STABILITY INDICATING RP-LC METHOD FOR DETERMINATION OF RASAGILINE MESYLATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT: An isocratic stability indicating liquid chromatographic method has been developed and validated for the determination of Rasagiline in bulk drug and its pharmaceutical dosage forms. Separation of the drug with degradation products was achieved using Puroshere Star, C18, 150 x 4.6mm; 5µm column as stationary phase and pH 7.0(±0.05) buffer: Acetonitrile (40:60,v/v) as mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 210 nm. The method is linear over the range of 4.8 – 150.5 µg/mL. The percent recovery of drug in dosage forms was ranged from 98.0 to 102.1. The method is simple, rapid, precise, selective and stability indicating and can be used for the assay in quality control and stability studies samples.

Key words: RPLC, Rasagiline mesylate, Stability, Dosage forms

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. 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)

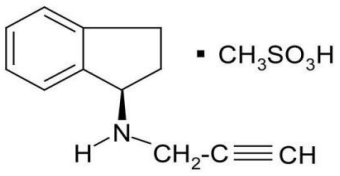


Figure-1 Chemical structure of Rasagiline mesylate

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 forms[7-20]. Hence, an LC procedure was developed to serve as a rapid and reliable method for the determination of Rasagiline in the presence of related impurities in bulk and pharmaceutical dosage forms. In the proposed method, related impurities were well separated and eluted with in 8 min. Finally the method was thoroughly validated for the assay of Rasagiline mesylate Tablets.

2.0 EXPERIMENTAL

2.1 Instrumentation

The Waters LC system equipped with 2489 pump and 2996 Photodiode array (PDA) detector was used. The output signal was monitored and integrated using Waters Empower 2 software.

2.2 Solutions

2.2.1 pH 7.0 buffer solution

Weighed accurately 2.73 g of potassium dihydrogen orthophosphate anhydrous and dissolved in 1000mL of milli-Q water and pH was adjusted to 7.0±0.05 with potassium hydroxide solution.

2.2.2 Mobile phase

A mixture of pH 7.0 buffer and acetonitrile in the ratio 40:60(v/v) was prepared and filtered through 0.45 µm nylon membrane filter and degassed for about 10 min.

2.2.3 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.

2.2.4 Standard solution (100µg/ml)

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.

2.2.5 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.

2.2.6 Preparation of Samples for Specificity Study

For **Acid degradation** Rasagiline sample was stressed with 1N HCl on mantel for 4 hours at 80°C and then neutralized by adjusting pH to 7.0 with 1 N NaOH. The solution was further diluted to the required concentration with the diluent.

For **Alkali degradation** Rasagiline sample was stressed with 2 N NaOH on mantel for 2 hours at 80°C and then neutralized by adjusting pH to 7.0 with 2N HCl. The solution was further diluted to required concentration with the diluent.

For **Oxidative degradation** Rasagiline sample was stressed with 1% H_2O_2 for 30 minutes on Bench top. The solution was further diluted to required concentration with the diluent.

For **Water degradation** Rasagiline sample was stressed with water by heating on mantel at 80ºC for 6 hours. The solution was further diluted to required concentration with the diluent.

For **Humidity degradation** Rasagiline sample was stressed at 25°C/90% RH for 288 hours.

For **Photolytic stress** the samples were exposed to UV light at 288 nm for 54 hours and visible light for 288hours meeting the specification of ICH i.e. UV (200 watt/m^2) and Visible (1.2 million Lux hours).

For **Thermal degradation** samples were exposed to temperature at 70ºC for 24 hrs.
The Photolytic, Humidity and Thermal stress sample solutions were prepared to required concentration with the diluent.

2.2.7 Chromatographic Conditions

A Puroshere Star, C18 (150 x 4.6 mm; 5 µm packing) column was used for analysis at column temperature 40ºC. The mobile phase was pumped through the column at a flow rate of 1.0 mL/min.The sample injection volume was 10µL. The photodiode array detector was set to a wavelength of 210 nm for the detection.

