RESEARCH ARTICLE

Development and Validation of RPHPLC method of Tacrine Hydrochloride in Nanoemulsion gel

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ABSTRACT

A simple, specific, precise and accurate RPHPLC method has been developed for determination of Tacrine hydrochloride in bulk and nanoemulsion gel using C18 column; 250 mm length, 4.6 mm internal diameter, 0.5µ particle size with UV visible detector (detection wavelength 243nm).Chromatographic separation was performed in an isocratic mode with mobile phase consisting of 0.05M triethylamine: acetonitrile (80: 20,); pH 3 using methanol as a diluent at a flow rate of 1.5 ml/minutes. Retention time was found to be 5.8 minutes. Linearity was established over a range of 20 to 160µg/ml. The method was rugged, robust with LOD of 0.00101µg/ml and LOQ of 0.0029 µg/ml. The percentage purity of tacrine hydrochloride in nanoemulsion gel was found to be 100.63%.

Keywords: Tacrine hydrochloride, nanoemulsion gel, RPHPLC, retention time

INTRODUCTION

Tacrine hydrochloride (9-amino-l, 2, 3, 4-tetrahydroacridine hydrochloride) is a reversible cholinesterase inhibitor which is used to treat mild to moderate Alzheimer's disease. [1] It undergoes extensive first-pass metabolism and is associated with reversible dose-dependent hepatotoxicity along with peripheral cholinergic side effects. Oral administration of tacrine (10 mg 4 times daily) results in poor bioavailability (17%±13%). [2] Transdermal delivery of tacrine hydrochloride by w/o nanoemulsion gel can provide benefits of improved patient compliance and avoidance of first pass metabolism along with reduction in dose related side effects. [3] Literature reports that Tacrine has been analyzed by spectrofluorimetry and by HPLC in pharmaceutical dosage forms and plasma. [4-7] However, there is unavailability of any method for its determination in lipophilic formulations. Hence the present study was aimed to develop and validate a simple, sensitive and accurate RP-HPLC method for w/o nanoemulsion gel of tacrine hydrochloride.

MATERIALS AND METHODS

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Instrumentation

The study was performed on a SHIMADZU HPLC instrument, SPINCHROME software using ODS, C18 column; 250 mm length, 4.6 mm internal diameter and 0.5µ particle size with UV visible detector. (SHIMADZU).

Reagents and materials

Tacrine hydrochloride was purchased from Sigma Aldrich. Labrail M, Transcutol P and Capryol 90 were gift samples form Gattefosse, France. HPLC grade solvents; methanol, triethylamine, acetonitrile, water were 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 contained 7mg Tacrine hydrochloride dissolved in 10% w/w distilled water, 30%w/w of Smix (Labrafil M and Transcutol P in 1:4 ratio) and 60% w/w of Capryol 90 thickened with 98.9 mg of ethyl cellulose(20 cps).

Chromatographic conditions

Isocratic elution of C18 column was done using mobile phase having composition of 0.05M triethylamine: acetonitrile in the ratio of 80: 20. 13.8 ml of triethylamine was dissolved in 1900 ml water; pH was adjusted to 3.0 with formic acid and volume made up to 2000 ml with HPLC grade water. The flow rate was optimized to 0.5ml/min; run time was set at 10 minutes with column temperature maintained at 25°C

taking injection volume as 10 µl. As the nanoemulsion gel was of lipophilic nature (w/o type), methanol was used as a diluent. The eluent was detected at 243nm.

Method development

Various solvent systems were tried for the development of suitable HPLC method.0.05M triethylamine: acetonitrile (80: 20), pH 3 was selected as the mobile phase while methanol was chosen as the diluent. 100µg/ml standard solution of Tacrine hydrochloride was prepared in methanol and scanned in UV region of 200 – 400nm for selection of detection wavelength.

PREPARATION OF STANDARD SOLUTION OF TACRINE HYDROCHLORIDE

100 mg Tacrine hydrochloride was weighed accurately and volume 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 tacrine 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". [8]

Specificity

Under the proposed chromatographic conditions, specificity was assessed by comparing the chromatograms obtained from solutions of blank, nanoemulsion gel and standard solution for tacrine hydrochloride.

