



ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Development of RP-HPLC method for the estimation of Rasagiline mesylate in bulk and tablet dosage forms

*Napa Delhi Raj¹, Abburu Rukmangada Rao¹, Chusena Narasimharaju Bhimanadhuni²

¹Nimra college of Pharmacy, Nimra nagar, Jupudi-521456, Vijayawada, Krishna (Dt), Andhra Pradesh, India

²Department of Pharmaceutical Analysis, Annabattuni Satya Narayana Pharmacy College, Burripalem Road, Tenali-522201, Guntur (Dt), Andhra Pradesh, India

ABSTRACT

A simple RP-HPLC method for the determination of Rasagiline Mesylate in bulk and tablet dosage form was developed. Numerous HPLC conditions were tested for determination of rasagiline. The best result was achieved by using Purosphere star RP-18, (150×4.6mm), 5µm column and a mobile phase consisting of Potassium Orthophosphate: Acetonitrile (60:40 v/v) adjusted to pH 7.0(±0.05) with Ammonia solution, a flow rate of 1.5 ml/min with ultraviolet detection at 210nm. The correlation coefficients for calibration curves within the detection range of 5-30µg/ml were 0.9993. The within and between-day precision was determined for both retention time and peak area. The retention time of rasagiline is 6.0 minutes.

Key Words: Rasagiline, Purosphere star RP-18, Tablets, Estimation, RP-HPLC, Validation, Method development.

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)-, methane-sulfonate. 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 enantiomers (Chen *et al.*, 2007). Literature survey reveals that only a few methods based on RP-HPLC method was developed and validated for the determination of rasagiline mesylate in pharmaceutical dosage forms (Vijayalakshmi *et al.*, 2010).

Stability indicating RP-LC method for determination of rasagiline mesylate in bulk and pharmaceutical dosage forms (Kumar *et al.*, 2010). LC-mass Spectrometry method for the determination of rasagiline mesylate in human plasma (Song *et al.*, 2007). LC-MS-MS method for the study of metabolism, excretion and pharmacokinetics of rasagiline in healthy human subjects (Ma *et al.*, 2003). Validated and stability indicating dissolution test with reverse phase-hplc analysis for rasagiline mesylate in tablet dosage form (Poongoothai *et al.*, 2011). Spectrophotometric method in ultraviolet region has been developed for the determination of Rasagiline in bulk and in pharmaceutical formulations (Rama *et al.*, 2010). Reverse phase liquid chromatography method for the quantification of rasagiline mesylate in biodegradable PLGA microspheres. (Fernandez *et al.*, 2008). The present investigation by the author describes a rapid, accurate and precise RP-HPLC method for the determination of rasagiline mesylate from bulk sample and pharmaceutical dosage form. Since this drug is being marketed in domestic and international market. The method was validated as per ICH guidelines.

*Corresponding Author:

Napa Delhi Raj, Associate Professor
Department of Pharmaceutical analysis
Nimra college of pharmacy, Nimra nagar, Jupudi- 521456
Vijayawada, Krishna (Dt), Andhra Pradesh, India.
E-mail: pharmaraj1981@gmail.com
Contact No.: +91 8985351361

Table 1: Analysis of pharmaceutical formulation.

Pharmaceutical formulation	Labelled amount (mg)	Assay found by proposed method (%) (mg) ^a	Recovery by proposed method (%) ^b
Rasagiline tablet	0.5	98±0.4	99.85±0.41
Rasagiline tablet	1.0	98±0.2	99.95±0.54

^aAverage ±SD (n=6)

^bRecovery of 0.5 and 1.0mg added to the pharmaceutical preparation (average of six determinations)

EXPERIMENTAL

The waters HPLC equipped with 2487 pump and UV-detector was used. The output signal was monitored and integrated using Waters Empower 2 software. The reference standard of Rasagiline was supplied by M/s Orchid Pharmaceuticals, Chennai, India and Rasagiline Tablets (Azilent 1mg) was purchased from local drug store. HPLC Acetonitrile was purchased from Merck (Mumbai, India) and potassium dihydrogen orthophosphate and ammonia solution was obtained from S.D Fine (Mumbai, India). All chemicals were of analytical grade. The column used in the development for determination is Purosphere star RP-18, (150×4.6mm), 5µm column.