RESULTS AND DISCUSSION

3.1 Method development

3.1.1 Separation of known degradation impurities

To develop a suitable rapid, rugged and robust LC method for the determination of Rasagiline, different mobile phases and columns were employed to achieve the best separation and resolution. The method development was started with a Peerless basic, C18 (150 x 4.6 mm; 5 µm packing) column using a mobile phase containing pH 7.0 buffer and Acetonitrile in the ratio 70:30 with 1.0 mL/min, where elution was found to be very broad. Early elution with slight separation was observed with mobile phase consisting of above components in the ratio 30:70. Finally the mobile phase with the ratio 40:60 was found to be appropriate with good separation and symmetrical peak shape to get Rasagiline peak RT about 3 min with 1.0 ml/min using Puroshere Star, C18 (150 x 4.6 mm; 5 µm packing) column Figure-2. Under the last condition all related compounds were eluted with in 8 min and well separated. The chromatogram of Rasagiline sample spiked with the related impurities using the proposed method is shown in Figure -3. In the proposed method the resolution is more than 2 between the Rasagiline and 1-Amino indane Rasagiline. System suitability results of the method are presented in Table 1. Rasagiline and its related compounds show significant UV absorbance at wavelength 210 nm. Hence this wavelength has been chosen for detection in the analysis of Rasagiline.

TABLE 1: SYSTEM SUITABILITY REPORT

Compound	Tailing Factor [a]	Theoretical plates[a]	Resolution[a]	%RSD[a]
Rasagiline	1.1	13890	9.5	0.2

[a] **Number of samples analyzed are six**

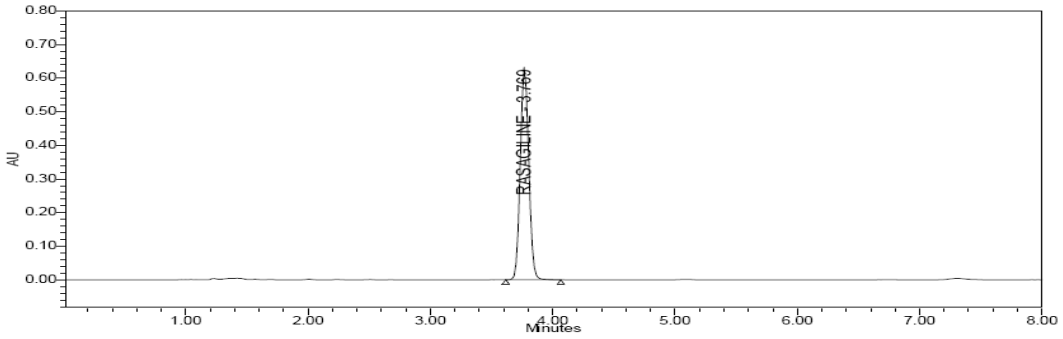


Figure -2: TYPICAL LC CHROMATOGRAM OF FORMULATED RASAGILINE (1mg)

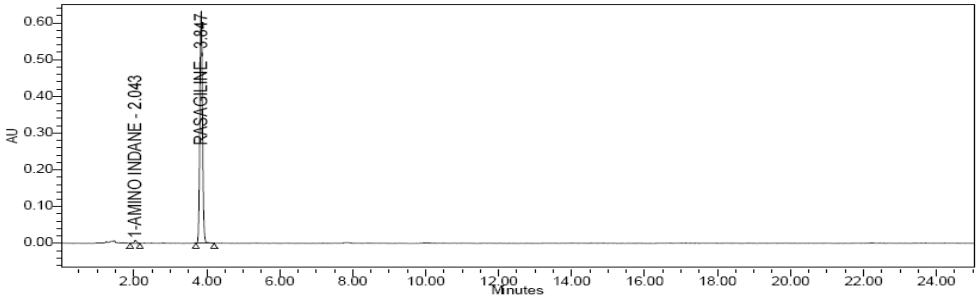


Figure -3: TYPICAL LC CHROMATOGRAM OF FORMULATED RASAGILINE SPIKED WITH IMPURITY -Impurity: 1-Amino indane

3.1.2 **Column Selection** (Merck Index 1996,2003)

Based on the retention and separation of the compounds Puroshere Star, C18 (150 x 4.6 mm; 5 µm packing) column was selected as suitable column for the analysis of Rasagiline.

