METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF NEW ANTIPSYCHOTIC AGENT RASAGILINE IN BULK AND IN PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, precise, rapid, reproducible, accurate and stability indicating reverse phase HPLC method has been developed for the determination of new antipsychotic agent Rasagiline in bulk and in pharmaceutical formulation. An enable develosil ODS mg-5, C_{18}, 150x4.6mm, I.D- 5µm particle size column was used with variable wavelength UV detector. The mobile phase consisting of pH 7.0 Buffer and Acetonitrile in the ratio of 60:40V/V was used. The flow rate was 1ml/min and the effluent was monitored at 210nm. The retention time of the drug was 4.306 minutes. The method was linear over the concentration range of 0.5-150µg/ml. the method precision for the determination of assay was below 2% RSD. The percentage recovery of Rasagiline was 98.2 – 102.1%. The validation of method was carried out utilizing ICH guidelines.

Keywords: Rasagiline, RP- HPLC, Development, Validation, Rasagiline tablets.

INTRODUCTION

Rasagiline (1-11) is an irreversible inhibitor of monoamine oxidase used as a monotherapy in early Parkinson's disease or as an adjunct therapy in more advanced cases [1-5]. It is selective for MAO type B over type A by a factor of fourteen. It was developed by Teva euroscience, initially investigated by Prof. Moussa Youdim and Prof. John Finberg of the Faculty of Medicine, Technion – Israel Institute of Technology. Parkinson's disease is caused by the progressive impairment or deterioration of neurons (nerve cells) in an area of the brain known as the substantia nigra. When functioning normally, these neurons produce a vital brain chemical known as dopamine. Dopamine serves as a chemical messenger allowing communication between the substantia nigra and another area of the brain called the corpus striatum [6-9].

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. 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 [10-11].

Figure 1. Chemical structure of Rasagiline mesylate

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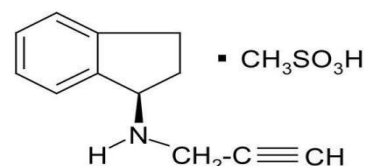
It is not official in any pharmacopoeia and till now, few liquid chromatographic (LC) procedures have been Reported for the determination of Rasagiline and its metabolites in biological fluids. However, there are limited publications on the LC analysis of Rasagiline in bulk and pharmaceutical dosage forms7-20. Hence, an LC rocedure was developed to serve as a rapid and reliable method for the determination of Rasagiline bulk and pharmaceutical dosage forms. In the proposed method, well separated and eluted with in 8 min. Finally the method was thoroughly validated for the assay of Rasagiline mesylate Tablets [12, 13].

MATERIALS AND METHODS
Table 1. Instrumentation

Equipment	Configuration/Model	Make
HPLC	Detector – DWD-2487 Injector – Rheodyne Empower software	WATERS
Column	C18, 150x4.6mm, 5μm	Develosil ODS MG-5
Precision balances	D – 4323 10040	Shimadzu
pH meter	LI/613	Elico
Sonicator	---	Spino tech

Table 2. Reagents and Chemicals

Chemicals	Manufacturers	Grade
Methanol	Qualigens	HPLC
potassium dihydrogen orthophosphate anhydrous	Merck	HPLC
Sodium hydroxide	Merck	HPLC
Acetonitrile (ACN)	Merck	HPLC
Milli Q Water	Milli Q	HPLC

Table 3. Chromatographic parameter

Instrument	Waters HPLC
Column	Develosil ODS MG-5 C18, 150x4.6mm, 5μm
Wavelength	210nm
Column Temperature	40°C
Flow rate	1.0 ml / min
Injection volume	20 μl
Mobile phase	Buffer : Acetonitrile (60:40)

Preparation of Mobile Phase
Mix 600 ml of above buffer and 400 ml of Acetonitrile and degas by filtering through 0.45 membranes.

Diluent
pH 7.0 buffer and acetonitrile mixed in the ratio 1:1(v/v) mixed well and filtered through 0.45μm nylon membrane filter.

Determination of retention time
Preparation of Rasagline standard solution
Weigh accurately about 79mg of Rasagiline working standard was transferred in to a 100 mL volumetric flask and to that 70 ml of diluent was added and sonicated to dissolve and diluted to volume with the diluent. Further 5 mL of the resulting solution was taken into 25 mL volumetric flask and made up to volume with the diluent. Solution was filtered through 0.45μm nylon membrane filter.. 20μl of this solution was injected and chromatogram was recorded.

