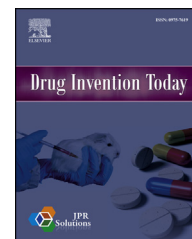




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Original Article

Rasagiline hemitartrate: Synthesis, characterization and RP-HPLC validation for its estimation in bulk form

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ABSTRACT

Objectives: To develop a reverse phase high performance liquid chromatographic method for validation and quantitative estimation of the synthesized drug rasagiline hemitartrate in bulk form.

Methods: Rasagiline hemitartrate was synthesized and characterized by spectral (Infrared, Proton Nuclear Magnetic Resonance and Mass) as well as elemental analysis. Chromatographic separation was conducted on Agilent TC-C18 (250 × 4.6 mm, 5 μm) column at ambient temperature using mixture of 20 mM potassium dihydrogen orthophosphate buffer (pH 7.0): methanol and acetonitrile in the ratio (30:30:40 v/v) as a mobile phase and at a flow rate of 1.0 mL/min, while UV detection was performed at 285 nm. In addition to LOD and LOQ, other analytical parameters viz., linearity, precision, accuracy, ruggedness and robustness were detected by following the ICH (International Conference on Harmonization) guidelines.

Results: The retention time for rasagiline hemitartrate was found to be 4.30 ± 0.05 min. The method was found to be linear in the range of 10–50 μg/mL. The limit of detection and quantization for rasagiline hemitartrate are found to be 0.651 and 1.972 μg/mL respectively. Analytical recovery was 100.47%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. Correlation coefficient was found to be 0.9952.

Conclusion: In this study, simple, sensitive, accurate and reliable RP-HPLC method was developed and validated as per the ICH guidelines for the determination of the synthesized drug rasagiline hemitartrate in bulk form.

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1. Introduction

R(+)-N-propargyl-1-aminoindan (rasagiline) 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.¹ It is a propargylamine-based drug

indicated for the treatment of idiopathic Parkinson's disease.² In addition to rasagiline base, its acid addition salts (viz., mesylate, maleate, fumarate, tartrate, hydrobromide, esylate, p-toluenesulfonate, benzoate, acetate, phosphate and sulfate) are pharmaceutically acceptable.³ Rasagiline was generally well tolerated in clinical trials as both monotherapy and when

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administered with other anti-parkinsonian drugs.⁴ Rasagiline mesylate is a highly potent, selective, irreversible, second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAOB)^{5,6} which has been evaluated for the treatment of Parkinson's disease. Preclinical studies have demonstrated that propargylamines can protect neurons from a variety of toxins in both in vitro and in vivo models.⁷

Literature collection shows various assay methods for both rasagiline and its mesylate. For analysis of rasagiline mesylate in pharmaceutical dosage form, a thin-layer chromatographic method was established.² Visible spectrophotometric methods were developed by using chromogens⁸ and by formation of colored ion-pair complexes in acidic medium.⁹ UV spectrophotometric method was developed for the estimation of rasagiline in bulk and pharmaceutical formulation.¹⁰ HPLC methods for the estimation of rasagiline in tablet dosage form were developed using different mobile phase mixtures.¹¹⁻¹⁶ LC-MS/MS method was used for determination of rasagiline mesylate in human plasma of healthy Chinese volunteers.¹⁷⁻¹⁹

Youdim et al²⁰ and Peskin et al²¹ have patented the preparation of rasagiline salts which are pharmaceutically acceptable. Patent work of Stephen et al²² shows the preparation and characterization of rasagiline derivative viz., rasagiline succinate, rasagiline L-hemitartrate, rasagiline hydrochloride and rasagiline besylate by the addition of concerned acid to rasagiline base. Out of the above salts, no further work was reported on rasagiline hemitartrate which is chemically (R)-N-2-Propynyl-1-indanamine hemitartrate and its chemical structure was shown in Fig. 1. It is a white crystalline powder with molecular formula of $C_{12}H_{13}N \cdot \frac{1}{2}C_4H_6O_6$.

In view of lack of commercial supplier and non-development of assay method till the date for rasagiline hemitartrate, the present study was aimed at its synthesis and characterization followed by development of RP-HPLC method for its validation in bulk form by conducting systematic trails.