3.2 **Method Validation** (ICH Switzerland 1995, Geneva 2003)

The developed LC method was extensively validated for assay of Rasagiline mesylate Tablets using the following parameters.

3.2.1 Specificity

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 the estimation of Rasagiline in Rasagiline mesylate tablets.

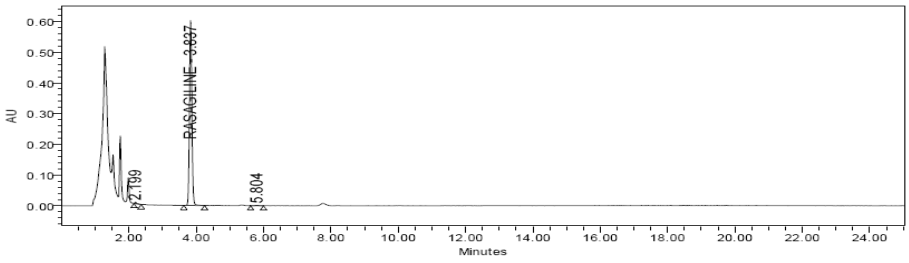
Interference from degradation products

A study was conducted to demonstrate the effective separation of degradants from Rasagiline peak. Separate portions of Drug product, Drug substance and Placebo were exposed to the following stress conditions to induce degradation. Stressed samples were injected into the HPLC system with PDA detector by following test method conditions. All degradant peaks were resolved from Rasagiline peak in the chromatograms of all samples. The chromatograms of the stressed samples were evaluated for peak purity of Rasagiline using Empower 2 software. In all the forced degradation samples, Rasagiline peak purity angle was less than purity threshold.

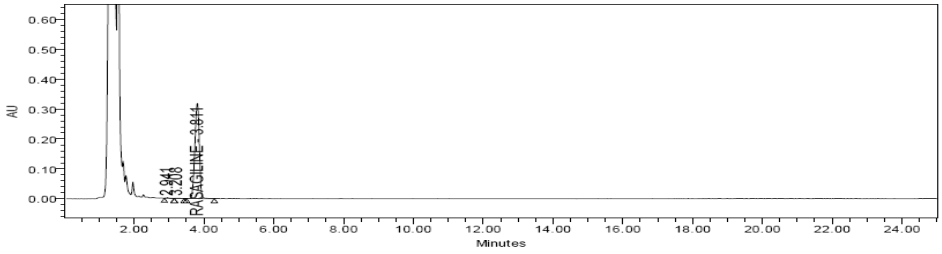
From the above results it is clear that the method can be used for determining the stability of Rasagiline as bulk and pharmaceutical formulations. Figure-4 shows the separation of Rasagiline from its degradation products.