Test Solution
The number of tablets equivalent to 10 mg of Rasagiline were weighed and transferred in to a 100 mL volumetric flask and about 70 ml of the diluent was added and swirled the flask to disintegrate, sonicated for 20 min and diluted to the volume with the diluent. The solution was filtered through 0.45μm nylon membrane filter prior to use.and 20μl of this solution was injected and chromatographic conditions described above and chromatogram recorded.

Amount of drug in each injection of Rasagline

$$= \frac{Spl.\ Area}{Std\ Area} \times \frac{Std\ dilution}{Spl\ dilution} \times \frac{Potency}{100} \times 100$$

Acceptance criteria: 90-100% w/w

Table 4. System Suitability Report

System Suitability Parameters	Observed Value	Acceptance Criteria
USP tailing factor for Rasagiline peak	1.1	NMT 2.0
USP Plate count for Rasagiline peak	13890	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution	0.2	NMT 2.0%

VALIDATION [14-23]
Validation of analytical method for the assay of Rasagiline
Validation of analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application.

1. System Precision and System Suitability
The standard solution, prepared by using Rasagiline Working standard as per the test method was injected 10 times into the HPLC system. The system suitability parameters were evaluated and found to be with

in limits. The RSD for peak areas of ten replicate injections of standard was found to be 0.4% for Rasagiline peak. The results are summarized in Table 5A & 5B.

Table 5A. System Suitability

System suitability parameters	Observed value	Acceptance criteria
USP tailing factor for Rasagiline peak	1.1	NMT 2.0
% RSD for Peak area of Rasagiline from five replicate injections of standard solution	0.5	NMT 2.0%

Table 5B. System Precision

Injection Number	Rasagiline peak Area
01	4954964
02	4951624
03	4956923
04	4945981
05	4956396
06	4968124
07	4988448
08	4964719
09	4971538
10	4965312
Average	4962403
%RSD	0.4

2. Specificity
a) Placebo Interference
A study to establish the interference of placebo was conducted. Assay was performed on Placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of placebo solutions showed no peaks at the retention time of Rasagiline peak. This indicates that the excipients used in the formulation do not interfere in estimation of Rasagiline in Rasagiline Tablets. The results are summarized in Table 6.

3. Linearity of Detector Response
Linearity of detector response was established by plotting a graph to concentration versus average area and determining the correlation coefficient. A series of solutions of Rasagiline standard were prepared in the concentration range of about 4.8 μg/ml to 150.5 μg/ml on X-axis versus response on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999.The results are summarized in Table 7.

4. Precision of Test Method
Repeatability
The precision of test method was conducted for 1mg tablets individually by assaying six samples of Rasagiline tablets. The average % assay of Rasagiline in Rasagiline tablets was found to be 100.5% with a RSD of 0.3% for 1mg strength. The results were summarized in Table 8.

5. Accuracy
The study of recovery of Rasagiline from spiked placebo was conducted. Samples were prepared by mixing placebo with Rasagiline raw material equivalent to about 5%, 25%, 50%, 75%, 100%and 150% of the target concentration of Rasagiline Tablets 1mg. Sample solutions were prepared in triplicate for each spike level and assayed as per test method. The percentage recovery was found to be within limits. The results are summarized in Table 9.

6. Linearity of Test Method
A graph is plotted to 'average mg of Rasagiline added' versus 'average mg of Rasagiline recovered' in Accuracy (Recovery) section. The correlation coefficient was found to be 0.999. From the above study, it was established that the method is linear from 5% to 150% of the target concentration shown in Table 10 and Figure 5.

7. Ruggedness
a) Analyst to Analyst Variability
Analyst to analyst variability study was conducted by two analysts by assaying six different test preparations of Rasagiline Tablets 10 mg. The average % assay obtained by both the analysts was found to be 100.5% and 100.7% with a relative standard deviation of 0.2% and 0.2 % respectively. The system suitability parameters were evaluated as per the test method by both the analysts and found to be with in the limits. The results are summarized in Table 11. Comparison of results obtained by both the analysts' shows that the assay method is meeting intermediate precision acceptance criteria.

SYstem to System Variability
System to system variability study was conducted by two system by assaying six different test preparations of Rasagiline Tablets 1 mg. The average % assay obtained by both the system was found to be 100.5% and 100.7% with a relative standard deviation of 0.2%and 0.2 % respectively. The system suitability parameters were evaluated as per the test method by both the system and found to be within the limits. The results are summarized in Table 12. Comparison of results obtained by both the analysts' shows that the assay method is meeting intermediate precision acceptance criteria.

