# A Validated Normal Phase LC Method for Enantiomeric Separation of Rasagiline Mesylate and Its (*S*)-Enantiomer on Cellulose Derivative-Based Chiral Stationary Phase

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*ABSTRACT* A simple, sensitive, and robust normal-phase isocratic HPLC-UV method was developed and validated for the enantiomeric separation of rasagiline mesylate and its (*S*)-enantiomer. The rasagiline and its (*S*)-enantiomer were resolved on a Chiralcel-OJ-H (4-methylbenzoate cellulose coated on silica) column using a mobile phase consisting of *n*-hexane:isopropyl alcohol:ethanol:diethyl amine (96:2:2:0.01) at a flow rate of 1.0 ml/min. The column temperature was maintained at 27 °C and elution was monitored at 215 nm. The resolution ($R_s$) between the enantiomers was found to be more than 2.0. The limit of detection and the limit of quantification of the (*S*)-enantiomer were found to be 0.35 and 1.05 μg/ml, respectively. The developed method was validated as per ICH guidelines with respect to linearity, limit of detection and quantification, accuracy, precision, and robustness—and satisfactory results were obtained. The sample solution and mobile phase were found to be stable up to 48 h. The method is useful for routine evaluation of the quality of rasagiline mesylate in bulk drug-manufacturing units. *Chirality 25:324–327, 2013.* 

*KEY WORDS:* rasagiline; validation; chiral HPLC; enantiomeric purity

## INTRODUCTION

Enantiomers of racemic drugs often show different behaviors in pharmacological action and metabolic process. In 1992, the U.S. Food and Drug Administration issued a policy statement for the development of new steroisomeric drugs that requires acceptable manufacturing control of synthesis and impurities, adequate pharmalogical and toxicological assessment, proper characterization of metabolism and distribution, and appropriate clinical evaluation.[1]

Rasagiline is a highly potent, selective, irreversible, second-generation monoamine oxidase inhibitor with selectivity for type B of the monoamine oxidase enzyme (MAO-B) and has been used for the treatment of idiopathic Parkinson's disease (PD). Its chemical designation is (*R*)-*N*-(prop-2-ynyl)-2,3-dihydro-1*H*-inden-1-amine methane sulfonate (Fig. 1).[2] The recomended dosage for initial monotherapy is 1 mg once daily. When rasagiline is used as adjunctive therapy with levodopa, the recommended initial dose is 0.5 mg/day and may be increased to 1 mg/day if the desired clinical effect is not achieved.[3,4] Rasagiline is produced as a single isomer, and (*S*)-enantiomer could be present as a chiral impurity. In general, the (*R*)-isomer (rasagiline) shows higher binding affinities than the corresponding compound in the *S* configuration (*S*-isomer), and this difference is more pronounced for MAO-B than for MAO-A.[5]

A literature survey reveals that few LC assay methods are reported for determination of rasagiline in bulk drug and pharmaceutical preparation.[6,7] The assay method by HPLC[8] describes the separation of degradation impurities from rasagiline formed during forced degradation studies, but it was out of our scope because it did not separate and determine the enantiomeric impurities. Estimation of rasagiline in human plasma by liquid chromatography-tandem mass spectrometry has been performed.[9,10] However, an extensive literature survey revealed that no LC method has been reported, including in major pharmacopoeias such as USP, EP and BP, for the quantitative determination of the enantiomeric impurity of rasagiline in bulk drugs. Therefore, it was felt necessary to develop an accurate, precise, and robust enantioselective normal-phase HPLC method for the separation and determination of the undesired (*S*)-enantiomer of rasagiline in bulk drugs.

The present work deals with the development and validation of a normal phase LC method to determine the enantiomeric purity of rasagiline using a new commercialized chiral stationary phase, namely, cellulose tris (4-methylbenzoate) coated on silica. The developed LC method was validated with respect to precision, accuracy, limits of detection and quantification, linearity, and robustness. These studies were performed in accordance with established ICH guidelines.

## EXPERIMENTAL

### *Chemicals and Reagents*

Rasagiline mesylate and its (*S*)-enantiomer (Fig. 1) were supplied by Dr. Reddy's Laboratories Ltd., India. HPLC-grade *n*-hexane and isopropyl alcohol were purchased from Rankem, India. Ethanol and diethyl amine were purchased from Merck, India.

### *Chromatographic Conditions*

Samples were analyzed on a Waters Alliance 2695 separation module (Waters Corporation, Milford, MA, USA) equipped with a 2489 UV/visible detector. The method was developed using a Chiralcel-OJ-H (250 mm × 4.6 mm, 5 μm) column with mobile phase consisting of *n*-hexane:isopropyl alcohol:ethanol:diethyl amine (96:2:2:0.01). The flow rate of the mobile phase was 1.0 ml/min.