Figure -4: HPLC CHROMATOGRAMS OF RASAGILINE AND ITS DEGRADATION PRODUCTS

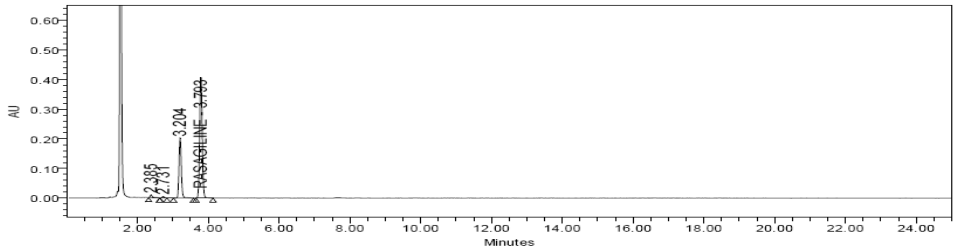
ACID DEGRADATION



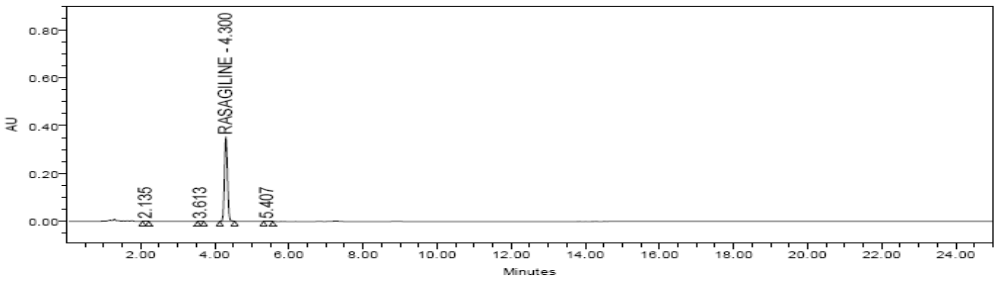
ALKALI DEGRADATION



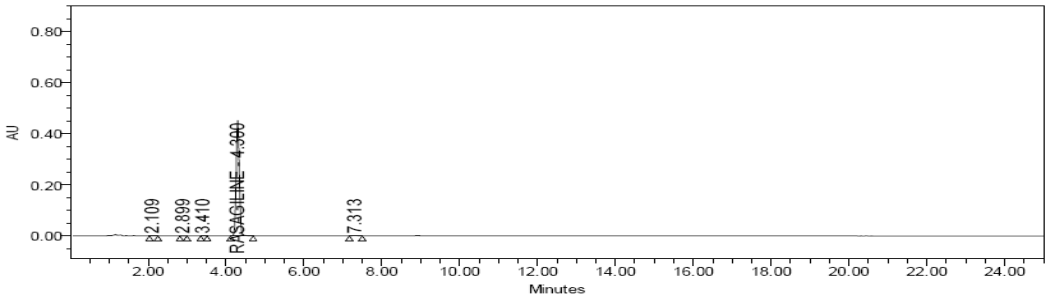
OXIDATIVE DEGRADATION



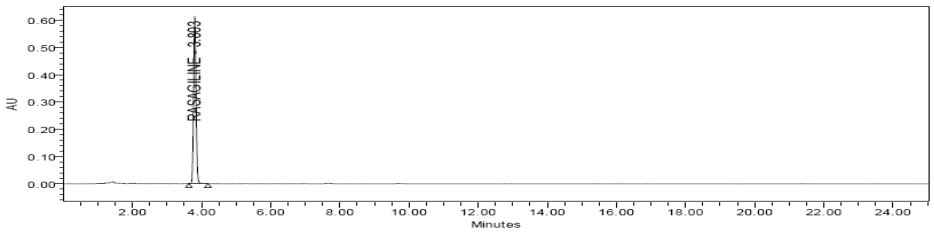
PHOTOLYTIC DEGRADATION



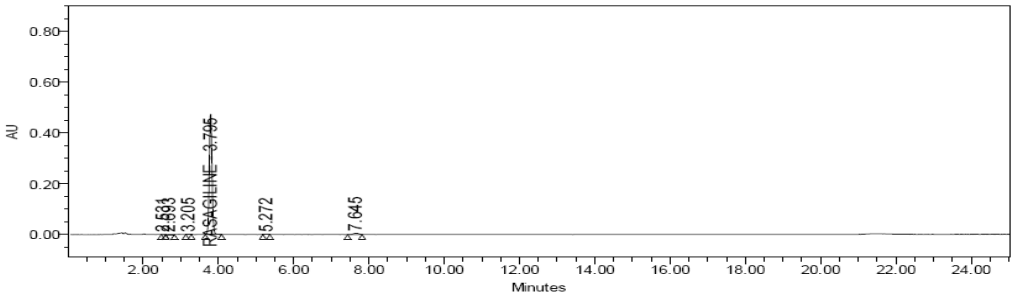
HUMIDITY DEGRADATION



HEAT DEGRADATION



WATER DEGRADATION



3.2.2 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 4.8155 µg/mL to 150.4845 µg/mL. A graph was plotted to concentration in µg/mL on the abscissa versus response on the ordinate. The detector response was found to be linear (Figure- 5) with a correlation coefficient of 0.999.

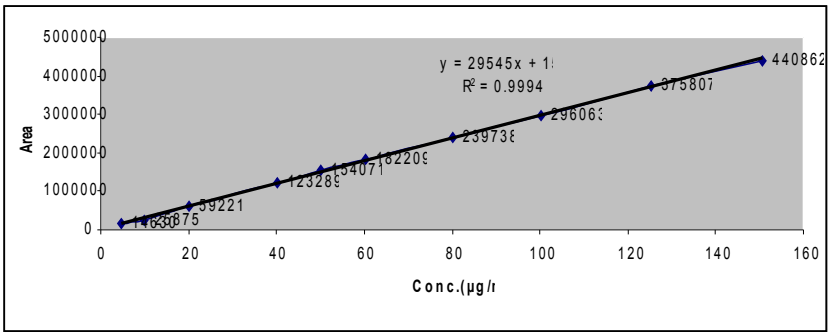


Figure 5: LINEARITY OF DETECTOR RESPONSE GRAPH

3.3.3) Precision of test Method

The precision of the test method was conducted by assaying six samples of Rasagiline mesylate tablets. The Average % assay of Rasagiline in Rasagiline mesylate tablets was found to be 100.5% with an RSD of 0.6%. The results are given in Table -2.

TABLE 2: RESULTS FOR PRECISION OF TEST METHOD

Sample No.	%Assay
1	100.3
2	100.2
3	100.8
4	100.3
5	101.5
6	99.7
MEAN	100.5
%RSD	0.6

3.3.4) Accuracy