Column to Column Variability
Column to column variability study was conducted by two column by assaying six different test preparations of Rasagiline Tablets 1 mg. The average %

assay obtained by both the system was found to be 100.5% and 101.3% with a relative standard deviation of 0.2% and 0.6% respectively. The system suitability parameters were evaluated as per the test method by both the system and found to be with in the limits. The results are summarized in Table 13. Comparison of results obtained by both the analysts' shows that the assay method is meeting intermediate precision acceptance criteria.

b) Bench Top Stability of Test Preparation and Standard Preparation

A study to establish the stability of Rasagiline in test preparation and standard preparation on bench top was conducted at initial, 1 day and 2 days. The assay of Rasagiline in test preparation and standard preparation were estimated against freshly prepared standard each time. The difference in % assay of Standard and Test preparations from initial to 1 day and 2 days was calculated and the results are summarized in Table 14. From the above study, it was established that the test preparation and the standard preparations are stable for a period of 2 days on bench top.

C) Refrigerator Stability of Test Preparation and Standard Preparation

A study to establish the stability of Rasagiline in test preparation and standard preparation in refrigerator was conducted over a period of 2 days. The % assay of Rasagiline in test preparation and standard preparation was estimated at initial, 1 day, 2 days against freshly prepared standard at each time. The difference in % assay of standard and test preparations from initial to 1 day and 2 days were found to be within the limits. The results are summarized in Table 15. From the above study, it was established that the test preparation and standard preparation are stable for a period of 2 days in the refrigerator.

8. Robustness
a) Effect of Variation in Mobile Phase Composition (Acetonitrile)

A study to establish the effect of variation in mobile phase composition was conducted. Three mobile phases were prepared having 90%, 100% and 110% of the method organic phase composition (Acetonitrile). Standard and test solutions prepared as per test method

were injected into HPLC system. The system suitability parameters and % assay were evaluated with the mobile phases as per test method and found to be within limits. The results are summarized in Table 16. From the above study, it was established that the allowable variation in organic phase composition (Acetonitrile) in mobile phase is from 90% to 110%.

a) Effect of Variation in Flow Rate

A study was conducted to determine the effect of variation in flow rate. Standard and test preparations were prepared as per test method was injected into the HPLC system with flow rate 0.8 mL/min, 1.0 mL/min and 1.2 mL/min. The system suitability parameters and difference from average assay values were evaluated as per test method for the flow rates and found to be with in limits. The results are summarized in Table 17. From the above study, it was established that the allowable variation in flow rate is from 0.8 mL/min to 1.2 mL/min.

Effect of Variation in pH of Buffer in Mobile Phase

A study to establish the effect of variation in pH of buffer in mobile phase was conducted. Three mobile phases were prepared having buffer of pH 6.8, pH 7.0 and pH 7.2. Standard and test solutions prepared as per the test method were injected into the HPLC system using three mobile phases. The system suitability parameters and % Assay were evaluated as per the test method and found to be with in the limits. The results are summarized in Table 18. From the above study, it was established that the allowable variation in p^H of Buffer in mobile phase is from 6.8 and 7.2.

Effect Of Using Different Filters (Filter Validation)

A study to establish the effect of using different filters in test solution and standard was conducted. Standard and test solutions prepared as per the test method were prepared and divided into three portion one portion centrifuged, second portion filtered through HNN filter and third portion filtered through HVF filter followed by injected into the HPLC system using three different condition. The system suitability parameters and % Assay were evaluated as per the test method and found to be within the limits. The results are summarized in Table 19. From the above study, it was established that both HNN and HVF filter suitable for filtration.

Table 6. Placebo Interference

Sample No	% Interference	
	0.5 mg	**1 mg**
1	NIL	NIL
2	NIL	NIL
3	NIL	NIL

Acceptance criteria: No interference from placebo shall be observed at the retention time of main peak.

Table 7. Linearity of Detector Response

S.No.	Concentration (µg/mL)	Average peak area
01	4.8155	146305
02	10.0323	258753
03	20.0646	592210
04	40.1292	1232893
05	50.1615	1540719
06	60.1240	1846719
07	80.2484	2397280
08	100.3230	2960631
09	125.4038	3758079
10	150.4845	4408624
Correlation coefficient	0.999	

Table 8. Repeatability (Precision)

Test No	0.5mg	1mg
01	100.5	100.4
02	101.5	100.5
03	101.2	100.8
04	100.9	100.3
05	101.5	100.2
06	100.9	100.8
Average	101.1	100.5
% RSD	0.4	0.3

