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Reverse Phase High Performance Liquid Chromatographic Method for Separation of R-Rasagiline Mesylate from S-Isomer

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High-performance liquid chromatographic method has been developed under reverse-phase conditions for the separation of enantiomeric R-rasagiline mesylate (chemically known as (R)-N-(prop-2-ynyl)-2,3-dihydro-1H-inden-1-amine mesylate) from its S-isomer. Chromatographic separation was carried out on amylase based CHIRALPAK AD-RH (150 × 4.6 mm, 5 μm) column using a mobile phase consisting of 20 mM potassium dihydrogen phosphate in water-acetonitrile (65:35, v/v) adjusted to pH 6.9 with 10 % potassium hydroxide solution. Flow rate was 0.5 mL min^{-1} and UV detection was at 210 nm. The conditions affording the best resolution (3.4) were found by selection and variation of the mobile-phase composition and the difference in separation capability of the method is noted. Relative standard deviation of retention time and peak areas were found to be 0.03 and 0.23, respectively for precision. R-rasagiline mesylate sample solution and mobile phase were found to be stable for 72 h.

Keywords: R-Rasagiline mesylate, Chiral HPLC, Reversed-phase liquid chromatography.

INTRODUCTION

R-Rasagiline mesylate (chemically known as (R)-N-(prop-2-ynyl)-2,3-dihydro-1H-inden-1-amine) is a selective, irreversible monoamine-oxidase B inhibitor[1]. It is used for the treatment of the signs and symptoms of idiopathic Parkinson's disease as initial monotherapy and as adjunct therapy to levodopa[2]. Rasagiline is a highly potent, selective and irreversible second-generation monoamine oxidase inhibitor with selectivity for type B of the enzyme (MAO-B). R-Rasagiline mesylate is the (R)-stereoisomer and it was developed by teva neuroscience. It was shown that R-rasagiline mesylate is more effective than S-rasagiline mesylate. 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 R-configuration which is produced as single enantiomer[3]. There are many methods reported in the literature for analysis of rasagiline mesylate, for example, GC-MS[4], HPLC methods[5-7], LC-MS/MS in human plasma[8,9] and spectrophotometric methods[10,11]. Process for the synthesis of enantiomerically pure R-rasagiline mesylate was reported[12,13]. For carrying out further research on R-rasagiline mesylate, there is a need for separation and quantification of enantiomers of rasagiline mesylate. Therefore, we developed a single, reliable and reverse-phase HPLC method for separation of R-rasagiline mesylate from its S-isomer.

EXPERIMENTAL

R-Rasagiline mesylate was obtained from the R&D department of Suven Life Sciences Limited (Hyderabad, India). Chemical structure is presented in Fig. 1. HPLC-grade acetonitrile and GR grade potassium dihydrogen phosphate and potassium hydroxide pellets were purchased from Merck (Mumbai, India). Ultrapure water was purified using an Elix 3 coupled to a Milli-Q Gradient A10 system (Millipore, Bedford, USA).

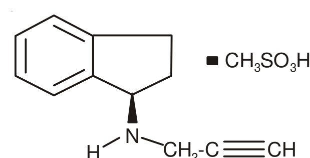

Fig. 1. Chemical structure of rasagiline mesylate

The analytical chiral separation was carried out using 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of a G1310A quaternary pump, a G1379A degasser, a G1313A auto sampler, a G1316A thermostatted column compartment, a G1315B diode array detector and was processed using Chemstation software Ver. A.10.02.

Chromatographic conditions: The chromatographic conditions were optimized using a chiral stationary phase

CHIRALPAK AD-RH (150 × 4.6 mm, 5 µm, Daicel, Japan) which was safeguarded with a 1 cm length guard column. Mobile phase system consists of 20 mM potassium dihydrogen phosphate in water, aetonitrile (65:35 v/v) adjusted to pH 6.9 with 10 % potassium hydroxide solution with flow rate of 0.5 mL/min, column temperature was maintained at 30 °C and eluent was monitored at a wavelength of 210 nm. The injection volume was 10 µL. R-Rasagiline mesylate in-house working standard solution was prepared in mobile phase and its concentration was fixed as 200 µg/mL. Different chiral columns were also employed during method development namely CHIRALCEL OJ-RH (250 × 4.6 mm, 5 µm, Daicel, Japan), CHIRALPAK AD-RH (250 × 4.6 mm, 5 µm, Daicel, Japan), CHIRALCEL OD-RH (150 × 4.6 mm, 10 µm, Daicel, Japan). Various experimental parameters were optimized for determination of S-rasagiline mesylate as an impurity in R-rasagiline mesylate.