System suitability

The prepared standard (100µg/ml) was injected 6 times to study the system suitability parameters. Peak area, theoretical plates and asymmetry were observed.

Linearity and range

Linearity was established over the concentration range of 20-160 µg/ml for Tacrine hydrochloride (n = 5). Mean peak areas(y) of Tacrine 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 is calculated from standard deviation and slope of response from linearity

$LOQ = 10\ \sigma / S \quad LOD = 3.3\ \sigma / S$

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 the same day and on three consecutive days, respectively. The precision was calculated as the %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 1.3ml/min and at 1.7ml/min. The column temperature was changed to 20°C and 30°C respectively %RSD was calculated.

Ruggedness:

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

Estimation of tacrine hydrochloride in nanoemulsion gel

539.31 mg of Tacrine hydrochloride nanoemulsion gel 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 a simple RPHPLC method for determination of Tacrine hydrochloride in w/o nanoemulsion gel. Although the formulation was lipophilic in nature, neither extraction procedure was involved nor was internal standard used. Methanol was used as a diluent and 0.05M triethylamine: acetonitrile (80: 20), pH 3 was selected as the mobile phase as it gave a good sharp peak with retention time of 5.8 minutes, with required symmetry and lack of tailing. 243nm was selected as wavelength on scanning as 100µg/ml solution of Tacrine hydrochloride showed maximum absorption at this wavelength. Optimized conditions are given in table 1.

Table 1: Optimized chromatographic conditions for Tacrine hydrochloride determination

Mode	Isocratic
Column	ODS, C18 column, 250 x 4.6 mm, 5 µ
Run time	10 min
Flow rate	1.5 ml/min
Detection wavelength	243 nm
Injection volume	10µl sample loop
Column temperature	25°C
Mobile phase	0.05M triethylamine: acetonitrile (80: 20)
Diluent	Methanol

Method validation

Typical chromatograms of tacrine hydrochloride standard and nanoemulsion gel are shown in Fig.1.and Fig.2. The absence of interference peak with blank and sample at the retention time of Tacrine hydrochloride ensured that the method was specific. System suitability met the acceptance criterion with % RSD as 0.4586, mean theoretical plates as 8385.391 and average tailing factor of 1.3005. (Table 2). The correlation coefficients (R2 = 0.9997) 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 0.001µg/ml and 0.003µg/ml respectively showing a high level of sensitivity in low concentration range. Results in Table 5 reflected that the developed method was precise and accurate. The intra-day precision was calculated ≤ 1.49% (n= 6) and interday precision over three successive days was found ≤ 1.63% (n=6). The average percentage recovery range for Tacrine hydrochloride was found to be between 98.46% and 101.41%. The method was found to be robust on changing the flow rate and column temperature as the % RSD values were below 0.32%. (Table 6) %RSD was found to be less than 0.36% on changing the analyst which demonstrated that the method developed was rugged.