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Received for publication 26 June 2012; Accepted 30 November 2012
DOI: 10.1002/chir.22150
Published online 9 May 2013 in Wiley Online Library (wileyonlinelibrary.com).

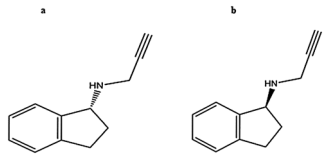

**Fig. 1.** Structures of (**a**) rasagiline and (**b**) its (*S*)-enantiomer.

The column temperature was maintained at 27 °C and the detection wavelength was 215 nm. The sample injection volume was 10 μl.

### *Preparation of System Suitability Solution*

Isopropyl alcohol was used as the diluent. A system suitability solution of rasagiline mesylate and its (*S*)-enantiomer was prepared by dissolving 1.0 mg of each substance in 2.5 ml of methanol and then diluting to 10 ml to obtain a final concentration of 0.1 mg/ml.

### *Preparation of Standard Solution*

A stock solution of rasagiline mesylate was prepared by dissolving 35.0 mg of the drug in 2.5 ml of methanol and then diluting to 10 ml (3.5 mg/ml). A working standard solution was prepared by diluting the above stock solution with diluent to obtain a final concentration of 3.5 μg/ml.

### *Preparation of Sample Solution*

To prepare a sample solution, 35.0 mg of rasagiline mesylate was dissolved in 2.5 ml of methanol with sonication and then diluting to 10 ml.

## METHOD VALIDATION

The proposed method was validated as per ICH guidelines.[11] The following validation characteristics were addressed: accuracy, precision, limit of detection and quantification, linearity, range, and robustness.

### *System Suitability*

System suitability was determined before sample analysis from six replicate injections of the standard solution containing 3.5 μg/ml of rasagiline mesylate. The acceptance criteria were: less than 5% relative standard deviation (RSD) for peak areas, the USP tailing factor less than 2.0 for the rasagiline peak from standard solution and from system suitability solution, and a minimum resolution of 2.5 between rasagiline and its (*S*)-enantiomer.

### *Precision*

The precision of the method was verified by repeatability and intermediate-precision studies. Repeatability was checked by injecting six individual preparations of rasagiline mesylate spiked with (*S*)-enantiomer at the 0.15% level. The %RSD of area for the (*S*)-enantiomer was calculated. The intermediate precision of the method was also evaluated using a different analyst and a different instrument and performing the analysis on different days.

### *Limits of Detection (LOD) and Quantification (LOQ)*

The LOD and LOQ for the (*S*)-enantiomer were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. A precision study was also carried out at the LOQ level by injecting six individual preparations of (*S*)-enantiomer and calculating the %RSD of the area.

### *Linearity*

Linearity test solutions were prepared by diluting the stock solutions to the required concentrations. The solutions were prepared at seven concentration levels from LOQ to 150% of specification level (i.e., 1.05, 1.33, 2.63, 3.96, 5.25, 6.58, and 7.88 μg/ml). A calibration curve was plotted between the responses of peak versus analyte concentration. The coefficient correlation, slope and *y*-intercept of the calibration curve and % bias are reported.

### *Accuracy*

The accuracy of the method for (*S*)-enantiomer was evaluated in triplicate, using four concentration levels from LOQ to 150% (i.e., 1.05, 2.63, 5.25, and 7.88 μg/ml). The percentage recovery of (*S*)-enantiomer was calculated at each level.

### *Robustness*

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between rasagiline and its (*S*)-enantiomer and system suitability parameters for the rasagiline standard were recorded. The variables evaluated in the study were column temperature (±5 °C), flow rate (±0.2 ml/min), and composition of organic solvents (isopropyl alcohol and ethanol) in the mobile phase (±10%).

### *Solution Stability and Mobile Phase Stability*

The solution stability of rasagiline mesylate spiked with its (*S*)-enantiomer was determined by leaving the test solution and standard solutions in tightly capped volumetric flasks at room temperature for 48 h and then by measuring the amount of rasagiline (*S*)-enantiomer at every 24-h interval against a freshly prepared standard solution. The stability of mobile phase was also determined by freshly prepared test solutions of rasagiline mesylate spiked with its (*S*)-enantiomer at 24-h intervals for 48 h. The mobile phase was not changed during the study.