A study of recovery of Rasagiline from spiked placebo was conducted at six different spike levels i.e.5%, 25%, 50%, 75%, 100% and 150%. Samples were prepared by mixing placebo with Rasagiline mesylate raw material equivalent to that of the target initial concentration of Rasagiline. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery and correlation coefficient were calculated and given in Table- 3. The mean recoveries of Rasagiline from spiked were found to be in the range of 98.0-102.1%.

TABLE 3: ACCURACY IN THE ASSAY DETERMINATION OF RASAGILINE

Sample No.	Spike level	'mg' added	'mg' found (recovered)	% Recovery	Average %recovery
1.	5%	0.50	0.51	102.0	102.0
2.		0.50	0.51	102.0	
3.		0.50	0.51	102.0	
4.	25%	2.55	2.60	102.0	102.1
5.		2.55	2.61	102.4	
6.		2.55	2.60	102.0	
7.	50%	4.99	5.04	101.0	100.9
8.		4.99	5.03	100.8	
9.		4.99	5.04	101.0	
10.	75%	7.49	7.59	101.3	101.5
11.		7.49	7.60	101.5	
12.		7.49	7.62	101.7	
13.	100%	9.94	10.00	100.6	100.2
14.		9.94	9.94	100.0	
15.		9.94	9.94	100.0	
16.	150%	14.81	14.52	98.0	98.0
17.		14.81	14.57	98.4	
18.		14.81	14.46	97.6	

3.3.5) Ruggedness

A study to establish the stability of Rasagiline in standard and test solutions were conducted on bench top and Refrigerator at Initial, 1 day and 2 days. The assay of Rasagiline in standard and test solutions was estimated against freshly prepared standard each time. The difference in % assay of standard and test solutions from initial to 1 day and 2 days was calculated and given in Table- 4.