Table 9. Accuracy

Sample No.	Spike level	'mg' added	'mg' found	% Recovery	Average %recovery
1		0.49	0.50	102.0	
2	5%	0.49	0.50	102.0	102.0
3		0.49	0.50	102.0	
4		2.53	2.60	102.8	
5	25%	2.53	2.59	102.4	102.1
6		2.53	2.60	102.8	
7		4.99	5.04	101.0	
8	50%	4.99	5.03	100.8	100.9
9		4.99	5.04	101.0	
10		7.49	7.59	101.3	
11	75%	7.49	7.60	101.5	101.5
12		7.49	7.62	101.7	
13		9.94	10.00	100.6	
14	100%	9.94	9.94	100.0	100.2
15		9.94	9.94	100.0	
16		14.81	14.61	98.6	
17	150%	14.81	14.67	99.1	98.9
18		14.81	14.66	99.0	

Table 10. Linearity of Test Method

Spike level	Average 'mg' of Rasagiline added	Average 'mg' of Rasagiline Recovered
5%	0.49	0.5
25%	2.53	2.6
50%	4.99	5.04
75%	7.49	7.60
100%	9.94	9.96
150%	14.81	14.65
Co-efficient of Correlation (r)		0.999

Table 11. Analyst to Analyst Variability

Analyst – 1		Analyst - 2	
N.Suresh kumar		S. Anupriya	
System Suitability Parameters	**Observed Value**		**Acceptance Criteria**
	Analyst-1	**Analyst-2**	
USP tailing factor for Rasagiline peak	1.1	1.1	NMT 2.0
USP plate count for Rasagiline peak	13890	10321	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution.	0.2	0.2	NMT 2.0%

Sample No.	% Assay	
	Analyst - 1	Analyst - 2
1	100.4	100.6
2	100.5	100.4
3	100.8	100.9
4	100.3	100.6
5	100.2	100.7
6	100.8	100.9
Mean	100.5	100.7
%RSD	0.2	0.2
Overall %RSD	0.2	

Table 12. System to System Variability

HPLC System		HPLC System - 1	
Instrument ID.No.		ADE 161	
System Suitability Parameters	**Observed Value**		**Acceptance Criteria**
	System-1	**System-2**	
USP tailing factor for Rasagiline peak	1.1	1.1	NMT 2.0
USP plate count for Rasagiline peak	13890	11883	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution.	0.2	0.3	NMT 2.0%

Sample No.	% Assay	
	System-1	System-2
1	100.4	100.6
2	100.5	100.1
3	100.8	100.1
4	100.3	101
5	100.2	101.4
6	100.8	101
Mean	100.5	100.7
%RSD	0.2	0.2
Overall %RSD	0.2	

Table 13. Column to Column Variability

HPLC Column	Column – 1		
Column ID.No.	CLC1000		
System Suitability Parameters	**Observed Value**		**Acceptance Criteria**
	Column-1	**Column-2**	
USP tailing factor for Rasagiline peak	1.1	1.0	NMT 2.0
USP plate count for Rasagiline peak	13890	13512	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution.	0.2	0.4	NMT 2.0%

Sample No.	% Assay	
	Column-1	**Column-2**
1	100.4	100.5
2	100.5	101.0
3	100.8	100.9
4	100.3	101.6
5	100.2	101.6
6	100.8	101.9
Mean	100.5	101.3
%RSD	0.2	0.6
Overall %RSD	0.6	

Table 14. Ruggedness – Bench top Stability of Standard and Test Preparation

Time in Days	% Assay of Standard preparation	Difference from Initial	% Assay of test preparation		Difference from Initial	
			Test - 1	**Test - 2**	**Test - 1**	**Test – 2**
Initial	63.9*	NA	101.8	100.1	NA	NA
1	63.5	0.4	101.3	100.5	0.5	0.4
2	64.1	0.2	102.9	100.9	1.1	0.8

* Potency of working standard as Rasagiline on as is basis.
NA = Not Applicable

Table 15. Ruggedness – Refrigerator stability Of Standard and Test Preparation

Time in Days	% Assay of Standard preparation	Difference from Initial	% Assay of test preparation		Difference from Initial	
			Test - 1	**Test - 2**	**Test - 1**	**Test - 2**
Initial	63.9*	NA	101.8	100.1	NA	NA
1	64.4	0.5	102.1	100.7	0.3	0.6
2	63.3	0.6	101.0	101.3	0.8	1.2

* Potency of working standard as Rasagiline on as is basis.
NA = Not Applicable

Table 16. Robustness - Effect of Variation In Mobile Phase Composition (Acetonitrile)