2. Materials and methods

Acetonitrile (HPLC grade) and potassium dihydrogen orthophosphate, ortho phosphoric acid, triethyl amine, methanol, isopropyl alcohol, L-tartaric acid (G.R.) used were products of Merck Pvt. Ltd., India. Milli-Q water was used for buffers and other reagents preparation. Buffer solutions were prepared according to US Pharmacopoeia.²³

2.1. Synthesis of rasagiline hemitartrate

About 1.71 g of rasagiline base (0.01 M) was dissolved in 12 mL of isopropyl alcohol and then 1.5 g of L-Tartaric acid (0.01 M)

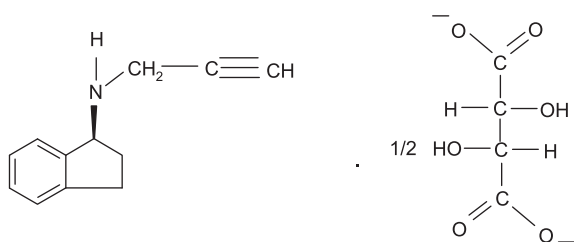


Fig. 1 – Structure of rasagiline hemitartrate.

was added. At warm condition (40 °C), the mixture was stirred continuously for 1 h. The contents were cooled to the room temperature and stirring was continued for 24 h at room temperature. The obtained rasagiline hemitartrate was filtered, washed with isopropyl alcohol. Then the crude product was recrystallized from a mixture of methanol and isopropyl alcohol (1:1). The wet solid dried under vacuum. 1.2 g of dry solid product in the form of white crystalline powder was obtained. The current procedure was modification to that of Stephen et al.²²

The synthesized rasagiline hemitartrate was freely soluble in water, ethanol, methanol, and acetonitrile and sparingly soluble in isopropyl alcohol. Its melting point was 212 °C and characterized by spectral studies using Perkin Elmer 1600 series Fourier Transformer-Infrared Spectrophotometer in KBr – Pellet method; Bruker 400 MHz NMR spectrometer (Bruker Bioscience, Billerica, MA, USA); Triple Quadrupole Mass Spectrometer (SHIMADZU, QMS-8030) and CHNS analyzer (SDCH-435, Hunan Sundry Science and Technology Development Co., Ltd.).

2.2. Instrument and conditions for HPLC determination

Agilent-1220 Infinity LC HPLC instrument (Japan) equipped with a Gradient pump was used to perform chromatography. A reverse phase Agilent TC-C18 (250 × 4.6 mm, 5 μm in particle size) column was used. The detection was achieved by SPD – 20A prominence UV-Visible detector (Japan). EZICHRON chromatographic software was used for data acquisition and processing. Sample was injected using a Rheodyne – 7725 injection valve via a 20 μL loop.

2.3. Method development

A number of parameters such as solvent, mobile phase composition and pH were varied in order to optimize the operating conditions for isocratic RP-HPLC detection of rasagiline hemitartrate by measuring the active ingredient at 285 nm. Two organic solvents (methanol and acetonitrile), water and KH_2PO_4 buffer were taken and their effects on the elution of rasagiline hemitartrate were investigated. Six trails were carried out with varying solvent/composition of mobile phase/pH of buffer (Water:Acetonitrile – 50:50 (v/v); Water:methanol:acetonitrile – 50:25:25 (v/v/v); 10 mM potassium dihydrogen orthophosphate:acetonitrile – 50:50 (v/v); 20 mM potassium dihydrogen orthophosphate:acetonitrile – 30:70 (v/v), 20 mM potassium dihydrogen orthophosphate (pH 6.5):methanol:acetonitrile – 30:30:40 (v/v/v) and 20 mM potassium dihydrogen orthophosphate (pH 7.0):methanol:acetonitrile – 30:30:40 (v/v/v)). The pH of the 20 mM potassium dihydrogen orthophosphate buffer was adjusted to 7.0 with diluted ortho phosphoric acid and 0.2% triethyl amine. A nylon membrane filter (0.45 μ) was used to filter the mobile phase and prior to use the mobile phase was degassed. The injection volume was 20 μL and from the solvent reservoir, the mobile phase was pumped to column at a flow rate of 1 mL/min.

Peaks with good shape and resolution were obtained by using a mixture of 20 mM potassium dihydrogen orthophosphate (pH 7.0), methanol and acetonitrile (30:30:40, v/v/v)

(Fig. 2). Hence, this mixture was used as mobile phase for the study and the retention time for rasagiline hemitartrate was 4.30 ± 0.05 min at UV detection point 285 nm.

2.4. System suitability studies

The standard solutions were injected under optimum chromatographic conditions. The chromatogram obtained was tested for its acceptance using below parameters (Table 1).