## RESULTS AND DISCUSSION

### *Method Development*

The main aim of the chromatographic method development was to achieve the separation of rasagiline and its (*S*)-enantiomer and accurate quantification of the (*S*)-enantiomer. A racemic mixture solution of rasagiline and (*S*)-enantiomer (0.1 mg/ml each) was prepared in methanol and isopropyl alcohol. To develop a robust and suitable HPLC method for the separation of the two enantiomers, different stationary phases and mobile phases were employed. The racemic mixture solution was subjected to separation by normal-phase LC on the Chiralpak IA (250 mm × 4.6 mm, 5-μm column) with *n*-hexane and isopropyl alcohol in a 96:4 ratio as a mobile phase. Broad peak shapes were observed with resolution less than 1.0. To separate both the components, the composition of the mobile phase was changed to *n*-hexane, isopropyl alcohol, and ethanol in the ratio of 96:2:2 and the Chiralcel-OJ-H (250 mm × 4.6 mm, 5 μm) column was selected for separation. Resolution was observed between the enantiomers but peak tailing was also observed. With the addition of 0.01% diethyl amine to mobile phase, rasagiline and its (*S*)-enantiomer were eluted with superior resolution and symmetrical peak shape. The final optimized conditions are described as follows.

The separation was achieved using a Chiralcel-OJ-H (250 mm × 4.6 mm, 5 μm) column with mobile phase consisting of *n*-hexane:isopropyl alcohol:ethanol:diethyl amine (96:2:2:0.01). The flow rate of the mobile phase was 1.0 ml/min. The column temperature was maintained at 27 °C and the detection wavelength was 215 nm. The sample injection volume was 10 μl.

## METHOD VALIDATION

### *System Suitability*

System suitability shall be checked for the conformance of the suitability and reproducibility of the chromatographic system for analysis. The system suitability was evaluated on the %RSD of the peak area and USP tailing factor for rasagiline peak from standard solution; and resolution between rasagiline and its (*S*)-enantiomer from system suitability solution (Fig. 2a). All critical parameters tested met the acceptance criteria (Table 1).

### *Precision*

The %RSD for the area of the (*S*)-enantiomer in the repeatability study was within 0.7% and in the intermediate precision study was within 1.3%, which confirms the good precision of the method.

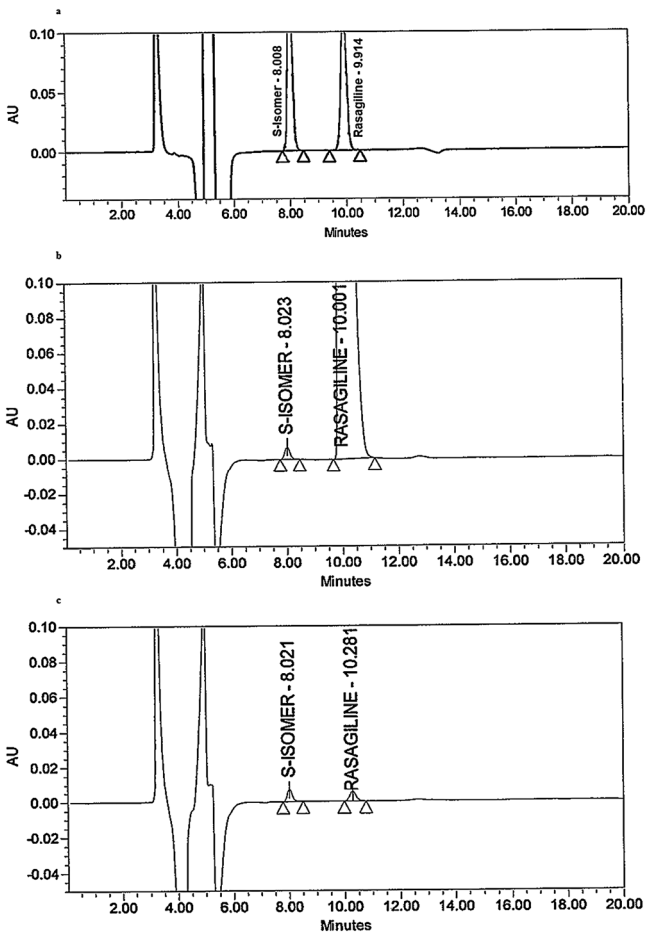


**Fig. 2.** Typical chromatograms for the resolution of (**a**) a racemic mixture of rasagiline and its (*S*)-enantiomer; (**b**) rasagiline spiked with its (*S*)-enantiomer at 0.15% level; and (**c**) a racemic mixture of rasagiline and its (*S*)-enantiomer at 0.15% concentration of the racemic solution. Chromatographic conditions: Chiralcel-OJ-H (250 mm × 4.6 mm, 5 μm) column at 27 °C; mobile phase: *n*-hexane:isopropyl alcohol:ethanol:diethyl amine (96:2:2:0.01); flow rate, 1.0 ml/min; wavelength, 215 nm.

