Asian Journal of Chemistry Vol. 15, Nos. 3 & 4 (2003), 1291–1296

Reverse-Phase HPLC Method for the Estimation of Selegiline Hydrochloride in Pharmaceutical Dosage Forms

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A rapid and sensitive high-performance liquid chromatographic method was developed for the estimation of selegiline hydrochloride in pharmaceutical dosage forms. Selegiline hydrochloride was chromatographed on a reverse phase C-18 column using ondansetron hydrochloride as internal standard in a mobile phase consisting of methanol and water in the ratio of 70:30 v/v. The mobile phase was pumped at a flow rate of 1 mL/min, and the eluents were monitored at 206 nm. The calibration curve was linear in the range of 0.2 to 20 μg/mL. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solution containing 5μg/mL was 98.47 ± 0.37% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining selegiline hydrochloride in bulk drug samples or in tablets.

Key Words: Estimation, Selegiline hydrochloride, Reverse-Phase HPLC.

INTRODUCTION

Chemically, selegeline is (R)-(-)-N,2-dimethyl-N-2-propynylphenethylamine hydrochloride. It is used in the treatment of Parkinson's disease and depression[1]. Several methods like potentiometric acidic-alkalimetric titration[2], LC-electrospray ionization mass spectrometry[3] and flourimetric[2] methods were reported. Some of the reported methods require specialized detectors and the process is considered tedious[2,3]. In the present study, a simple, sensitive, specific, precise and accurate HPLC method has been developed for the estimation of selegiline hydrochloride in bulk drug samples and pharmaceutical dosage forms using RP-C18 column.

EXPERIMENTAL

Selegiline hydrochloride and ondansetron hydrochloride were gift samples from M/s Sun Pharmaceutical Industries Ltd, Ahmedabad, India and M/s Natco

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Fine Pharmaceuticals Ltd., Hyderabad, India respectively. Methanol and water used were of HPLC grade (Qualigens). All other reagents (potassium dihydrogen orthophosphate) used in the study were of AR grade (Qualigens).

A gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP-C18 column (250 mm×4.6 mm I.D., particle size 5 μm, YMC, Inc., Wilmington, NC 28403, U.S.A) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

Preparation of stock solution of internal standard: Ondansetron hydrochloride was used as internal standard for the estimation of selegiline hydrochloride. About 100 mg of ondansetron hydrochloride was accurately weighed, transferred to 100 mL volumetric flask, dissolved in methanol and made up to volume with methanol so as to give a stock solution of 1000 μg/mL (Stock-I). 2 mL of this stock solution was diluted to 100 mL with 0.02 M potassium dihydrogen orthophosphate to give 20 μg/mL solution (Stock-II). 1 mL of stock-II solution was added to standard selegiline hydrochloride sample solutions.

Preparation of stock solutions of selegiline hydrochloride: About 100 mg of selegiline hydrochloride was accurately weighed and transferred to 100 mL volumetric flask. It was dissolved in water and the solution was made up to volume with water. Each mL of this stock solution (Stock-I) contained 1000 μg of selegiline hydrochloride. 10 mL of stock-I solution (1000 μg) were diluted to 100 mL with 0.02 M potassium dihydrogen orthophosphate to give a stock solution containing 100 μg/mL (Stock-II).

Chromatographic conditions: Both methanol and 0.02 M potassium dihydrogen orthophosphate were filtered before use through 0.4 μm membrane filter. The flow rate of the mobile phase was maintained at 1.0 mL/min in the ratio of 70:30 (methanol : 0.02-M potassium dihydrogen orthophosphate). The column temperature was maintained at 40°C and concentration of drug was detected by UV detector at 206 nm. The data were acquired, stored and analyzed with the software "Class-VP series version 5.03 (Shimadzu)".