Mobile phase

Accurately 1.36g of potassium orthophosphate was weighed and dissolved in 1000ml of milli-Q water. The pH of the above solution was adjusted to 7.0±0.05 with Ammonia solution. Phosphate buffer solution and Acetonitrile was mixed in the ratio of 600:400 v/v, filtered through solution through 0.45µm nylon membrane filter and degassed for about 10 min. The mobile phase was used as diluents. The mobile phase was filtered through 0.05µm membrane filter and sonicated by using Power Sonicator, model no. 405, Hwashin Technology, Korea before use. The detector wavelength was set at 210nm. A flow rate of 1.5ml/min was used for the determination of rasagiline. The samples and standards were dissolved in the mobile phase and 20µl samples were injected into the HPLC system at the column and sample temperature of 30°C.

Preparation of Standard and Sample solutions

Accurately 39.0mg of Rasagiline mesylate was weighed and transferred about into a 100ml volumetric flask and added about 60ml of diluent, sonicated to dissolve, maintained the solution to room temperature and diluted to the volume with diluent. 2.0ml of the above solution was transferred into a 50ml volumetric flask and diluted to the

volume with diluent. The Solution was filtered through 0.45µm nylon membrane filter.

Ten tablets of rasagiline were weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 5mg of rasagiline was transferred into a 200ml volumetric flask. 120ml of diluent was added, stirred for 10minutes on Orbital shaker and Sonicated for 20minutes with occasional stirring, maintained the solution to room temperature and diluted to the volume with diluent, filtered the solution through 0.45µm filter. Transferred 10ml of the above filtered solution into a 25ml volumetric flask & dilute to the volume with diluent. The solution was filtered through 0.45µm nylon membrane filter.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive precise and accurate HPLC method for the analysis of rasagiline in tablets. In order to achieve optimum separation of the component peaks, mixtures of buffer with Acetonitrile in different combinations were tested as mobile phase on a Purosphere Star, RP-18, (150 x 4.6 mm), 5 µm column using with mobile phase of Potassium Orthophosphate: Acetonitrile (60:40) adjusted to pH 7.0±0.05 with Ammonia solution that good for the determination of rasagiline in tablets. The retention time of the compound was found to be 6 min (Fig.1). The main

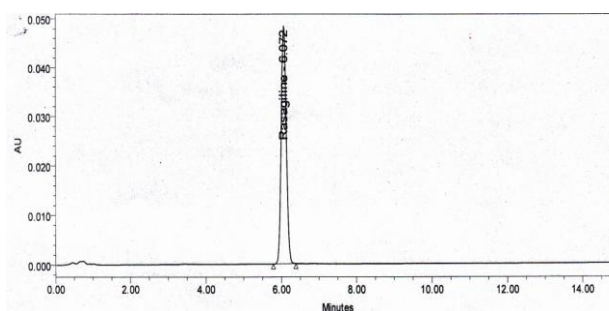


Figure 1: Chromatogram of Rasagiline.

Table 2: Stability.

Experiment	Percentage degradation	Purity angle	Purity threshold
Photolytic (2-day in Sun Light)	3.71	0.120	1.349
Thermal (2-day in 110°C)	38.23	0.145	1.158
Alkali Deg (2-N NaOH 1ml)	21.48	0.890	2.335
Acid Deg (2-N HCL 1ml)	45.19	0.152	1.180
H ₂ O ₂ 50%w/w (1ml)	29.19	0.140	1.136

advantage of this method is that it is simpler to carry out with regard to the preparation of samples and conditions used and thus it is less time consuming and less costly with the use of Purosphere Star, RP-18, (150 x 4.6 mm), 5 µm column.

