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Original Article

RP-HPLC method development and validation of Selegiline hydrochloride in nanoemulsion formulation

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ABSTRACT

A simple, specific, precise and accurate RPHPLC method has been developed for determination of Selegiline hydrochloride in bulk and nanoemulsion formulation using C18 column;250 mm length, 4.6 mm internal diameter,0.5µ particle size with UV visible detector (detection wavelength206nm). The elution was performed in an isocratic mode with mobile phase consisting of methanol: phosphate buffer (95:5) at a flow rate of 1ml/minute. Methanol was used as a diluent and retention time was found to be 2.9 minutes. The method was found to be linear (20 to 160µg/ml), rugged and robust with LOQ of 0.00819 µg/ml. The percentage purity of Selegiline hydrochloride in nanoemulsion formulation was found to be 98.9%.

Key words: Selegiline hydrochloride, nanoemulsion, RPHPLC, retention time

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder characterized by tremors, muscle rigidity, changes in speech and gait caused due to reduction of dopamine level in brain. At low doses, Selegiline hydrochloride selectively and irreversibly inhibits monoamine oxidase, type B which is responsible for the metabolism of dopamine. Selegiline hydrochloride therefore acts as a neuroprotective and enhances the action of dopamine by preventing its metabolism. [1] It is used as an adjunct to levodopa/carbidopa in treating early-stage Parkinson's disease. When administered orally(5 mg twice a day), Selegiline hydrochloride metabolizes to L-amphetamine and L-methamphetamine that causes various side effects such as dizziness, dry mouth, insomnia, muscle pain, rash, nausea and constipation. Oral bioavailability of Selegiline hydrochloride is 4.4% due to extensive first pass metabolism. Transdermal delivery of Selegiline can provide benefits of improved patient compliance, controlled release, avoidance of hepatic first pass effect and reduction in side effects.[3] Nanoemulsions is one of the technique widely used in improving the transdermal permeation of lipophilic and hydrophilic drugs.

Literature reports that Selegiline has been analyzed by HPLC with UV/fluorescence detection and by spectrophotometric method in pharmaceutical dosage forms. [4,5,6]Analytical methods such as LCMS, fluorimetry and gas chromatography have been used for the estimation of Selegiline hydrochloride or its metabolites in human plasma by.[7,8,9] However, no method is available for its determination in nanostructured delivery systems such as nanoemulsions. Therefore, the main objective of the present study was to develop and validate a simple and accurate RPHPLC method for the quantitative estimation of Selegiline hydrochloride in w/o nanoemulsion formulation meant for transdermal administration.

MATERIALS AND METHODS

Instrumentation: The study was performed on a SHIMADZU HPLC instrument, SPINCHROME software using ODS, C18column;250mm length,4.6 mm internal diameter and 0.5µ particle size with UV visible detector.

Reagents and materials: Selegiline hydrochloride was received as a gift sample from Intas Pharma (Ahmedabad) and Viscup160® from Lonza (USA). HPLC grade solvents; methanol and water were

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used in the study. (S.D. Fine Chem. Ltd, Mumbai). Buffers and other chemicals were of analytical-reagent (AR) grade.1 gram of w/o nanoemulsion formulation contained Selegiline hydrochloride (0.5mg)dissolved in 3% w/w distilled water, 21%w/w of Smix (Span 85, PEG 400 and polysorbate 80) and 36% w/w of isopropyl myristate thickened with 40%w/w Viscup160®.

Chromatographic conditions: Isocratic elution of C18 column was done using mobile phase having composition of methanol: phosphate buffer (95:5). 2.7215 g of monobasic potassium phosphate was weighed and dissolved in 1000 ml of water. The resulting solution was filtered through 0.45µm filter. The flow rate was optimized to 1ml/min; run time was set at 15 minutes with column temperature maintained at 25ºC taking injection volume as 20 µl. Methanol was used as a diluent and the eluent was detected at 206nm.

Method development: Various solvent systems were tried for the development of suitable HPLC method. Methanol: phosphate buffer (95:5) was selected as the mobile phase. 100µg/ml standard solution of Selegiline hydrochloride was prepared in diluent and scanned in UV region of 200 – 400nm for selection of detection wavelength.