Validation of the method

Specificity: The specificity/selectivity of the analytical method was investigated by confirming the complete separation and resolution of the desired isomer of the analyte in mobile phase, spiked standard, blank and standard 1:1 mixture (20 µg/mL) of both enantiomers.

Linearity: The linearity of the method was established by preparing and analyzing five linear solutions of the S-rasagiline mesylate (concentration from 0.5 to 4 µg/mL) in diluent. Linear regression curve was applied and slope (a), intercept (b), correlation coefficient (r) and obtained by plotting peak area versus concentration using the least squares method. The percentage relative standard deviation of the slope (a) and intercept (b) of the calibration curve were determined (Table-1).

TABLE-1
REGRESSION CHARACTERISTICS OF S-RASAGILINE MESYLATE AND R-RASAGILINE MESYLATE

Parameter	S-Rasagiline mesylate	R-Rasagiline mesylate
Regression equation	y = 46.51x + 0.2542	y = 52.721x - 3.5784
Slope (a)	46.51	52.721
y-Intercept (b)	0.2542	3.5784
Linearity Range (µg/mL)	0.5–4.0	0.5-4.0
Correlation coefficient (r)	0.999	0.999

Precision: Precision was determined both in terms of measuring repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility) and reproducibility for retention time and peak areas of R-rasagiline mesylate. In order to determine the repeatability of the method, replicate injections (n = 6) of standard was carried out from single preparation. Expressed as mean, SD and % RSD calculated from the data obtained. The reproducibility of the method was determined by analyzing six different preparations of test solutions containing R-rasagiline mesylate (0.2 mg/mL). Expressed as mean, SD and % RSD calculated from the data obtained. The % RSD values for the both intra-day and inter-days were less than 2.0 %, which indicates that the proposed method is precise.

Accuracy: Accuracy was determined in terms of percentage of recovery. Standard addition and recovery experiments were conducted to determine the accuracy of the method for quantification of the S-enantiomer in samples. The accuracy of the method was determined by spiking S-enantiomer to R-rasagiline mesylate at 0.5 µg/mL (25 %) to 4 µg/mL (200 %) levels. The percentage recoveries were ranging from 99.06 to 100.87 in samples of R-rasagiline mesylate.

$$\%\ \text{Recovery} = \left(\frac{A}{B}\right) \times 100$$

where A is the response of the analyte with respect to the sample, B is the response of the analyte with respect to Standard.

Limit of detection and limit of quantification: Detection and quantification limits were determined through dilution method using slope and styex approach by injecting a 10 µL sample. The slope was estimated from the calibration curve of the analyte. The styex was estimated from the calibration curve of the analyte limit of detection was considered as the minimum concentration with a signal, while limit of quantification was taken as a minimum concentration.

Stability: The solution stability studies of R-rasagiline mesylate was carried out over a period of 72 h at ambient condition.

Robustness: The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate (± 10 %), mobile phase composition (± 10 %), pH change (± 0.2) and column temperature (± 5 °C). To determine robustness of the method experimental conditions were purposely altered and chromatographic resolution between (R and S) enantiomers was evaluated.

The flow rate of the mobile phase was set as 0.5 mL/min, to study the effect of flow rate on the resolution of enantiomers and altered it between 0.45 to 0.55 mL/min. The effect of change in percent of buffer and acetonitrile on resolution was studied by varying from -10 to +10 %. The effect of change in pH of the mobile phase (± 0.2) on resolution of enantiomers was studied. The effect of column temperature on resolution was studied at 25 and 35 °C instead of 30 °C, while the other mobile phase compositions were held constant.

