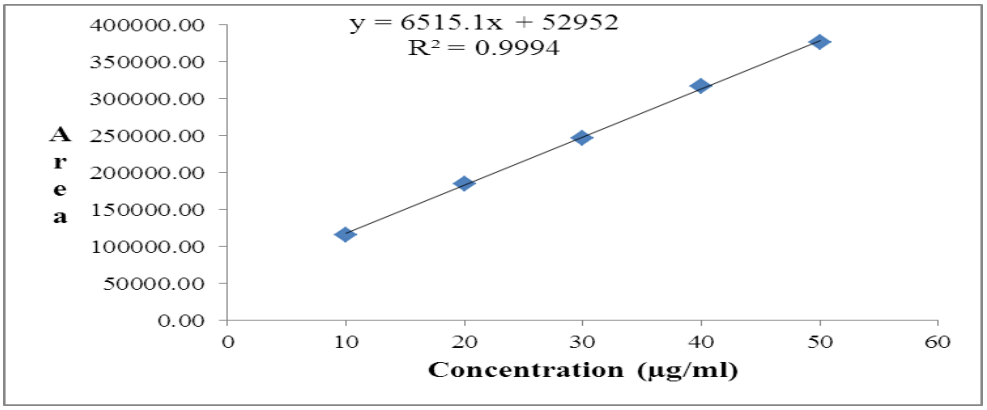

Fig. 5: Linearity graph of Rasagiline.

Table 2: Data of regression analysis of Rasagiline.

Drug	Straight line equation of calibration curve	R^2	Correlation coefficient (r)
Rasagiline	y = 6515.1x + 52952	0.9994	0.9997

Accuracy (% Recovery, n = 3)

The accuracy of the method was determined by calculating recovery of Rasagiline by using standard addition method at 80, 100, 120% level. Percentage recovery was calculated by injecting of each solution three times and % recovery was calculated with help of regression equation. Recovery for Rasagaline was obtained in the range of 99.03 – 102.30% which indicates accuracy of the method, as shown in table 3.

Table 3: Accuracy Study Data for Rasagiline.

% Level	Conc. Added (µg/mL)	Total Amount (µg/mL)	Total Amount Recovered (µg/mL)	Mean Area ± SD	% Recovery	% CV
80	16	36	36.83	185146.00 ± 902.76	102.30	0.933
100	20	40	39.61	296563.67 ± 2766.07	99.03	1.161
120	24	44	44.34	346237.00 ± 3149.65	100.76	0.910

Precision

The repeatability of method was checked by analyzing (n = 6) Rasagaline solutions and response was recorded.

The intra-day (3 times on the same day) and inter-day (3 different days over a period of 1 week) a precision of the proposed method was checked by measuring the responses for 3 different concentration of Rasagaline. The result of precesion for Rasagiline is as shown in Table 4. The % CV for repeatability of Rasagiline was found to be 0.592. The % CV for intra-day precision was found to be in the range of 0.593 – 0.770% while inter-day precision was found to be in the range of 0.671 – 0.793% for Rasagiline, which indicates the method is precise.

Table 4: Precesion (n = 7).

	Parameters	Result
Precision (% CV)	Repeatability (n = 6)	0.592
	Interday (n = 3)	0.671 – 0.793
	Intraday (n = 3)	0.593 – 0.770

LOD and LOQ

The Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated by the equation,

LOD = 3.3 x (SD/Slope)

LOQ = 10 x (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the calibration curve.

Slope = Mean slope of the calibration curve.

The limit of detection (LOD) & limit of quantification (LOQ) was calculated by using its equation and it is shown below, which indicate that the method is sensitive.

Table 5: LOD & LOQ Data for Rasagiline.

Drug	LOD	LOQ
	Concentration (µg/ml)	
Rasagiline	0.550	1.667

Robustness

Results of robustness studies of RP – HPLC method are shown in table 6. The method was found to be in term of variation in composition and flow rate, pH of the mobile phase and column temperature. As % CV for all parameter was found to be less than 2%, which indicates robustness of method.

Table 6: Robustness Data for Rasagiline (n = 3).

Chromatographic condition		Mean peak Area ± SD	% CV
Flow rate ± 0.2	0.8	184383.33 ± 1084.31	0.588
	1.0 (optimum)	185073.67 ± 1320.50	0.713
	1.2	186810.67 ± 1553.13	0.831
Mobile phase pH ± 0.2	3.5	186170.00 ± 1003.28	0.539
	3.7 (optimum)	184680.67 ± 886.91	0.480
	3.9	186637.67 ± 811.68	0.435

System suitability parameters

The results of system suitability test parameters are listed in table 7 and % CV for all parameter was found to be less than 2%.

Table 7: System suitability parameters Data for Rasagiline (n = 6).

Sr. No.	Retention time, min	Asymmetery	Theoretical Plates
1	4.66	1.18	3247
2	4.70	1.17	3236
3	4.65	1.16	3251
4	4.70	1.17	3209
5	4.64	1.19	3244
6	4.66	1.18	3269
Mean	4.67	1.18	3242.67
SD	0.03	0.010	19.81
% CV	0.549	0.893	0.611

Summary of Validation And System Suitability Parameters

All the validation and system suitability parameters are shown in Table 8.

Table 8: Summary of validation parameters & System suitability Parameters.

Validation parameters		
Linearity (n = 5)		10-30 µg/ml
Accuracy (% Recovery) (n = 3)		99.02 – 102.30
Precision (% CV)	Repeatability (n = 6)	0.592
	Interday (n = 3)	0.671 – 0.793
	Intraday (n = 3)	0.593 – 0.770
LOD		0.550 µg/ml
LOQ		1.67 µg/ml
Robustness	pH	Complies
	Temperature	Complies
	Flow rate	Complies
System suitability Parameters		
Retention time (min)		4.67
Theoretical plates		3242.67
Tailing factor		1.18

Estimation of Rasagaline in the marketed formulation by proposed RP-HPLC method: (n=5)

10 tablets were accurately weighed and transferred into a 100ml volumetric flask, about 50ml of methanol was added, and sonicated for 15 min with intermittent shaking to disperse the tablet completely, The sample was allowed to cooled RT and diluted up to the mark with methanol and mixed well. Sample was centrifuged at 3000rpm for 15 min, Then Pipette out

2ml of this solution in 10 ml V.F. volume was made up with mobile phase. Filtered through 0.45µ nylon filter. The sample was injected into the chromatographic system and the % labeled claim was calculated. Label claim of Rasagiline was 1 mg and average weight of 10 tablets was found to be 195 mg.

Table 9: Data of sample preparation for assay (n=5).

Sr. No.	Area of Rasagaline
1	187385
2	185786
3	186465
4	184976
5	185095
Mean Area ± SD	185941.40 ± 1003.83
% CV	0.540
% Mean Assay ± SD	100.52 ± 0.759

3. CONCLUSION

Based on the results of the above studies, it is concluded that the method for determination of assay of Rasagaline is precise, linear over the concentration range, stability indicating and robust. The method is specific for the quantization of assay of Rasagaline in pharmaceutical formulation. So the developed method can be easily applied for routine analysis of Rasagaline in its bulk form and dosage form.

The method was found to be simple accurate economical and rapid and it can be applied for routine analysis in laboratories and suitable for the quality control of bulk and pharmaceutical formulations.

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