Preparation of Standard Solution of Selegiline hydrochloride: 100 mg Selegiline hydrochloride was weighed accurately and volume was made up to 100 ml with methanol.(concentration of standard stock sultion:1000µg/ml)5ml of it was transferred into a 50 ml volumetric flask and volume was made up to 50 ml with methanol.(Concentration of working standard solution: 100µg/ml)The quality control samples of Selegiline hydrochloride at three different levels were prepared at low concentration (20µg/ml), medium concentration (100µg/ml) and high concentration. (160µg/ml)

***Method validation*:** The validation of the method was done according to the ICH guidelines "Q2 (R1): Validation of Analytical Methods". [10]

Specificity: Specificity was assessed by comparing the chromatograms obtained from solutions of blank, nanoemulsion formulation and standard solution for Selegiline hydrochloride.

System suitability: Working standard solution (100µg/ml)was injected 6 times to assess the system suitability parameters. Peak area, theoretical plates and asymmetry were observed.

Linearity and range: Linearity was established over a concentration range of 20-160 µg/ml for Selegiline hydrochloride (n = 5). Mean peak areas(y) of Selegiline hydrochloride were plotted against their respective concentrations (x) and linear regression analysis was performed on the resulting calibration curve.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ was calculated from standard deviation and slope of response from linearity

$LOQ = 10\ \sigma / S$

$LOD = 3.3\ \sigma / S$

Where, σ is standard deviation from response, S is slope from calibration curve.

Intra and Inter-Day Precision and accuracy: Intra-day: repeatability, inter-day precision and accuracy of the developed method were determined by six replicate analyses of quality control samples prepared at concentrations of low QC (20µg/ml), medium QC (100µg/ml) and high QC(160µg/ml) on same day and on three consecutive days, respectively. The precision was calculated as %RSD of measured concentrations for each calibration level. Accuracy study was carried out by adding a known quantity of drug to quality control samples and reanalyzing contents by proposed method to find % recovery.

Robustness: The prepared standard (100µg/ml) was injected with change in the flow rate at 0.8ml/min and at 1.2ml/min. The column temperature was changed to 20ºC and 30ºC respectively. %RSD was calculated.

Ruggedness: The ruggedness of the method was determined by carrying out the experiment by different operators on the same instrument.

Estimation of Selegiline hydrochloride in nanoemulsion formulation: 515.06 mg of Selegiline hydrochloride nanoemulsion formulation was accurately weighed and volume made up to 10 ml with diluent. The solution was filtered through 0.45µ syringe filter.

RESULTS AND DISCUSSION

Method development: The study was aimed to develop and validate RPHPLC method for the determination of Selegiline hydrochloride in w/o nanoemulsion formulation. Methanol: phosphate buffer (95:5) was chosen as the mobile phase as it gave a sharp peak having retention time of 2.9 minutes, with required symmetry and lack of tailing. Lipophilic formulations usually require an extraction procedure or use of internal standard for analysis. However, in order to keep the analytical method simple and prevent drug precipitation, methanol was used as a diluent. 206 nm was

selected as wavelength on scanning as100µg/ml solution of Selegiline hydrochloride showed maximum absorption at this wavelength. Optimized conditions are given in table 1.

Method validation: Typical chromatograms of Selegiline hydrochloride standard and nanoemulsion formulation are shown in Fig.1.and Fig.2. The absence of interference peak with blank and sample at the retention time of Selegiline hydrochloride (2.9 minutes) ensured the specificity of the method. System suitability ensures that the system used for determination is sufficiently sensitive, specific and reproducible for the current analytical run. The acceptance criterion was met for system suitability as % RSD was found to be 1.031 with mean theoretical plates as 4733.302and average tailing factor of 1.249.(Table 2)The correlation coefficients (R^2 =0.9999) of the calibration plot reflected good linearity in the range of 20 µg/ml to 160 µg/ml (Fig.3.) Results of linearity and statistical data from calibration curve are shown in table 3 and table 4 respectively. LOD and LOQ obtained for the developed method were 0.0027µg/mland0.00819µg/ml respectively showed the sensitivity of the method at low concentrations and suggested its applicability in nanoemulsion formulation development. Results in Table 5 reflected that the developed method was precise and accurate. The intra-day precision was calculated RSD ≤ 0.240881 % (n= 6) and inter-day precision over three successive days was found RSD ≤1.199199% (n=6).The average percentage recovery range for Selegiline hydrochloride was found to be between99.4 % and 102.55%.Low %RSD values and good accuracy result (Table 5) proclaimed the superiority of the developed method. On changing the flow rate and column temperature, % RSD values were found to be below %0.34suggesting that the developed method was sufficiently robust for normal expected variations in chromatographic conditions. (Table6)%RSD was found to be less than 0.38% on changing the analyst which demonstrated that the method developed was rugged.

Estimation of Selegiline hydrochloride in nanoemulsion formulation: Absence of interfering peaks from excipients (Fig.2.) and drug content of 98.9% of the labeled claim (Table 7) reflected that the developed method could be used for the determination of Selegiline hydrochloride in nanoemulsion formulation.