Procedure: From stock-II solution of selegiline hydrochloride, 0.05 to 1 mL of solution were transferred to 10 mL volumetric flasks. To these solutions 1 mL of ondansetron hydrochloride (internal standard) containing 20 μg/mL was added and volume was made up to volume with 0.02 M potassium dihydrogen orthophosphate to get 0.2, 0.5, 1, 2, 4, 6, 8, 10 and 20 μg/mL. The standard solutions prepared as above were filtered through 0.4 μm membrane filter and the filtrate was injected five times into the column at a flow rate of 1 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of selegiline hydrochloride either in pharmaceutical formulations or in validation study.

Assay of selegiline in tablets: Ten tablets (each containing 5 mg) were weighed, finely powdered and an accurately weighed sample of powdered tablets

equivalent to 1 mg of selegiline hydrochloride was placed in a 100 mL volumetric flask. 70 mL of methanol was added and the flask was allowed to stand for 5 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then made up to 100 mL with water, thoroughly mixed, and filtered through a 0.2 μm membrane filter. An aliquot of this filtrate (2 mL) was transferred to a volumetric flask along with appropriate volume of ondansetron hydrochloride solution and made up to volume with methanol to give an expected concentration 10 μg/mL of selegiline hydrochloride and 2 μg/mL of ondansetron hydrochloride (internal standard). All determinations were conducted in triplicate. The same procedure was used to estimate the concentration of the drug in two more commercial brands of selegiline hydrochloride tablets.

Precision: The precision of the assay was determined in terms of intra- and inter-day variation in the peak area ratio for a set of drug solutions (10 or 20 μg/mL) on three different days (n = 5). The intra- and inter-day variation in the peak area ratio was calculated in terms of coefficient of variation (CV), and obtained by multiplying the ratio of standard deviation to the mean with 100 [CV = (SD/mean) × 100].

Accuracy: The accuracy of the HPLC assay method was assessed by adding known amount (5 or 10 μg) of the drug to a drug solution of known concentration (5 μg/mL) along with 2 μg/mL internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (5 or 10 μg) was added to the volumetric flask containing the powder sample of the tablet formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of selegiline hydrochloride in tablet formulations. In both the cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery, and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the per cent recovery.

RESULTS AND DISCUSSION

The present study was carried out to develop a specific, sensitive, precise and accurate HPLC method for the analysis of selegiline hydrochloride in pharmaceutical tablet dosage forms. The run time of the method was set at 10 min. The retention times of selegiline hydrochloride and ondansetron hydrochloride (internal standard) were 7.8 min and 3.6 min respectively (Fig. 1). When the same drug solution was injected 5 times, the retention time of the drug and internal standard were the same. This indicates that the present HPLC method is rapid, which in turn shows that the method consumes fewer amounts of expensive HPLC solvents. Table-1 shows the mean peak area ratios of selegiline hydrochloride solutions for 5 such determinations. When the concentration of selegiline hydrochloride and its respective peak area ratios were subjected to regression analysis by least squares method, a high correlation coefficient was observed ($r = 0.9995 \pm 0.058$) in the range of 0.2 to 20 μg/mL. The regression of selegiline hydrochloride concentration over its peak area ratio was found to be $Y = -0.1407 + 2.5363X$ where 'Y' is the peak area ratio and 'X' is the concentration of selegiline

hydrochloride. This regression equation was used to estimate the amount of selegiline hydrochloride either in tablet formulations or in validation study (precision and accuracy).