2.5. Method for the standard graph

Ten mg of rasagiline hemitartrate was weighed accurately and transferred in to a 10 mL standard volumetric flask. Rasagiline hemitartrate was dissolved in HPLC grade acetonitrile by sonicating for about 30 min. One mL of this solution was diluted with 10 mL of acetonitrile to give a working standard solution containing of 100 $\mu\text{g/mL}$ of rasagiline hemitartrate (stock solution-A). Different aliquots (1, 2, 3, 4 and 5 mL) of the above rasagiline hemitartrate standard stock-A solution were transferred into a series of 10 mM standard volumetric flasks. Then the solutions were diluted to the mark with mobile phase to get concentrations of 10, 20, 30, 40 and 50 $\mu\text{g/mL}$. The prepared standard solutions were filtered through 0.45 μ membrane filter. Prior to the injection of rasagiline hemitartrate solution, the mobile phase was flown through the system to equilibrate the column for at least 30–45 min. A steady base line was recorded under the optimized chromatographic conditions. All the standard solutions were injected separately using rheodyne injector after stabilization for 30 min and the chromatograms were recorded. The concentration of drug was taken on X-axis and peak area of drug on Y-axis to plot the standard graph.

3. Results

3.1. Instrumental analysis of rasagiline hemitartrate

Spectral (IR, ^1H NMR and mass) as well as elemental analysis was carried out for characterization of the synthesized rasagiline hemitartrate.

Table 1 – System suitability parameters and chromatographic conditions.

Parameters	Values
Retention time (min)	4.30 ± 0.05
Flow rate (mL/min)	1.0
Theoretical plates/m	19,054.00
Capacity factor	20.36667
Asymmetry factor	2.01603
LOD ($\mu\text{g/mL}$)	0.651
LOQ ($\mu\text{g/mL}$)	1.972
Correlation coefficient	0.9991
UV detection (nm)	285

3.2. Method for validation

According to the International Conference on Harmonization Q2 (R1) (2005) guidelines, the proposed method was validated.

3.2.1. Linearity

To assess the linearity, a standard curve for rasagiline hemitartrate was constructed by plotting concentrations ($\mu\text{g/mL}$) versus absolute area (mV s) and shows good linearity on the 0–50.0 $\mu\text{g/mL}$ range (Table 2). Linear regression analysis was used to evaluate the linearity of the method. The representative linear equation was $y = 780371x$, where x is concentration and y is the peak absolute area. The correlation coefficient was $r^2 = 0.9952$, indicating good linearity.

3.2.2. Precision

Repeatability (intra-day) and intermediate precision (inter-day) determine the precision of the assay which was reported as % RSD. The peak area of drug solution containing 30 $\mu\text{g/mL}$ and 40 $\mu\text{g/mL}$ of rasagiline hemitartrate for intra and inter-day variation was calculated in terms of SD (Standard Deviation) and RSD (Relative Standard Deviation) (Table 3).

3.2.3. Accuracy

The presence of analyte recovered by assay from a known added amount represents accuracy. Accuracy of the RP-HPLC method was carried out by adding known amount of the drug (20 $\mu\text{g/mL}$ rasagiline hemitartrate) to the known

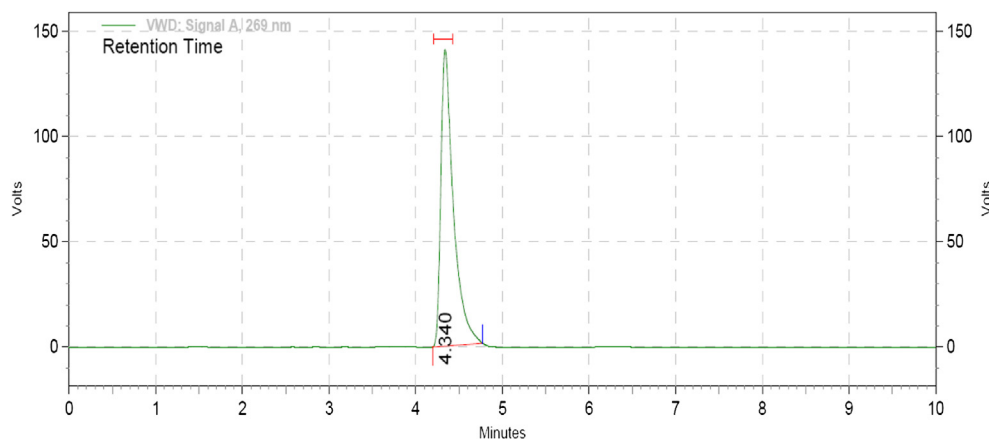


Fig. 2 – Chromatogram of rasagiline hemitartrate.

Table 2 – Calibration values for rasagiline hemitartrate.

Concentration ($\mu\text{g/mL}$)	Peak area (mVs)
0	0
10	8,614,197
20	16,154,535
30	23,717,305
40	29,423,432
50	39,887,016

concentrations of drug solution (50, 100 and 150%). All these solutions were prepared and analyzed in triplicate (Table 4).