CONCLUSION

The developed method was found to be linear over a range of 20µg/ml to160 µg/ml with LOD of 0.0027µg/ml and LOQ of 0.00819µg/ml. It was found to be precise, accurate, robust and rugged (suggested by low value of % RSD) and can be used for determination of Selegiline hydrochloride in bulk and w/o nanoemulsion formulation successfully.

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TABLE1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Chromatographic parameters	Optimized conditions
Mode	Isocratic
Column	ODS, C18 column, 250 x 4.6 mm, 5 µ
Run time	15 min
Flow rate	1ml/min
Detection wavelength	206nm
Injection volume	20µl sample loop
Column temperature	25ºC
Mobile phase	Methanol: phosphate buffer(95:5)
Diluent	Methanol

Optimized chromatographic conditions for the determination of Selegiline hydrochloride in nanoemulsion formulation

TABLE 2: RESULTS OF SYSTEM SUITABILITY

S.No	Area	Theoretical plates	Asymmetry
Standard 1	5294986	4673.963	1.257
Standard 2	5292711	4671.087	1.256
Standard 3	5288793	4690.289	1.254
Standard 4	5305300	4690.655	1.253
Standard 5	5308875	4683.657	1.255
Standard 6	5431267	4990.161	1.220
Average	5320322	4733.302	1.249
SD	54888.7	-	-
% RSD	1.031	-	-

System suitability meets the acceptance criteria (%RSD<2).

TABLE 3: RESULTS FOR LINEARITY FOR SELEGILINE HYDROCHLORIDE

Concentration (µg/mL)	Peak Area	Retention Time(minutes)	Asymmetry	Theoretical Plates
20	1366571	2.937	1.009	4106.851
40	2348036	2.941	1.140	4503.612
100	5287541	2.939	1.251	4711.076
120	6281762	2.940	1.268	4771.001
160	8152361	2.940	1.288	4743.211

Linearity observed in the range of 20 µg/ml to 160 µg/ml.

TABLE 4: STATISTICAL DATA OF CALIBRATION CURVE OF SELEGILINE HYDROCHLORIDE

Parameters	Selegiline hydrochloride
Linearity	20-160 µg/ml
Linearity equation	48630x+ 407828
Correlation Coefficient(R^2)	0.9999
Slope	48630
Intercept	407828

Linearity observed in the range of 20 µg/ml to 160 µg/ml with R^2*<1.*

TABLE 5: RESULTS OF ACCURACY AND PRECISION STUDIES

Parameters	Concentration taken (µg/ml)	Concentration added (µg/ml)	Concentration found (µg/ml)	Precision RSD (%)	Accuracy (%)
Intraday precision	20	5.5	25.34±0.054	0.214203	99.4
	100	5.5	106.78±0.091	0.089432	101.22
	160	5.5	169.71±0.40	0.240881	102.55
Inter-day precision	20	5.5	25.37±0.08	0.331575	99.46
	100	5.5	105.573±1.26	1.199199	99.01
	160	5.5	168.55±1.583	0.939452	101.82

Precision meets the acceptance criteria (%RSD<2.)Accuracy found between 99.4 &102.55%.

TABLE 6: ROBUSTNESS OF DEVELOPED METHOD FOR SELEGILINE HYDROCHLORIDE

Parameters	Variation	Area	Theoretical plates	Trailing factor	%RSD
Standard flow rate 1	-	5298133	4682	1.26	0.16
Flow rate	0.8ml/min	6741960	5304	1.22	0.17
	1.2ml/min	4524832	4400	1.25	0.34
Temperature	20°C	5312480	4740	1.26	0.09
	30°C	5374047	4749	1.24	0.34

Robustness meets acceptance criteria (%RSD<0.34).

TABLE 7: RESULTS OF ASSAY OF NANOEMULSION FORMULATION

Pharmaceutical dosage form	Labeled claim(mg)	Amount found(mg)	% Assay
Selegiline hydrochloride nanoemulsion formulation	0.5	0.494	98.9

Transdermal nanoemulsion formulation contained 0.5mg of Selegiline hydrochloride /ml.

