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Stability Indicating Analytical Method Development and Validation For Estimation of Donepezil HCL and It's Pharmaceutical Dosage Form

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ABSTRACT

A simple and sensitive high performance thin layer chromatography (HPTLC) method has been developed for the quantitative estimation of Donepezil HCL in its bulk and tablet dose tablet formulation (5 mg). Donepezil HCL was chromatographed on silica gel 60 F254 TLC plate using methanol : toluene (2:8, v/v) as mobile phase. Donepezil HCL showed Rf value 0.54 + 0.008 and scanned at 245 nm using a camag TLC scanner 3. The method was validated in terms of linearity (100 – 800 ng/spot), precision (system precision = 0.0123 and method precision = 0.0084), accuracy (100.3 ± 0.76) and specificity. The limit of detection and limit of quantification for Donepezil HCL were found to be 1.72 ng/spot and 2.07 ng/spot, respectively. The developed method was successfully used for the assay of Donepezil HCL tablet formulation. This method also contain forced degradation studies for standard and tablet. The method was found to be simple, sensitive, specific, accurate and precise and can be used for the routine quality control testing of Donepezil HCL in tablet dosage form.

Keywords: Donepezil HCL , HPTLC, tablet, validation, forced degradation studies.

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INTRODUCTION

Donepezil HCl is chemically 2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1H-inden-1-one hydrochloride[1]. It is centrally acting reversible acetyl cholinesterase inhibitor. It is used in the management of Alzheimer's disease where it is used to increase cortical acetylcholine. Enantioselective LC–MS–MS[2], High-performance liquid chromatography [3, 4], UV and Spectrofluorimetric method [5] has been reported for Donepezil HCl. There is only one method has so far been reported for the estimation of Donepezil HCl by HPTLC in pharmaceutical dosage forms but that paper not contain forced degradation studies. The aim of present study is to develop a HPTLC method for the estimation of Donepezil HCl in bulk and in tablets with the forced degradation studies. The present paper describes simple, sensitive, accurate, precise and specific HPTLC method for the estimation of Donepezil HCL in bulk and tablet dose form including forced degradation studies.

MATERIALS AND METHOD

Apparatus

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag (Muttenz, Switzerland) flat bottom and twin-trough developing chamber (10 × 10 cm), UV cabinet with dual wavelength UV lamp, Camag winCATS software, Hamilton syringe (100 µl), Sartorius CP224S analytical balance (Germany), Ultrasonic bath (Frontline FS-4, Mumbai, India) were used in the study.

Reagents and Materials

Pharmaceutical grade of Donepezil HCL pure powder was kindly supplied as a gift sample from Zydus Cadila Healthcare Ltd., Maharashtra, India. Silica Gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. The pharmaceutical tablet formulation containing 5 mg of Donepezil HCL was procured from the local pharmacy. Methanol and Toluene (HPLC grade, Rankem, India) were used for mobile phase preparation and as solvents.

Preparation of standard solution

A standard solution of Donepezil HCL (500 µg/ml) was prepared by accurately weighing Donepezil HCL (5 mg) and transferred in 10 ml volumetric flask, dissolved in and diluted up to mark with methanol.

Preparation of sample solution

Twenty tablets were weighed, their average weight was determined, and crushed in mortar. Powder equivalent to 105 mg of Donepezil HCL was weighed and transferred to 25 ml volumetric flask. The drug from powder were dissolved and makeup volume with methanol. To ensure complete dissolved of drugs it was ultrasonicated for 10 min. The extract was filtered through Whatman filter paper No. 41 and residue was washed with methanol. The extract and washing were pooled and transferred to another 25 ml volumetric flask and volume was made with methanol. Four microlitres of this solution was applied to the HPTLC plate and followed by development.

Chromatographic conditions

The chromatographic estimations were performed using following condition; stationary phase, precoated Silica Gel 60 F254 aluminum sheets (10 × 10 cm) (pre-washed with methanol and dried in air); mobile phase, methanol : toluene (2:8, v/v); chamber saturation time, 15 min; temperature, 25 ± 2 , migration distance, 80 mm; wavelength of detection, 254 nm; slit dimensions, 5 × 0.45 mm; scanning speed, 10 mm/s. Following spotting parameter were used - band width, 6 mm; distance from the plate edge, 10 mm; space between two bands, 10 mm and spraying rate, 1 µl/s.

Chromatographic separation

Four microlitres of standard solution of Donepezil HCL (500 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of methanol : toluene (2:8, v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 15 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 254 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

Validation of the proposed method

The proposed method is validated according to the International Conference on Harmonization (ICH) guidelines[12] .