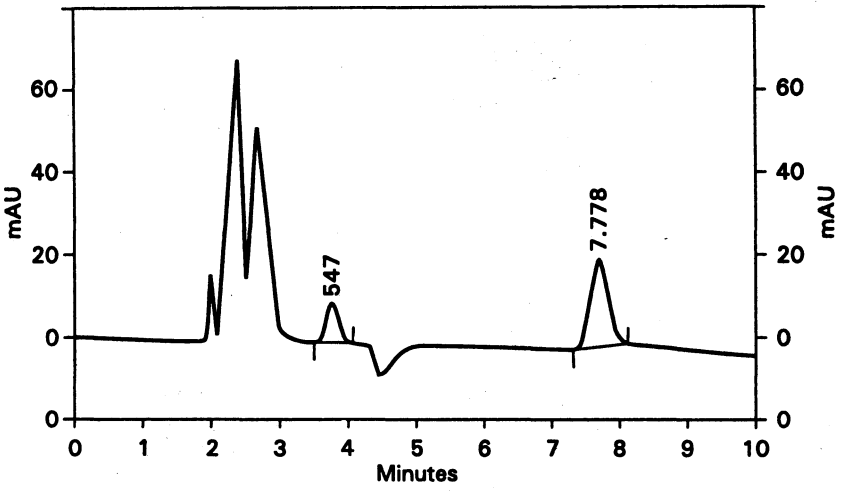

Fig. 1. Typical chromatogram for selegeline hydrochloride

TABLE-1
CALIBRATION OF THE HPLC METHOD FOR THE ESTIMATION OF SELEGILINE HYDROCHLORIDE

Concentration of selegiline hydrochloride (μg/mL)	Mean (±s.d) peak-area ratio (n = 5)	CV (%)
0	0	0
0.2	0.3666	1.98
0.5	1.1137	1.11
1	2.2309	1.25
2	4.4554	1.98
4	10.4865	1.96
6	15.3319	1.88
8	20.2035	1.57
10	24.9464	2.94
20	48.5856	1.78

Regression equation (from 0.2 to 20 μg/ mL):

$$Y = -0.14071 + 2.53632X \quad (r = 0.9995)$$

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 10 or 20 μg/mL of selegiline hydrochloride along with 2μg/mL of ondansetron hydrochloride were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area ratio

of the drug for five replicate injections was found to be less than 1.5%. Also, the inter-day variation (3 days and five injections) was found to be less than 3% (Table-2). Thus, the results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (5 or 10 μg) was added to a preanalyzed sample of drug solution (5 μg/mL), there was a high recovery (98.47 ± 0.37%) of selegiline hydrochloride indicating that the proposed HPLC method is highly accurate.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Selegiline hydrochloride concentration (μg/mL)	Concentration of selegiline hydrochloride (μg/mL) found on			
	Intra-day		Inter-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
10	10.12	1.09	10.05	1.47
20	20.19	1.21	20.09	2.50

TABLE-3
RECOVERY OF SELEGILINE HYDROCHLORIDE

Amount of drug added (μg)	Mean (±s.d.) amount (μg) recovered (n = 5)	Mean (±s.d.) % of recovery (n = 5)
5	4.93 ± 0.12	98.60 ± 0.51
10	9.89 ± 0.09	98.90 ± 0.02

The HPLC method, developed in the present study has also been used to quantify selegiline hydrochloride in tablet dosage forms. Selegiline hydrochloride tablets (containing 5 mg of the drug) were analyzed as per the procedure described above. The average drug content was found to be 97% of the labeled amount (Table-4).

TABLE-4
MEAN (±s.d) AMOUNT OFSELEGILINE HYDROCHLORIDE IN TABLET DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the tablet	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AA	5	4.81 ± 0. 19	96.20 ± 2.83
BB	5	4.68 ± 0.28	93.60 ± 1.41
CC	5	4.98 ± 0.10	99.60 ± 0.98

No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed HPLC method. A known amount of the drug solution was added to the powder sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method. There was a high recovery of selegiline hydrochloride (98.69 ± 0.31%) indicating that the proposed procedure for the determination of selegiline hydrochloride in the tablet dosage forms is highly accurate.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from Government of India, Department of Science and Technology (DST) and All India Council for Technical Education (under MODROBS) in establishing the infrastructure for HPLC. The authors thank M/s Sun Pharmaceutical Industries Ltd., Ahmedabad and M/s Natco Fine Pharmaceuticals Ltd., Hyderabad, India for providing gift samples of selegiline hydrochloride and ondansetron hydrochloride respectively.

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(*Received*: 21 November 2002; *Accepted*:26 March 2003) AJC-3021