TABLE 4: RUGGEDNESS
STABILITY DATA OF RASAGILINE IN STANDARD AND TEST SOLUTIONS

BENCH TOP STABILITY						
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		NA	102.8	102.1	NA	NA
1	64.2	0.3	101.3	102.5	1.5	0.4
2	63.9	0.0	103.9	101.3	1.1	0.8
REFRIGERTOR STABILITY						
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		NA	102.8	102.1	NA	NA
1	64.3	0.4	102.1	100.7	0.7	1.4
2	63.1	0.8	101.0	101.3	1.8	0.8

*** Potency of Rasagiline on as is basis**

From this study, it was established that the test preparation and standard are stable for a period of 2 days on bench top and Refrigerator

3.3.6) Robustness

A study to establish the effect of variation in mobile phase organic phase composition, variation in mobile phase pH of buffer solution, variation in column oven temperature and variation in Flow rate was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated and given in Table-(5A-5D). From the above study the proposed method was found to be Robust.

ACKNOWLEDGEMENTS

The authors wish to thank the Orchid Healthcare for providing the samples of Rasagiline

TABLE 5: ROBUSTNESS DATA OF RASAGILINE IN TEST SOLUTIONS

TABLE: 5A

Effect of variation in Mobile phase composition(Acetonitrile)				
System Suitability	**Organic phase ratio (Acetonitrile)**			
	100%	90%	110%	**Acceptance criteria**
USP Tailing factor for Rasagiline Peak	1.1	1.0	1.1	NMT 2.0
USP Plate count for Rasagiline Peak	14094	13852	13438	NLT 2500
%RSD of Rasagiline peak area from five replicate injections of standard solution	0.5	0.3	0.1	NMT 2.0%

Organic phase ratio (Acetonitrile)	**% Assay of test preparation**		**Avg. Assay**	**Difference**
	Trail - 1	**Trail - 2**		
100%(Organic phase)	99.6	98.6	99.1	NA
90%(Organic phase)	100.5	99.9	100.2	1.1
110%(Organic phase)	100.0	99.4	99.7	0.6

TABLE: 5B

Effect of variation in pH of Mobile phase				
System Suitability	**Variation in buffer solution pH**			
	pH 6.8	**pH 7.0**	**pH 7.2**	**Acceptance criteria**
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 of Rasagiline peak area from five replicate injections of standard solution	0.1	0.3	0.2	NMT 2.0%

Variation in buffer solution pH	**% Assay of test preparation**		**Avg. Assay**	**Difference**
	Trail - 1	**Trail - 2**		
pH 7.0	99.8	99.4	99.6	NA
pH 6.8	100.1	100.6	100.4	0.8
pH 7.2	100.3	100.2	100.3	0.7

TABLE: 5C

Effect of variation in Flow rate					
System Suitability	**Variation in Flow rate (mL/min)**				
	0.8	**1.0**	**1.2**	**Acceptance criteria**	
USP Tailing factor for Rasagiline Peak	1.1	1.1	1.1	NMT 2.0	
USP Plate count for Rasagiline Peak	14548	14024	13149	NLT 2500	
%RSD of Rasagiline peak area from five replicate injections of standard solution	0.1	0.8	0.2	NMT 2.0%	
Variation in Flow rate (mL/min)	**% Assay of test preparation**		**Avg. Assay**	**Difference**	
	Trail - 1	**Trail - 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	