System Suitability Parameters	Organic phase ratio (Acetonitrile)			Acceptance Criteria
	100%	**90%**	**110%**	
USP tailing factor for Rasagiline peak	1.1	1.0	1.1	NMT 2.0
USP plate count for Rasagiline peak	15094	14852	14438	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution.	0.5	0.3	0.1	NMT 2.0%

Organic phase ratio (Acetonitrile)	% Assay		Average %Assay	Difference
	Trial-1	**Trial-2**		
100%	100.5	100.9	100.7	NA
90%	99.6	100.7	100.2	0.5
110%	100.0	99.4	99.3	1.4

NA = Not applicable

Table 17. Robustness - Effect of Variation In Flow Rate

System Suitability Parameters	Flow Rate (ml/min)			Acceptance Criteria
	1.0	**0.8**	**1.2**	
USP tailing factor for Rasagiline peak	1.1	1.1	1.1	NMT 2.0
USP plate count for Rasagiline peak	15022	15541	15142	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution.	0.8	0.1	0.2	NMT 2.0%

Flow Rate (mL/min)	% Assay		Average %Assay	Difference
	Trial-1	**Trial-2**		
1.0	98.9	100.8	99.9	NA
0.8	97.5	99.1	98.3	1.6
1.2	97.3	99.0	98.2	1.7

NA = Not applicable

Table 18. Robustness – Effect of Variation in pH

System Suitability Parameters	Observed value at			Acceptance Criteria
	pH 6.8	**pH 7.0**	**pH 7.2**	
USP tailing factor for Rasagiline peak	1.1	1.1	1.0	NMT 2.0
USP plate count for Rasagiline peak	11749	12156	12687	NLT 2500
%RSD for Peak area of Rasagiline from five replicate injections of standard solution.	0.1	0.3	0.2	NMT 2.0%

pH	% Assay		Average %Assay	Difference
	Trial-1	**Trial-2**		
6.8	100.1	100.6	100.4	0.8
7.0	99.8	99.4	99.6	NA
7.2	100.3	100.2	100.3	0.7

NA = Not applicable

Table 19. Robustness – Filter Variation

Filter Description	Filters	
	HNN	**HVF**
Manufacturers Name	Advanced Microdevices	Advanced Microdevices
Lot No.	NB169695	420 / 720 /2
Size	0.45μm	0.45μm

	Centrifuged			Sample filtered through HNN filter			Sample filtered through HVF filter		
	1	2	3	1	2	3	1	2	3
%ASSAY	101.4	101.7	101.1	100.9	101.0	101.3	101.0	101.2	101.2
DIFF	NA	NA	NA	0.5	0.7	0.2	0.4	0.5	0.1

NA = Not applicable

Figure 2A. Typical chromatogram for placebo of Rasagiline Tablets (Purosphere star C$_{18}$, 150mm X 4.6 mm, 5 μm)
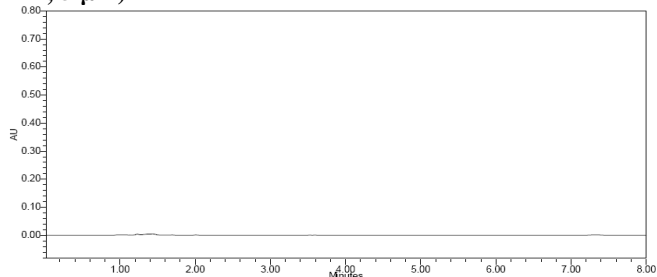

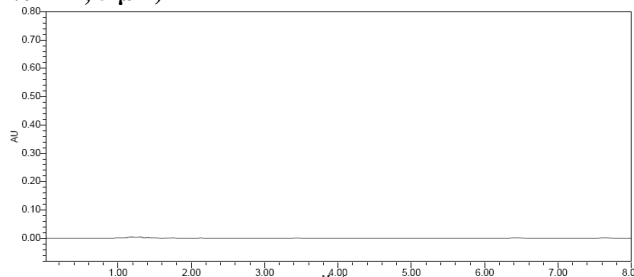**Figure 2B. Typical chromatogram for placebo of Rasagiline Tablets (Develosil ODS MG-5, C$_{18}$, 150mm X 4.6 mm, 5 μm)**
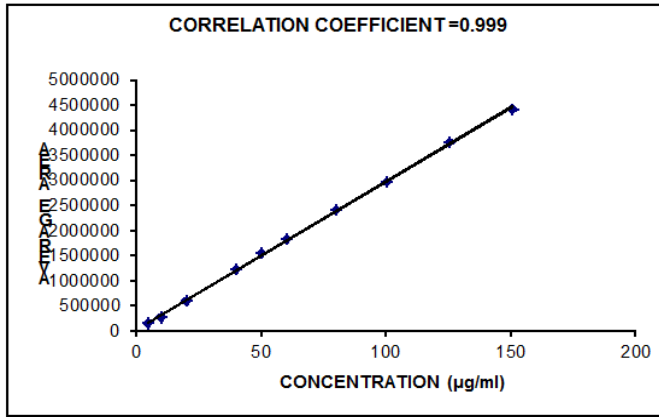
Fig 3. Linearity of Detector Response Graph