To validate the RP-HPLC method, a series of tests were made using the most promising conditions. A calibration curve was made and concentration examined within the detection range of 5-30µg/ml for rasagiline the correlation coefficient was found to be 0.9993. The within-day, precision (expressed as the relative standard deviation (R.S.D)) for area under the curve (AUC) and retention times was determined for rasagiline for repeated analysis (n=6) was found to be 0.17 and 0.79 respectively. Average within-day R.S.D values for between-day precision obtained for AUC were 0.41%. The assay values obtained by proposed method and recovery experiment values obtained were performed by adding a fixed amount of drug to preanalyzed formulation summarized in Table 1. The stability of sample was checked by forced degradation in different conditions and percentage of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The values in Table 2 indicate that the any other impurity is not merging with the main peak. The analyte solution was stable up to 14hrs. The reliability of the method was determined by made small deliberate variations in method parameters and the RSD values in Table 3, an indication of its reliability on normal usage.

CONCLUSION

A method was developed for the determination for rasagiline in tablets which is simple, quick, reliable,

Table 3: Robustness.

Sl. No.	Condition	%RSD
1	Flow (+10%)	0.20
2	Flow (-10%)	0.09
3	Temperature (35°C)	0.38
4	Organic (+2%Abs)	0.57
5	Organic (-2%Abs)	0.13
6	Wavelength (+5nm)	0.16
7	Wavelength (-5nm)	0.16
8	pH of the buffer (+0.2)	0.32
9	Robustness – pH of the buffer (-0.2)	0.55

inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

ACKNOWLEDGEMENT

The authors are thankful to Principal, Dr. S. Duraivel, Nimra College of Pharmacy, Vijayawada, Andhra Pradesh for encouragement and providing laboratory facilities to carry out this research work.

REFERENCES

- Chen, J.J., Swope, D.M. and Dashtipour, K. (2007) Comprehensive review of rasagiline, a second-generation monoamine oxidase inhibitor, for the treatment of Parkinson's Disease, *Clinical Therapeutics* .2007, vol. 29, no. 9, 1825–1849. [\[DOI\]](#)
- Fernandez M., Barcia E., Negro S. (2009) Development and validation of a reverse phase liquid chromatography method for the quantification of rasagiline mesylate in biodegradable PLGA microspheres. *J Pharm Biomed Anal.*, 49(5), 1185-1191. [\[DOI\]](#)
- Kumar NR, Rao NG. and Naidu PY (2010) Stability indicating RP-LC method for determination of rasagiline mesylate in bulk and pharmaceutical dosage forms, *International Journal of Applied Biology and Pharmaceutical Technology*, vol. 1,no. 2,247-259.
- Ma, J., Chen, X., Duan, X., Deng, P., Wang, H., Zhong, D. (2008) Validated LC-MS/MS method for quantitative determination of rasagiline in human plasma and its application to a pharmacokinetic study. *J Chromatogr B*, 873, 203- 208.
- Rama, B., Preeti, K. (2010) UV Spectrophotometric method for the determination of rasagiline mesylate in bulk and pharmaceutical formulations,"*International Journal of Pharmaceutical Sciences Review and Research*, vol. 5, no. 1, pp. 5–7.
- Song, M., Wang, L., Zhao, H., Hang T.J., Wen, AD, Yang L, Jia L. (2008) Rapid and sensitive liquid chromatography-tandem mass spectrometry: Assay development validation and application to a human pharmacokinetic study. *J Chromatogr B*, 875, 515-521.
- Sundaramurthy Poongothai, Varadarajan Balaji, Rajasekhar Reddy, Raju Ilavarasan (2011) *International Journal of Pharmaceutical Sciences Review and Research*.2011, Volume 10, Issue 2, 18-23.
- Vijayalakshmi, M., Seshagiri Rao, J.V.L.N. and A. Lakshmana Rao (2010) Development and validation of RP-HPLC method for the estimation of rasagiline tablet dosage forms, *Rasayan Journal of Chemistry*, vol. 3, no. 4, pp. 621–624.