Linearity (Calibration curve)

A liquots of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of standard Donepezil HCL solution (500 µg/ml) were spotted on precoated TLC plate using semiautomatic spotter under nitrogen stream. The TLC plate was developed and photometrically analyzed as described under chromatographic separation. Each concentration was spotted five times on the TLC plate. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Donepezil HCL by the standard addition method. Known amounts of standard solutions of Donepezil HCL was added at 80, 100 and 120 % level to prequantified sample solutions of Donepezil HCL. The amount of Donepezil HCL was estimated by applying obtained values to the regression line equation.

Precision

1.Method Precision

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of Donepezil HCL (500 ng/spot) without changing the parameters of the proposed method.

2. System Precision

The system precisions of the proposed methods were determined by estimating the corresponding responses for concentration of standard solution of Donepezil HCL for the proposed method. The results were reported in terms of relative standard deviation (% RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and the LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines12 .

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma /S$

Where σ = Standard deviation of the response

S = Slope of calibration curve

Specificity

The specificity of the method was ascertained by analyzing standard drugs and the sample. The spots for deflazacort in the samples were confirmed by comparing the Rf and spectra of the spots with that of the standards.

Analysis of Donepezil HCL in Tablet

Four microlitres of sample solution was applied to the TLC plate to get 500 ng/spot and followed by development and scanning as described earlier. Analysis was carried out in triplicate, peak areas were measured at 254 nm and sample concentrations calculated. The amount of Donepezil HCL present in the sample solution was determined by fitting area values of peak corresponding to Donepezil HCL into the equation of line representing calibration curve of Donepezil HCL. The potential interference from excipients was also examined.

Pharmaceutical Preparation Assay, Precision Evolution

The amount of Donepezil HCL was found by number of replicates of the pharmaceutical preparations. The assay results were reported in the table. Precision studies were performed by

using standard solution containing Donepezil HCL, the concentration of drug covering the entire calibration range. The precision of the method in terms of system and method precision by analyzing the standard solution. The result of the precision studies are as showed in table. No.1

Table 1: Result of Donepezil HCL in Pharmaceutical Formulation (n=5)

Brand	Sample No.	Amount of Donepezil HCL (Lable Claim 5 mg per Tablet)
Donepezil HCL	1	5.10
	2	5.12
	3	4.98
	4	5.05
	5	4.87
Mean Assay		5.04 ± 0.50
(%) Mean Assay		100.4 ± 1.24
(%) R.S.D.		0.894

*n is number of replication

RESULTS AND DISSCUSION

Donepezil HCL is soluble in methanol; therefore methanol was selected as solvent. Several mobile phases were tried to accomplish good separation of Donepezil HCL. Using the mobile phase methanol : toluene (2:8, v/v) and 10 × 10 cm silica gel 60F254 aluminum-backed plates, good separation was attained with retardation factor (Rf) values of 0.54± 0.008 for Donepezil HCL (Figure 1 and 2). A wavelength of 254 nm was used for quantification of the drug. Even saturation of TLC chamber with mobile phase for 15 min assured better reproducibility and better resolution. Linearity range for Donepezil HCL was found in the concentration range of 100 to 800 ng/spot, with a correlation coefficient of 0.9877. The average linear regression equation was represented as Y = 2214.7X + 1099.2, where X = concentration of Donepezil HCL in ng/spot and Y = peak area. The limit of detection and limit of quantification for Donepezil HCL were found to be 1.72 ng/spot and 2.07 ng/spot, respectively indicate sensitivity of the method.

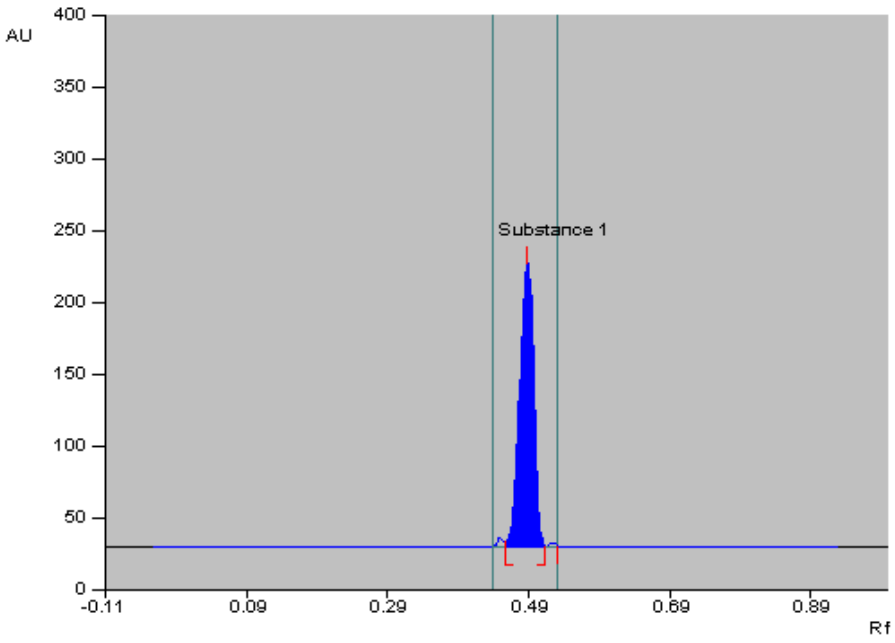

Figure 1: HPTLC Chromatogram of Donepezil HCL With Corresponding Rf Value at 254nm.