CORRELATION COEFFICIENT =0.999

Fig. 4. Typical Chromatogram of Rasagiline tablets
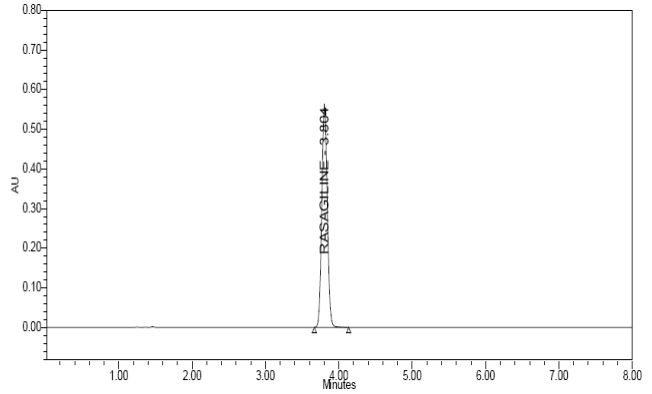
Fig. 5. Linearity of Test Method Graph

CORRELATION COEFFICIENT =0.999

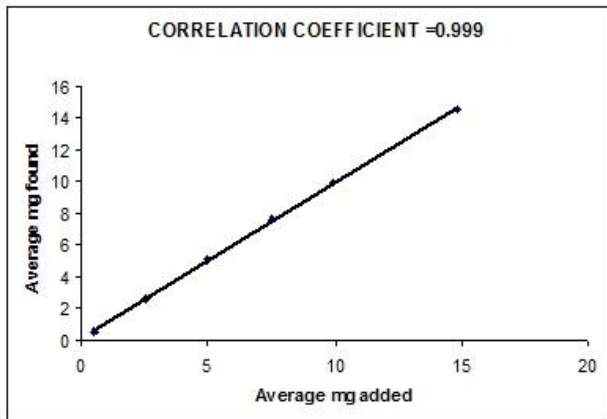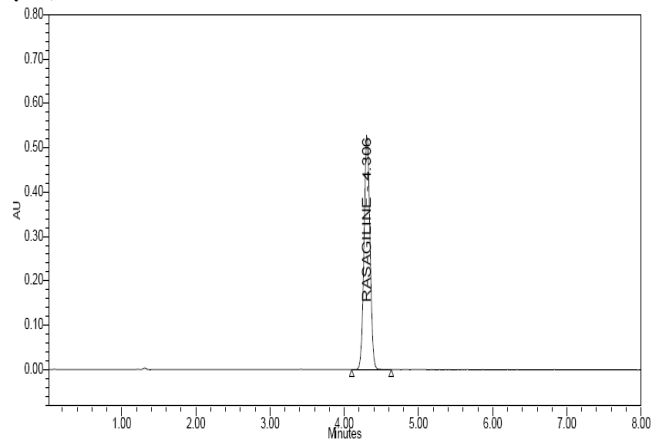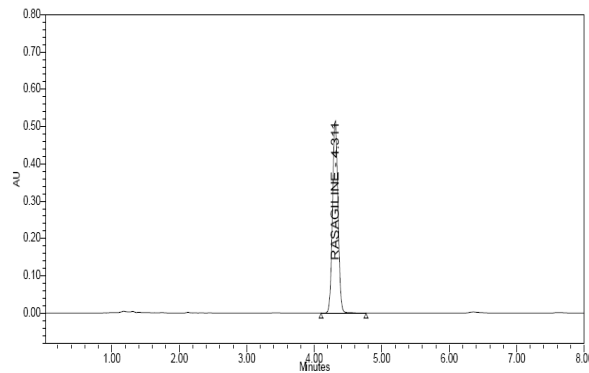**Figure 6A: Typical chromatogram of Rasagiline Standard (Develosil ODS MG-5, C$_{18}$, 150mm X 4.6mm, 5µm)**

Figure 6B: Typical chromatogram of Rasagiline Tablets Test preparation (Develosil ODS MG-5, C$_{18}$, 150mm X 4.6mm, 5µm)

RESULTS AND DISCUSSION

As there is no official method for the estimation of Rasagiline in pharmacopoeia, hence it was felt necessary to develop a sensitive method for estimation of Rasagiline and literature review doesn't show any effective and precise method determination in this dosage formulation.