**TABLE 1. System suitability test results**

| | | Observed values | |
|---|---|---|---|
| Parameters | Specification | precision | intermediate precision |
| Resolution[a] | > 2.5 | 5.8 | 5.4 |
| % RSD | < 5.0% | 1.7 | 1.4 |
| USP Tailing | < 2.0 | 1.3 | 1.3 |

[a]Resolution between rasagiline and its (*S*)-enanatiomer.

### *Limits of Detection (LOD) and Quantification (LOQ)*

The limit of detection and limit of quantification for (*S*)-enantiomer were found to be 0.35 and 1.05 μg/ml. The %RSD value of precision at LOQ level was 2.2.

### *Accuracy*

The percentage recovery of (*S*)-enantiomer in rasagiline samples varied from 98.3 to 101.9%. The LC chromatogram of rasagiline sample spiked with (*S*)-enantiomer at 0.15% level is shown in Fig. 2b. The recovery values for (*S*)-enantiomer are presented in Table 2.

### *Linearity*

The linearity calibration plot for the (*S*)-enantiomer was obtained over the calibration ranges tested, i.e., LOQ to 150% of specification level (Fig. 2c). The correlation coefficient obtained was greater than 0.999. The equation of the calibration curve for the (*S*)-enantiomer was $y = 16{,}848.4x - 1594.1$. The % bias was found to be 1.8%. The above result shows that an excellent correlation existed between peak area and concentration of the (*S*)-enantiomer over a range of 1.05 to 7.88 μg/ml.

### *Robustness*

In all the deliberate varied chromatographic conditions (flow rate, column temperature, and composition of organic solvent), both analytes were adequately resolved and the elution order remained unchanged. The resolution between rasagiline and its (*S*)-enantiomer was greater than 2.5, the % RSD for rasagiline peak from the standard solution was less than 3.8, and the tailing factor was less than 1.4. The results are reported in Table 3.

### *Solution Stability and Mobile Phase Stability*

The variability in the estimation of (*S*)-enantiomer was within ±10% during solution stability. The results from the solution stability and mobile phase stability experiments confirmed that mobile phase, sample solution, and standard solutions were stable up to 48 h.

**TABLE 2. Recovery results for (*S*)-enantiomers (*n* = 3)**

| Spiked levels | Added (μg/ml) | Recovered (μg/ml) | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| LOQ | 1.05 | 1.07 | 101.9 | 4.3 |
| 50% | 2.63 | 2.60 | 98.9 | 1.9 |
| 100% | 5.25 | 5.16 | 98.3 | 0.5 |
| 150% | 7.88 | 7.93 | 100.6 | 0.8 |

**TABLE 3. Robustness results of HPLC method**

| Variation in chromatographic condition | Observed system suitability parameters | | |
|---|---|---|---|
| | resolution[a] > 2.5 | USP tailing < 2.0 | % RSD < 5.0 (*n* = 6) |
| Column temperature 22 °C | 5.3 | 1.3 | 1.8 |
| Column temperature 32 °C | 5.6 | 1.2 | 1.2 |
| Flow rate 0.8 ml/min | 6.0 | 1.3 | 2.1 |
| Flow rate 1.2 ml/min | 5.6 | 1.3 | 1.9 |
| *n*-hexane: isopropyl alcohol: ethanol: diethyl amine (960:20:18:0.1 ml) | 5.9 | 1.3 | 1.5 |
| *n*-hexane: isopropyl alcohol: ethanol: diethyl amine (960:20:22:0.1 ml) | 4.9 | 1.4 | 2.5 |
| *n*-hexane: isopropyl alcohol: ethanol: diethyl amine (960:18:20:0.1 ml) | 4.7 | 1.4 | 2.3 |
| *n*-hexane: isopropyl alcohol: ethanol: diethyl amine (960:22:20:0.1 mL) | 5.0 | 1.3 | 1.7 |

[a]Resolution between rasagiline and its (*S*)-enanatiomer. Actual chromatographic conditions: Chiralcel-OJ-H (250 mm × 4.6 mm, 5 μm) column at 27 °C; mobile phase: *n*-hexane:isopropyl alcohol:ethanol:diethyl amine (96:2:2:0.1 ml); 1.0 ml/min of flow rate; wavelength of 215 nm.

## CONCLUSIONS

A simple, accurate, and efficient normal-phase chiral HPLC method was developed and validated for quantitative analysis of the (*S*)-enantiomer from rasagiline. A cellulose-based chiral column, the Chiralcel OJ-H column, was found to be selective for rasagiline and its (*S*)-enantiomer. Satisfactory results were obtained from method validation and the method found to be precise, accurate, linear, and robust during validation. The developed method can be conveniently used by quality control departments for the quantitative determination of enantiomeric impurity in rasagiline as a bulk drug.

## ACKNOWLEDGMENT

The authors are thankful to the management of Dr. Reddy's Laboratories Ltd., Hyderabad for providing facilities to carry out this work.

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