Selection of mobile phase: Different mobile phases were tested for their suitability for the separation of the enantiomers on the CHIRALPAK AD-RH (150 mm × 4.6 mm, 5 µm) column. For each determination, capacity, selectivity and resolution factors were measured.

k^1 (capacity factor of the first eluted enantiomer)
= [retention time of the first eluted enantiomer-dead volume]/dead volume

k^2 (capacity factor of the second eluted enantiomer)
= [retention time of the second eluted enantiomer - dead volume]/ dead volume

α: selectivity factor, $\alpha = \frac{k^2}{k^1}$

RS: Resolution factor, RS = 2 × [difference of retention time of (S) and (R) enantiomers]/[the bandwidths of the two peaks].

RESULTS AND DISCUSSION

Selection of suitable column: The aim of this work is to separate the R-rasagiline mesylate and quantify accurately.

Enantiomeric mixture solution of 20 μg/mL was prepared in diluent and used in the method development. To develop a rugged and suitable liquid chromatography method for the separation of R-rasagiline mesylate enantiomers, different mobile phases and stationary phase were employed. The main target of the chromatographic method is to separate the enantiomeric mixture of rasagiline mesylate into individual enantiomers using single mobile phase. Various reverse-phase chiral columns namely CHIRALCEL OJ-RH (150 × 4.6 mm, 5 μm, Daicel, Japan), CHIRALPAK AD-RH (150 × 4.6 mm, 5 μm, Daicel, Japan), CHIRALCEL OD-RH (150 × 4.6 mm, 5 μm, Daicel, Japan) were used with different possible mobile phases for separation. There is an indication of separation in CHIRALPAK AD-RH column using a mobile phase composition of 20 mM potassium dihydrogen phosphate in water and acetonitrile adjusted to pH 6.9 with 10 % potassium hydroxide solution in various compositions.

Optimization of chromatographic conditions: Initially, the mobile phase consisting of 20 mM potassium hydrogen phosphate in water and acetonitrile adjusted to pH 6.9 with 10 % potassium hydroxide solution in various compositions. The two enantiomers were started merging and separation of enantiomers was disturbed. Several buffers and organic solvents were tried for the separation of enantiomers. Finally good resolution (> 3.3) and peak shape was achieved using a mobile phase composition of 20 mM potassium dihydrogen phosphate in water and acetonitrile (65:35 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution.

In the optimized method, the typical retention times of S-rasagiline mesylate and R-rasagiline mesylate were about 12.8 and 15.2 min, respectively. The separation of rasagiline mesylate on CHIRALPAK AD-RH column was shown in Fig. 2. Baseline separation of S-rasagiline mesylate ($k^1 = 2.45$) and R-rasagiline mesylate ($k^2 = 3.05$) was obtained with a total run time of 25 min.

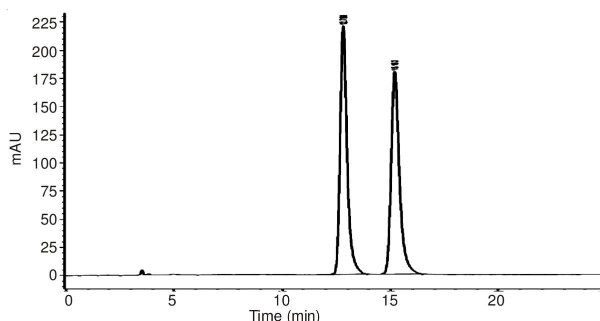

Fig. 2. Enantiomeric resolution of S-rasagiline mesylate and R-rasagiline mesylate on CHIRALPAK AD-RH column

Validation of method: In the repeatability study, % relative standard deviation (% RSD) was 0.03 % for the retention times, 0.23 % for peak areas of the S-enantiomer and 0.03 % for the retention times and 0.51 % for peak areas of the R-enantiomer. In reproducibility studies, the results show that % RSD values were in the same order of magnitude as those obtained for repeatability (Table-2).