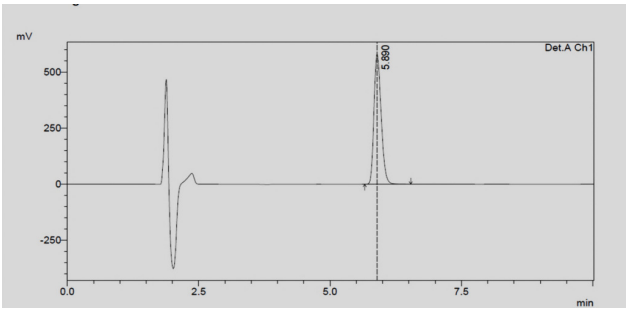

Fig.1: Chromatogram of standard of Tacrine hydrochloride

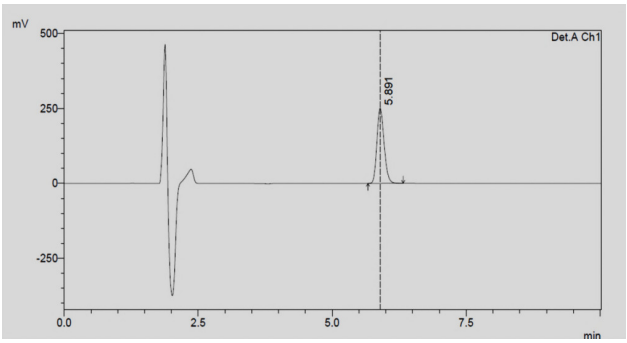

Fig.2: Chromatogram of sample of Tacrine hydrochloride

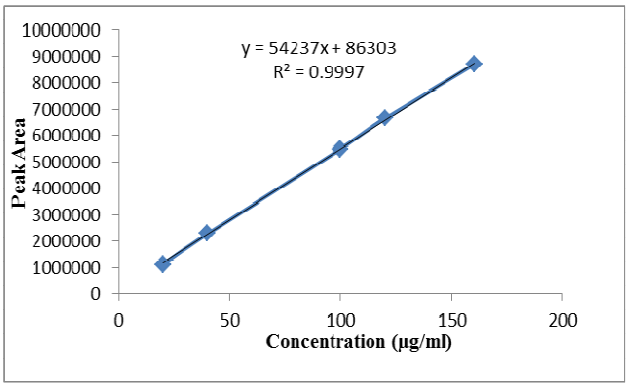

Fig.3: Standard curve for Tacrine hydrochloride

Table 2: Results of system suitability

S. No	Area	Theoretical plates	Asymmetry
Standard 1	5516234	8370.481	1.301
Standard 2	5496310	8405.523	1.301
Standard 3	5497745	8410.304	1.299
Standard 4	5513685	8410.07	1.301
Standard 5	5519886	8348.502	1.299
Standard6	5565929	8367.464	1.302
Average	5518298	8385.391	1.3005
Standard Deviation	25308.78	-	-
% RSD	0.458634	-	-

Table 3: Results for linearity for Tacrine hydrochloride

Concentration (µg/ml)	Peak Area	Retention Time (minutes)	Asymmetry	Theoretical Plates
20	1126298	5.904	1.209	8761.277
40	2284077	5.899	1.231	8721.704
100	5508842	5.885	1.300	8399.126
120	6668181	5.878	1.326	8250.628
160	8708369	5.870	1.371	7956.224

Table 4: Statistical data of calibration curve of Tacrine hydrochloride

Parameters	Tacrine hydrochloride
Linearity	20-160 µg/ml
Linearity equation	Y=54237x+86303
Correlation Coefficient(R^2)	0.9997
Slope	54237
Intercept	86303

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	20	39.88 ± 0.483	1.21	99.7
	100	20	119.08 ±1.156	0.97	99.23
	160	20	177.23 ±2.64	1.49	98.46
Interday precision	20	20	40.566 ±0.574	1.41	101.41
	100	20	123.366 ±1.87	1.55	100.60
	160	20	180.533 ±2.94	1.63	100.29

Table 6: Robustness of developed method for Tacrine hydrochloride

Parameters	Variation	Area	Theoretical plates	Trailing factor	%RSD
Standard flow rate 1.5 ml/min and temperature 25°C	-	5518298	8385.391	1.3005	0.45
Flow rate	**1.3ml/min**	6459634	8976	1.315	0.14
	1.7ml/min	4985507	7718	1.297	0.32
Temperature	**20°C**	5700249	8267	1.299	0.14
	30°C	5705530	8324	1.313	0.24

Estimation of Tacrine hydrochloride in nanoemulsion gel

The drug content was found to be 100.63% of the labeled claim in the assay of nanoemulsion gel of tacrine hydrochloride. (Table 7)

Table 7: Results of assay of nanoemulsion gel

Formulation	Labeled claim(mg)	Amount found(mg)	% Assay
Tacrine hydrochloride nanoemulsion gel	7	7.04	100.63

CONCLUSION

The developed method was found to be linear over a range of 20µg/ml to160 µg/ml with LOD of 0.001µg/ml and LOQ of 0.003µg/ml. It was found to be precise, accurate, robust and rugged (suggested by low value of % RSD) and can be used for determination of Tacrine hydrochloride in bulk and w/o nanoemulsion gel successfully.

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