A method was developed with mobile phase system of buffer and Acetonitrile (60:40) v/v with flow rate 1.0ml/min on Develosil ODS MG-5 C$_{18}$, 150x4.6mm, 5µm particle size with UV Visible detector 210nm gave a satisfactory chromatogram with Rasagiline of retention time 3.804 min. It is shown in fig.10.

Thus the present proposed system provides shorter analysis time and conserves mobile phase system. The method was validated based on United States pharmacopoeia and ICH parameters. The parameters are System Precision and System Suitability, Specificity, Linearity, Precision, Accuracy, ruggedness and robustness.

Specificity

The specificity of the method was confirmed by injecting the placebo and observed that there was no interference due to placebo. These chromatograms are shown in fig. 8A & 8B and in Table 6.

Acceptance criteria: No interference from placebo shall be observed at the retention time of main peak.

Linearity of Detector Response

The data regarding linearity of Rasagiline are given in table 8 and calibration graph are shown in fig. 9. Chromatograms are shown in fig. 10. From the linearity studies the specified range was determined for Rasagiline 4.8155 – 150.4845 μg/mL and Correlation coefficient was found to be 0.999.

Acceptance Criteria: The Correlation coefficient should be not less than 0.999.

Precision

The precision of the method was determined by replicate injections standard solution. The percentage of RSD of assay was to be 0.3 for Rasagiline which was with in the acceptance criteria of 2%. Thus the proposed method was found to be providing high degree of precision and reproducibility. The chromatograms are shown in fig. 10 and data are reported in table 8.

Acceptance criteria:

1. The relative standard deviation of % assay results should be not more than   2.0%.
2. The assay of Rasagiline should be not less than 97.0% and not more than 103.0%.

Accuracy

The validation of the proposed reverse phase HPLC method was further verified by recovery studies. The percentage recovery was found to be within 98.9 – 102.1 %w/w of Rasagiline. This serves a good index of accuracy and reproducibility of the proposed method showed in Table 10. For Linearity of Test Method, a graph is plotted to 'average mg of Rasagiline added' versus 'average mg of Rasagiline recovered' in Accuracy (Recovery) section. The correlation coefficient was found to be 0.999. From the above study, it was established that the method is linear from 5% to 150% of the target concentration shown in Table 10 and Figure 11.

Acceptance criteria: The average recovery of Rasagiline at each spike level should be not less than 97.0% and not more than 103.0%.

Acceptance criteria: Coefficient of correlation should be not less than 0.999.

Ruggedness

a) Analyst to Analyst Variability

The ruggedness of the method was determined by performing by the same assay by different analyst and performing the assay a different day to check the reproducibility. The average % assay obtained by both the analysts was found to be 100.5% and 100.7% with a relative standard deviation of 0.2% and 0.2 % respectively. The system suitability parameters were evaluated as per the test method by both the analysts and found to be within the limits. The results are summarized in Table 11.

Acceptance criteria:

1. The Assay of Rasagiline should be not less than 97.0% and not more than 103.0%.
2. The relative standard deviation of % assay results should be not more than 2.0%.

System to System Variability

System to system variability study was conducted by two system by assaying six different test preparations of Rasagiline Tablets 1 mg. The average % assay obtained by both the system was found to be 100.5% and 100.7% with a relative standard deviation of 0.2%and 0.2 % respectively. The system suitability parameters were evaluated as per the test method by both the system and found to be with in the limits. The results are summarized in Table 12.

Acceptance criteria:

1. The Assay of Rasagiline should be not less than 97.0% and not more than 103.0%.
2. The relative standard deviation of % assay results should be not more than 2.0%.

Column to Column Variability

Column to column variability study was conducted by two column by assaying six different test preparations of Rasagiline Tablets 1 mg.

The average % assay obtained by both the system was found to be 100.5% and 101.3% with a relative standard deviation of 0.2% and 0.6% respectively. The system suitability parameters were evaluated as per the test method by both the system and found to be with in the limits. The results are summarized in Table 13.

Acceptance criteria:

1. The Assay of Rasagiline should be not less than 97.0% and not more than 103.0%.
2. The relative standard deviation of % assay results should be not more than 2.0%.

b) Bench Top Stability of Test Preparation and Standard Preparation

A study to establish the stability of Rasagiline in test preparation and standard preparation on bench top was conducted at initial, 1 day and 2 days. The assay of Rasagiline in test preparation and standard preparation were estimated against freshly prepared standard each time. The difference in % assay of Standard and Test preparations from initial to 1 day and 2 days was calculated and the results are summarized in Table 14.