Coefficient of correlation between concentration and detector response 0.999 for the first eluting S-rasagiline mesylate shows that the method is linear over the concentration range of 0.5 to 4 μg/mL. The limit of detection (LOD), determined as the amount for S-rasagiline mesylate, was 0.16 μg/mL.

TABLE-2
S-RASAGILINE MESYLATE CALIBRATION RANGE, LINEARITY AND SENSITIVITY ACCURACY OF METHOD

R-Rasagiline mesylate	Accuracy (mean % recovery ± SD)
Spiked concentration 0.5 μg/mL	101.10 ± 0.27
Spiked concentration 1.0 μg/mL	99.31 ± 0.54
Spiked concentration 2.0 μg/mL	100.87 ± 0.67
Spiked concentration 3.0 μg/mL	100.30 ± 0.23
Spiked concentration 4.0 μg/mL	99.64 ± 0.84
S-Rasagiline mesylate recovery (% RSD for accuracy)	
Spiked concentration 0.5 μg/mL	0.97
Spiked concentration 1.0 μg/mL	1.09
Spiked concentration 2.0 μg/mL	0.68
Spiked concentration 3.0 μg/mL	0.16
Spiked concentration 4.0 μg/mL	0.42
Calibration range (μg/mL)	0.50-4.0
Linearity (S-Rasagiline mesylate)	
Slope[a]	46.51
Intercept[b]	0.2542
Correlation coefficient (r)	0.999
Standard error (Es)	0.56
S-Rasagiline mesylate spiked in R-rasagiline mesylate (25 to 200 %)	
Slope[a]	49.607
Intercept[b]	-0.413
Correlation coefficient (r)	0.999
Standard error (Es)	0.84
Repeatability (S-rasagiline mesylate)	
Injection repeatability (mean; % RSD)[c]	15.11; 0.03
Injection repeatability (mean; % RSD)[d]	9337.39; 0.23
Analysis repeatability (mean; % RSD)[e]	15.08; 0.03
Analysis repeatability (mean; % RSD)[f]	9268.98; 0.51
Sensitivity (S-rasagiline mesylate)	
Limit of detection, LOD (μg/mL)	0.16
Limit of quantification, LOQ (μg/mL)	0.49

(a) n=3; (b) n= 5; (c & e) Retention time (min); (d & f) Peak area

A HPLC chromatogram of S-Rasagiline mesylate and R-Rasagiline mesylate was shown in (Fig. 2). The chromatographic resolution of S-Rasagiline mesylate and R-rasagiline mesylate peaks were used to evaluate the method robustness under modified conditions. The resolution between S-rasagiline mesylate and R-rasagiline mesylate was > 3.3, under all separation conditions tested, demonstrating sufficient robustness (Table-3).

Conclusion

A simple, rapid and accurate reverse-phase chiral liquid chromatography method for separation of R-rasagiline mesylate from its s-isomer has been developed and validated. Amylase based CHIRALPAK AD-RH column was found to be suitable for enantiomers separation of Rasagiline mesylate. The method was completely validated, demonstrating satisfactory data for all the method validation parameters tested. The developed method is suitable for separation and quantification of enantiomers of rasagiline mesylate.

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TABLE-3
ROBUSTNESS PARAMETERS

Flow change	Flow rate (mL/min)	Selectivity (α)	R_S
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	0.5	1.24	3.59
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	0.45	1.25	3.68
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	0.55	1.24	3.55
Solvent ratio change			
20 mM potassium hydrogen phosphate in water, aetonitrile (685:315 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	0.50	1.24	3.73
20 mM potassium hydrogen phosphate in water, aetonitrile (615:385 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	0.50	1.24	3.49
pH change			
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 6.7 with 10 % potassium hydroxide solution	0.50	1.24	3.33
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 7.1 with 10 % potassium hydroxide solution	0.50	1.25	3.82
Temparature change temperature			
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	25 °C	1.27	3.78
20 mM potassium hydrogen phosphate in water, aetonitrile (650:350 % v/v), adjusted to pH 6.9 with 10 % potassium hydroxide solution	35 °C	1.22	3.32

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