3.2.4. Robustness and ruggedness

By carrying out small variations in the HPLC conditions such as change in the composition of mobile phase, change in pH of the buffer by ± 0.1 units and change in flow rate by ± 0.05 mL/min, robustness of the method was determined. Results were unaffected by small variations in these parameters. Over an acceptable working range of HPLC operational parameters, the method was found to be robust. By the varying the instrument, column and analyst, ruggedness was determined. For different analysts and instruments % RSD of the obtained results was found to be less than 1.0. A number of statistical values such as peak asymmetry, theoretical plates and HETP were calculated for the observed readings in order to ascertain the system suitability for the proposed method. The results act in accordance with in specification limits.

3.2.5. Limit of detection (LOD) and limit of quantification (LOQ)

For determination of sensitivity of the proposed method LOD and LOQ were calculated. Based on the signal to noise ratio they were quantified. The lowest detectable concentration of the analyte by the method was LOD where as the minimum quantifiable concentration was LOQ. LOD and LOQ for rasagiline hemitartrate were calculated according to the ICH guidelines by using S (relative standard deviation of the response) and σ (slope of the calibration curve).

$$\text{LOD} = 3.3 \times \sigma/S = 0.651 \mu\text{g/ml and}$$

$$\text{LOQ} = 10 \times \sigma/S = 1.972 \mu\text{g/ml}$$

4. Discussion

4.1. Characterization of the synthesized rasagiline hemitartrate

The elemental analysis of the synthesized compound shows that the % of (calculated, experimental) values for C, H and N

are (68.274, 67.99), (6.548, 6.274) and (5.687, 5.65) respectively, which confirms the molecular formula as $\text{C}_{12}\text{H}_{13}\text{N} \cdot \frac{1}{2}\text{C}_4\text{H}_6\text{O}_6$. For characterization of the synthesized rasagiline hemitartrate, IR, NMR and Mass Spectral analysis was carried out.

4.1.1. Interpretation of IR bands

IR (KBr, cm^{-1}): 3697.54 (O–H Str); 3396.64 (N–H Str), 3278.99 (C–H Str in acetylene), 3228.84 (OH Str in Alcohol), 3045.60 (C–H Str in Aromatic), 2968.45 (CH Str in Cyclic CH_2), 2929.87 (CH Str in acyclic CH_2), 2574.97 (b) (OH Str in COOH), 2349.30 (Asymmetrical stretching of COO^-), 2127.48 ($\text{C}\equiv\text{C}$ Str), 1938.46 (Aromatic Ring substitution over tone), 1732.08 (Aromatic Ring substitution over tone), 1629.85 (C=O Str of Tartaric acid), 1558.48; 1519.91; 1462.04; 1440.83 (C=C Aromatic Str), 1400.83 (C–H scissoring and bending), 1367.53 (C–N Str), 1315.45 (C–O Str in COOH), 1118.71 (C–O Str in C–OH), 1056.99 (O–H in plane bending), 758.02 (o-disubstituted aromatic ring, out of plane C–H bending) and 688.59 (C–H bend of alkynes).

4.1.2. Interpretation of ^1H NMR peaks

^1H NMR (DMSO- d_6 , MeOD, δ ppm): Corresponding to rasagiline: 2.95 (1H, s, $\equiv\text{CH}$); 7.30–7.54 (4H, m, ArH); 3.07 (2H, s, Propargyl CH_2); 3.19 (1H, t, CH); 2.22 (2H, m, Indane CH_2 nearer to imine group); 2.51 (2H, m, Indane CH_2 attached to Ar) and 4.36 (1H, s, NH). Corresponding to tartrate: 4.79 (2H, s, OH) and 3.84 (2H, s, CH).

4.1.3. Interpretation of mass spectra peaks

The peaks values obtained from both negative and positive modes of mass spectra were interpreted (Table 5) by taking molecular weights of Rasagiline Hemitartrate (RT), Rasagiline (R) and Tartaric Acid (T) as 246.29, 171.24 and 150.09 respectively.

4.2. HPLC method for validation of rasagiline hemitartrate

The proposed HPLC method for validation of rasagiline hemitartrate is first of its kind and has equal and/or better chromatographic properties compared to rasagiline mesylate, the equivalent drug available in the market. For estimation of rasagiline hemitartrate, mobile phase used in this method was 20 mM potassium dihydrogen orthophosphate: methanol and acetonitrile (30:30:40 v/v), which was cost effective due to usage of lower volume ratio of acetonitrile compared to the methods suggested by others for estimation of equivalent drug – rasagiline mesylate.^{11,12,14}

Neutral pH was maintained in this method similar to the method proposed by Kumar et al,¹² whereas, acidic pH was

Table 3 – Precision of the proposed RP-HPLC method for rasagiline hemitartrate.