TABLE: 5D

Effect of variation in Column oven temperature					
System Suitability	**Variation in Column oven temperature**				
	40°C	35°C	45°C	**Acceptance criteria**	
USP Tailing factor for Rasagiline Peak	1.1	1.1	1.0	NMT 2.0	
USP Plate count for Rasagiline Peak	14094	13589	13470	NLT 2500	
%RSD of Rasagiline peak area from five replicate injections of standard solution	0.5	0.3	0.2	NMT 2.0%	
Variation in Column oven temperature	**% Assay of test preparation**		**Avg. Assay**	**Difference**	
	Trail - 1	**Trail - 2**			
40°C	99.6	98.6	99.1	NA	
35°C	100.1	99.2	99.7	0.6	
45°C	99.4	98.0	98.7	0.4	

REFERENCES

British Pharmacopeia, Vol. I & II and Vet 1993, HMSO, London, 1999.
Comprehensive review of rasagiline, a second-generation monoamine oxidase inhibitor, for the treatment of Parkinson's Disease. Clinical Therapeutics, Volume 29, Issue 9, September 2007,Pages 1825-1849, Jack J. Chen, David M. Swope and Khashayar Dashtipour.
Chattan, L.G., Ed., Pharmaceutical Chemistry, Vol I & II, Moral Dekker Inc, New York, 1966.
C.S.P. Sastry, D.G. Sankar, M.N. Reddy and M. Aruna, Indian J. Pharm. Sci., 50, 1998, 178
European Pharmacopeia, 3rd Edition, 1997 and Supplement, Council of Europe, Strasbourg, 1999.
Goodman, L.S. and Gilman, A.G., The Pharmacological Basis of Therapeutics, 9th Edn By Hardman, J.G.Limbard, L.E.; Editors in Chief, McGraw – Hill, 1996.
Higuchi, T., Brochman Hansen, E., Edt., Pharmaceutical Analysis, Interscience, London, 1961.
ICH stability testing of new drug substances and products (Q1AR2), International Conference on Harmonization, IFPMA, Geneva, 2003
ICH Draft Guidelines on Validation of Analytical Procedures: Text and methodology (Q2R1), IFPMA, Switzerland, 1995 .
Indian Pharmacopeia, Vol I, II, 1996 & Addendum 2000 and Vet, Government of India, Ministry of Health and Family Welfare, Controller of Publications, Delhi, 2000.
Japan Pharmacopoeia, XIIIth Edition, 1996.
Journal of Pharmaceutical and Biomedical Analysis, Volume 49, Issue 5, 12 July 2009, Pages 1185-1191 Marcos Fernández, Emilia Barcia and Sofía Negro .
Journal of Chromatography B, Volume 873, Issue 2, 1 October 2008, Page i ,Water Research, Volume 22, Issue 7 , July 1988, Pages 873-881, Yu-Ping Chin, Carole Sue Peven, Walter J.Weber Jr
Reynolds, J.E.F.; Martindale, The Complete Drug Reference (Extra Pharmacopeia) 30th Edition, The Pharmaceutical Press, London, 1993.
Rapid and sensitive liquid chromatography-tandem mass spectrometry: assay development, validation and application to a human pharmacokinetic study. Song M, Wang L, Zhao H, Hang T, Wen A, Yang L, Jia L. Department of Pharmaceutical Analysis, China pharmaceutical University
The Merck Index, 12th Edition, Merck & Co Inc, New York, 1996.
The Merck index, 13th edition**,** Merck Research Laboratories., Division of Merck and CO, INC**,** White House station, NJ, 2003
The Drugs and Cosmetics Act and Rules, Government of India Publications, 1984
United States Pharmacopeia USP XXIV & NF IXX, 2000 and 4 Addendum, USP convention Inc., Rockville, 2002.
Validated LC–MS/MS method for quantitative determination of rasagiline in human plasma and its application to a pharmacokinetic study . Journal of Chromatography B,Volume 873,Issue 2, 1 October2008, Pages203-208,Jinfei Ma, Xiaoyan Chen, Xiaotao Duan, Pan Deng, Hui Wang and Dafang Zhong Nanjing 210009,China
Wolff, M.E., Ed., Burger's Medicinal Chemistry, Part IV, 4th Edn., Wiley Interscience, New York, 1981.

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