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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF RASAGILINE TABLET DOSAGE FORMS

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ABSTRACT

An accurate and precise HPLC method was developed for the determination of rasagiline. Separation of the drug was achieved on a reverse phase C_{18} column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 50:50 v/v. The flow rate was 0.5 ml/min and the detection wavelength was 210 nm. The linearity was observed in the range of 10-125 µg/ml with a correlation coefficient of 1.000. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of rasagiline in tablet dosage forms.

Keywords: Rasagiline, Estimation, RP-HPLC, Validation, Tablets.

INTRODUCTION

Rasagiline mesylate[1], a propargylamine based drug is indicated in the treatment of idiopathic parkinson's disease. Chemically[2], it is 1*H*-inden-1-amine,2,3-dihydro-N-2-propynyl-(1R)- methane sulfonate (Fig.-1). Rasagiline is also used as an adjunct therapy to levodopa. Rasagiline is a chemical inhibitor of the enzyme monoamine oxidase (MAO) type B which has a major role in the inactivation of biogenic and diet-derived amines in the central nervous system. MAO has two isozymes (types A and B) and the type B is responsible for metabolising dopamine in the central nervous system. As dopamine deficiency is the main contributing factor to the clinical manifestations of parkinson's disease, inhibition of MAO-B should tend to restore dopamine levels towards normal values and this improves the condition. A few HPLC[3] and LC-MS[4-5] methods were reported earlier for the determination of rasagiline in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of rasagiline in bulk samples and in tablet dosage forms.

EXPERIMENTAL

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase chromatopak C_{18} column (250mmx4.6mm; 5µm), a 2695 binary pump, a 20 µl injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and solvents

The reference sample of rasagiline was supplied by Sun Pharmaceutical Industries Ltd., Baroda. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer (pH 3.0)

Two grams of KH_2PO_4 was weighed into a 1000ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethyl amine was added and pH adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

500 ml of the phosphate buffer was mixed with 500 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum.

Procedure

A mixture of buffer and acetonitrile in the ratio of 50:50 v/v was found to be the most suitable mobile phase for ideal separation of rasagiline. The solvent mixture was filtered through a 0.45µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.5 ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 210 nm. The run time was set at 8min. Under these optimized chromatographic conditions the retention time obtained for the drug was 4.62 min. A typical chromatogram showing the separation of the drug is given in Fig. 2.

Calibration plot

About 25 mg of rasagiline was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:50 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 250 µg/ml solution. From this, a working standard solution of the drug (50µg/ml) was prepared by diluting 2 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 10-125 µg/ml were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 0.5 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 10-125 µg/ml of the drug. The relevant data are furnished in Table-1. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of rasagiline in tablets dosage forms.

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of rasagiline. Solutions containing 25, 50 and 75 µg/ml of rasagiline were subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2. The accuracy of the HPLC method was assessed by analyzing solutions of rasagiline at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Estimation of rasagiline in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate rasagiline in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of rasagiline was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 50:50 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

RESULTS AND DISCUSSION

In the proposed method, the retention time of rasagiline was found to be 4.624 min. Quantification was linear in the concentration range of 10-125 µg/ml. The regression equation of the linearity plot of concentration of rasagiline over its peak area was found to be $Y=126238.15+74148.32X$ ($r^2=1.000$), where X is the concentration of rasagiline (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 4640, which indicates efficient performance of the column. The limit of

detection and limit of quantification were found to be 0.010 µg/ml and 0.028 µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 50:50 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

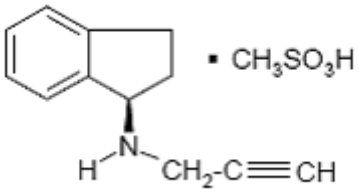

Fig.-1: Chemical structure of rasagiline mesylate

Table-1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=6)
10	813094
25	2070391
50	3820978
75	5688099
100	7467134
125	9444838

Table-2: Precision of the proposed HPLC method

Concentration of rasagiline (µg/ml)	Measured concentration of rasagiline (µg/ml)			
	Intra-day		Inter-day	
	Mean (n=3)	% C.V.	Mean (n=3)	%C.V.
25	25.30	0.16	24.30	0.18
50	48.97	0.32	48.25	0.25
75	74.26	0.36	74.24	0.35

Table-3: Accuracy studies

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	12.3	12.47	101.40	
100%	24.8	24.29	98.70	99.71
150%	37.6	37.23	99.00	

Table-4: System suitability parameters

Parameter	Result
Linearity ((µg/ml)	10-125
Correlation coefficient	1.000
Theoretical plates (N)	4640

Tailing factor	1.72
LOD (µg/ml)	0.010
LOQ (µg/ml)	0.028

Table-5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Rasalect	0.5	0.5001	100.02
Rasipron	1	0.9920	99.20

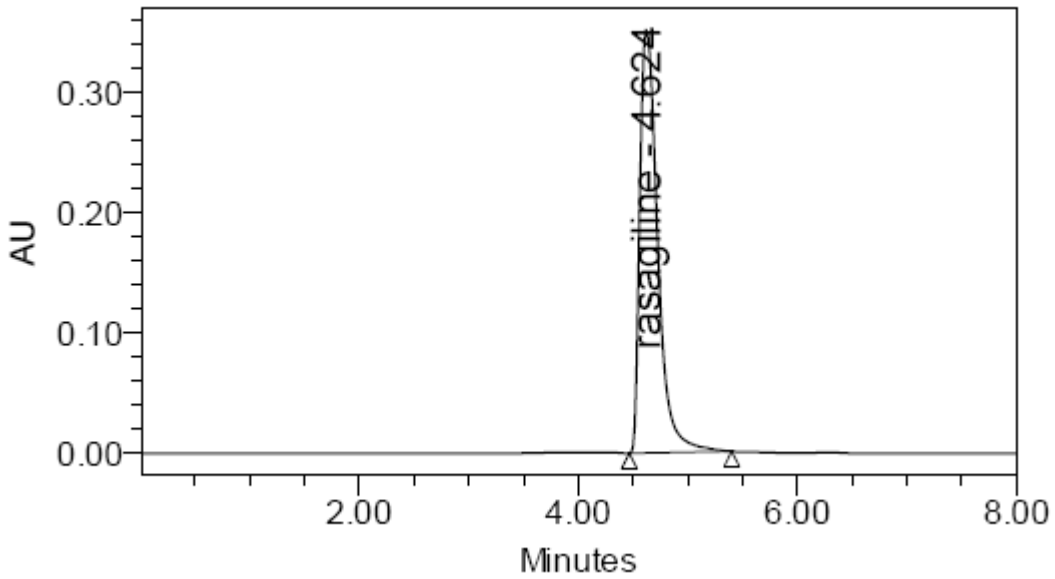

Fig.-2: Typical chromatogram of rasagiline

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of rasagiline and can be reliably adopted for routine quality control analysis of rasagiline in its tablet dosage forms.

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