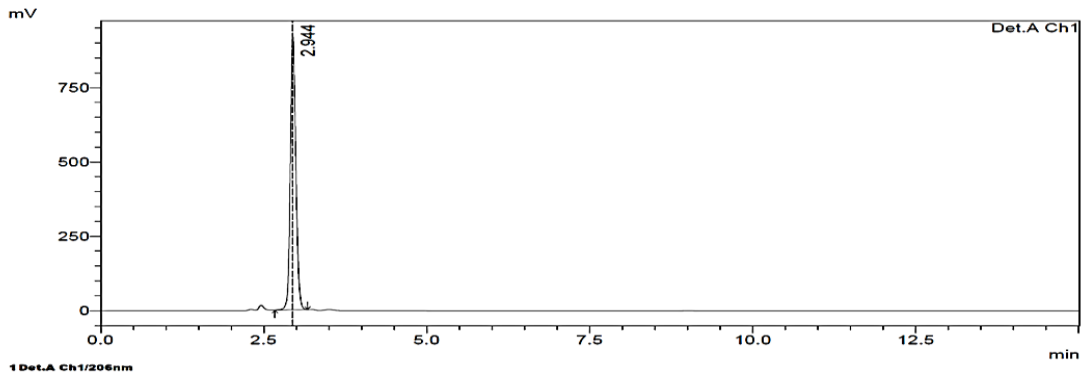

Fig.1.Chromatogram of standard of Selegiline hydrochloride

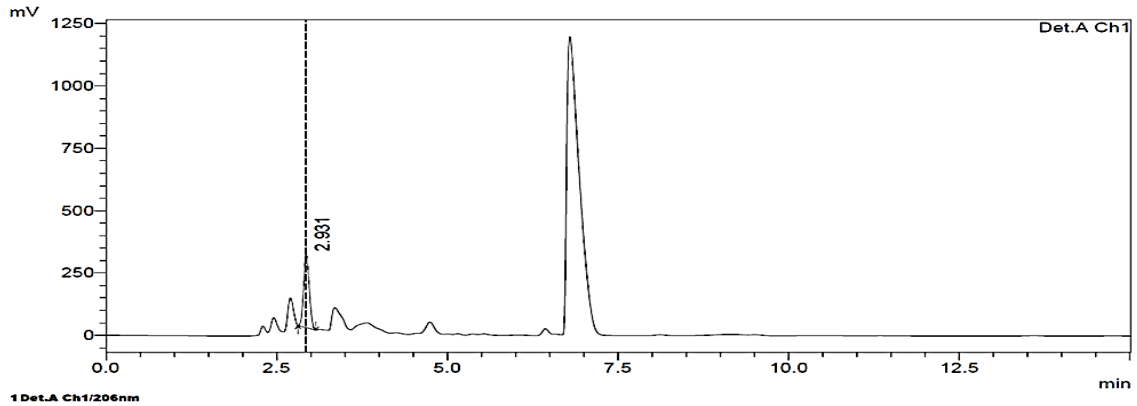

Fig.2.Chromatogram of sample of Selegiline hydrochloride

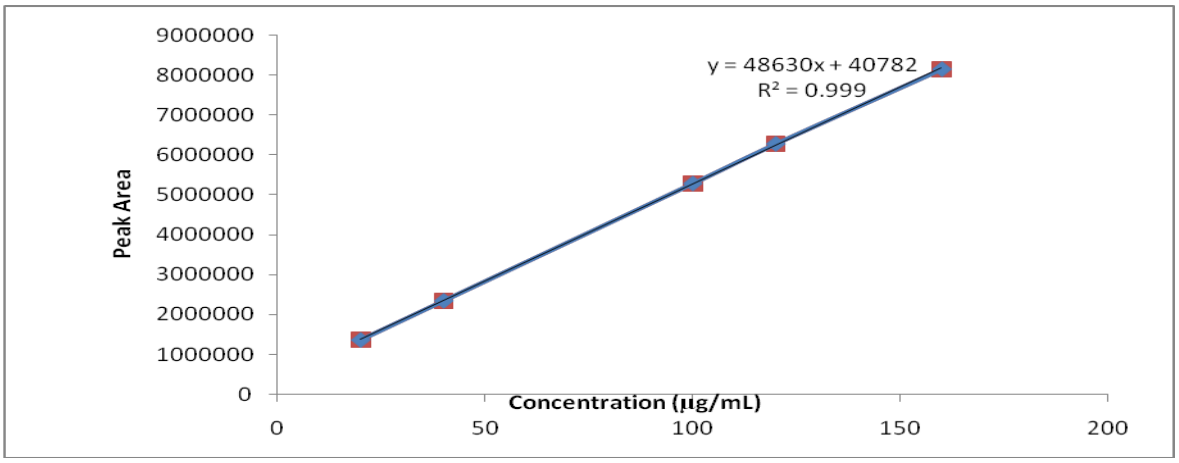

Fig.3.Standard curve for Selegiline hydrochloride

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