Acceptance Criteria: The % assay of Rasagiline in standard and test preparation should not differ by more than 2.0 from initial value.

C) Refrigerator Stability of Test Preparation and Standard Preparation

A study to establish the stability of Rasagiline in test preparation and standard preparation in refrigerator was conducted over a period of 2 days. The % assay of Rasagiline in test preparation and standard preparation was estimated at initial, 1 day, 2 days against freshly prepared standard at each time. The difference in % assay of standard and test preparations from initial to 1 day and 2 days were found to be within the limits. The results are summarized in Table 15.

Acceptance Criteria: The % assay of Rasagiline in standard and test preparation should not differ by more than 2.0 from initial value.

Robustness
a) Effect of Variation in Mobile Phase Composition (Acetonitrile)

A study to establish the effect of variation in mobile phase composition was conducted. Three mobile phases were prepared having 90%, 100% and 110% of the method organic phase composition (Acetonitrile). Standard and test solutions prepared as per test method were injected into HPLC system. The system suitability parameters and % assay were evaluated with the mobile phases as per test method and found to be within limits. The results are summarized in Table 16. From the above study, it was established that the allowable variation in organic phase composition (Acetonitrile) in mobile phase is from 90% to 110%.

Acceptance criteria:
1. All the system suitability parameters shall meet the requirements as per the test method.
2. The Average %Assay values should not differ by more than 2.0 when compared with that of the test method values.
3. %Assay should be NLT 97.0% and NMT 103.0%.

b) Effect of Variation in Flow Rate

A study was conducted to determine the effect of variation in flow rate. Standard and test preparations were prepared as per test method was injected into the HPLC system with flow rate 0.8 mL/min, 1.0 mL/min and 1.2 mL/min. The system suitability parameters and

difference from average assay values were evaluated as per test method for the flow rates and found to be within limits. The results are summarized in Table 17.

From the above study, it was established that the allowable variation in flow rate is from 0.8 mL/min to 1.2 mL/min.

Acceptance criteria:
1. All the system suitability parameters shall meet the requirements as per the test method.
2. The Average %Assay values should not differ by more than 2.0 when compared with that of the test method values.
3. %Assay should be NLT 97.0% and NMT 103.0%.

EFFECT OF VARIATION IN pH OF BUFFER IN MOBILE PHASE

A study to establish the effect of variation in pH of buffer in mobile phase was conducted. Three mobile phases were prepared having buffer of pH 6.8, pH 7.0 and pH 7.2. Standard and test solutions prepared as per the test method were injected into the HPLC system using three mobile phases. The system suitability parameters and % Assay were evaluated as per the test method and found to be within the limits. The results are summarized in Table 18.

From the above study, it was established that the allowable variation in p^H of Buffer in mobile phase is from 6.8 and 7.2.

Acceptance criteria:
1. All the system suitability parameters shall meet the requirements as per the test method.
2. The Average %Assay values should not differ by more than 2.0 when compared with that of the test method values.
3. %Assay should be NLT 97.0% and NMT 103.0%

EFFECT OF USING DIFFERENT FILTERS (Filter Validation)

A study to establish the effect of using different filters in test solution and standard was conducted. Standard and test solutions prepared as per the test method were prepared and divided into three portion one portion centrifuged, second portion filtered through HNN filter and third portion filtered through HVF filter followed by injected into the HPLC system using three different condition. The system suitability parameters and % Assay were evaluated as per the test method and found to be within the limits. The results are summarized in Table 19.

From the above study, it was established that both HNN and HVF filter suitable for filtration.

Acceptance Criteria:
The difference of % assay result from centrifuged sample to filtered samples should be not more than 2.0.

CONCLUSION

A HPLC method was developed for the estimation of Rasagiline tablet dosage form using RP-HPLC. A

method was developed with mobile phase system of Buffer : Acetonitrile (60:40) v/v with flow rate 1.0 ml/min on Develosil ODS MG-5 C_{18}, 150x4.6mm, 5µm particle size with UV Visible detector 210nm gave a satisfactory chromatogram with Rasagiline. The peaks of Rasagiline are found well separated at 3.804min. The developed method is validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, ruggedness and robustness. The results obtained are within the acceptance criteria. The proposed method is applied for determination of Rasagiline in marketed formulation. Hence the proposed method is found to be satisfactory and could be used for the routine analysis of Rasagiline in tablet dosage form.

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