Concentration ($\mu\text{g/mL}$)	Intra-day variation ^a			Inter-day variation ^a		
	Peak area mean	SD	% RSD	Peak area mean	SD	% RSD
30	24,266,936	271,556	1.119	24,214,974.8	96,212.093	0.397
40	29,692,742.000	295,315.914	0.995	29,646,983.667	260,287.057	0.878

a Average of six determinations.

Table 4 – Recovery of rasagiline hemitartrate using the proposed RP-HPLC method.

S. no.	Level of recovery (%)	Nominal concentration used ($\mu\text{g/mL}$) (a)	Amount of drug spiked ($\mu\text{g/mL}$) (b)	Theoretical amount ($\mu\text{g/mL}$) (a + b)	Amount of drug recovered ($\mu\text{g/mL}$) (mean \pm SD) ^a	% Of recovery (mean \pm SD) ^a	% RSD
1.	50	20	10	30	30.19 \pm 0.22	100.63 \pm 0.74	0.73
2.	100	20	20	40	40.23 \pm 0.13	100.57 \pm 0.31	0.31
3.	150	20	30	50	50.24 \pm 0.16	100.47 \pm 0.31	0.31

a Average of three determinations.

Table 5 – Interpretation of fragmentation.

m/z value	Interpretation	Fragment
Positive mode		
56.4	171.24 – ($-\text{CH}_2-\text{C}\equiv\text{CH}$) – (C_6H_4)	$(-\text{CH}_2-\text{CH}_2-\text{CH}-\text{NH})^+$
118.3	171.24 + 1 – 54	$(\text{R} + \text{H}-\text{NH}-\text{CH}_2-\text{C}\equiv\text{CH})^+$
117.3	171.24 – 54	$(\text{R}-\text{NH}-\text{CH}_2-\text{C}\equiv\text{CH})^+$
172.3	171.24 + 1	$(\text{R} + \text{H})^+$
Negative Mode		
149.2	150.2 – 1	$(\text{T}-\text{H})^-$
150.2	150.2	T^-
306.2	RT + 59.91	$[\text{RT} + -\text{CH}\cdot\text{OH}-\text{CH}\cdot\text{OH}-]^-$
307.2	RT + 60.91	$[\text{RT} + \text{H} + -\text{CH}\cdot\text{OH}-\text{CH}\cdot\text{OH}-]^-$

Mol. wt of rasagiline hemitartrate (RT) = 246.29; Mol. wt of rasagiline (R) = 171.24; Mol. wt of tartaric acid (T) = 150.09; $(-\text{CH}\cdot\text{OH}-\text{CH}\cdot\text{OH}-)$ = tartaric acid – 2COOH.

maintained by Kullai Reddy et al.¹⁶ UV detection for rasagiline mesylate in other methods was performed mostly at 210 nm, where as in this method it was at higher wavelength, i.e., 285 nm which can be explained based on the higher polarity of the mobile phase. It is well known that in presence of a polar solvent, the more polar II* orbital will be more stabilized than the II orbital leading to a net decrease in the transition energy, which result in an increase in transition wavelength or a bathochromic shift/red shift.²⁴

Retention time for rasagiline hemitartrate in this method was 4.3 ± 0.05 min whereas for rasagiline mesylate the retention time mostly ranges from 4.36 to 6 min. Though, the retention time in the method proposed by Kumar et al¹¹ for rasagiline mesylate estimation was lower, its linearity range covers only upto 25 $\mu\text{g/mL}$, but in this method, it ranges up to 50 $\mu\text{g/mL}$. Better % of analytical recovery was achieved in this method (100.47) compared to other methods, where it varies from 99.38 to 99.71%. The percentages of RSD for precision and accuracy of the proposed method were found to be in the ranges 0.397–0.878 and 0.31–0.73 respectively, which were very much within the allowable limit²⁵ of 2%.

5. Conclusion

The developed RP-HPLC method for the synthesized rasagiline hemitartrate was precise, accurate and validated statistically. For routine estimation of rasagiline hemitartrate in bulk forms,

this method can be applied in view of its simplicity, fastness and robust nature by employing a mobile phase comprising of potassium dihydrogen orthophosphate (20 mM) (pH 7.0), methanol and acetonitrile in the volume ratio of 30:30:40 with UV detection at 285 nm and flow rate of 1 mL/min.

Conflicts of interest

All authors have none to declare.

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