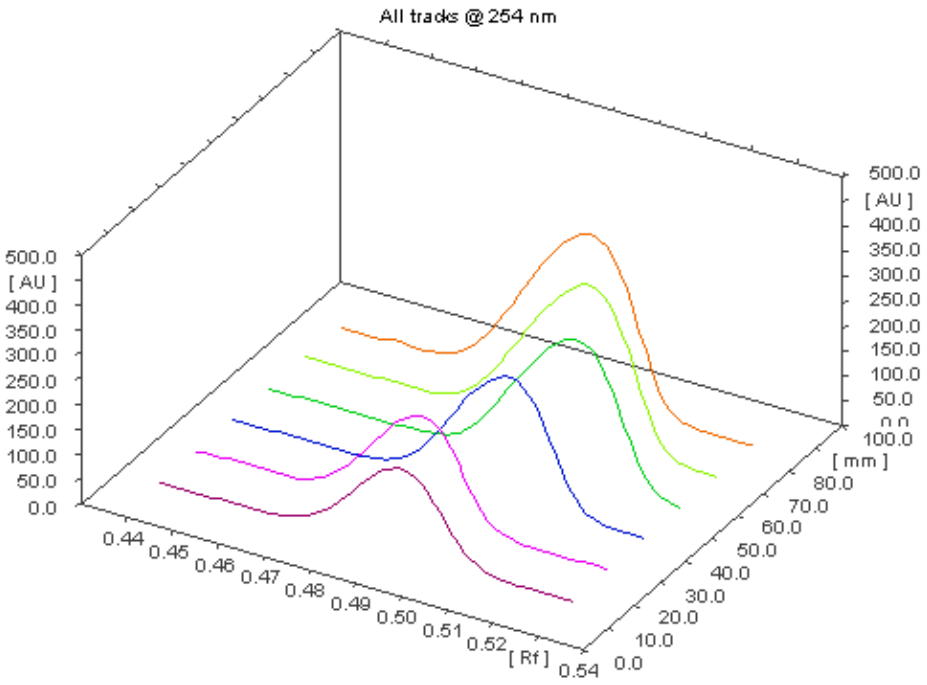

Figure 2: 3D-Chromatogram Shows Peaks of Donepezil HCL in Different Concentrations at 254nm.

The System precision (% RSD) was calculated for standard Donepezil HCL solutions for 3 times on the same day. The Method precision (% RSD) was calculated for standard Donepezil HCL solutions for 3 times over a period of one week. The System and Method precision variation (%

RSD) were found to be in the range of 0.0123 and 0.0084, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (500 ng/spot) of Donepezil HCL six times without changing position of the plate and % RSD for measurement of peak area was found to be 0.24. The % RSD for measurement of peak area ensures proper functioning of HPTLC system indicates repeatability of the proposed method. Different validation parameters for the proposed HPTLC method for determining Donepezil HCL content are summarized in Table. 3

Table 2: Result From Precision Evaluation

Donepezil HCL	System Precision	Method Precision
Mean Peak	4638.74	4608.32
S.D.	0.0124	0.009954
R.S.D. (%)	0.0123	0.0084

Table 3: Regression Analysis Data and Summary of Validation Parameters for Proposed HPTLC Method

Parameters	Results
Linearity Range (ug)	0.5-3ug
Slope	2214.7
Intercept	1099.2
Correlation Co-efficient (r2)	0.9877
Precision (% RSD) :	
System Precision (% RSD)	0.0123
Method Precision (% RSD)	0.0084
Accuracy (% Recovery) (n = 5)	100.3 ± 0.76
Limit of Detection (LOD) (ng/spot)	1.72
Limit of Quantification (LOQ) (ng/spot)	2.07
Specificity	Specific

*n is number of determination and RSD is relative standard deviation.

Accuracy of the method was evaluated by calculating recovery of Donepezil HCL by standard addition method at 3 different levels of the calibration curve (n = 5). The % mean recovery was found to be 100.3 ± 0.76 ensuring that the method is accurate (Table4).

Table 4: Recovery Data for the Proposed Method

Type of Recovery	%Label Added	Amount Claim	%Amount of Recovered	Recovery
80		4	4.98	100.3
100	5	5	5.05	100.25
120		6	6.08	98.2

*mean of six determinations.

The method was found to be specific for Donepezil HCL. The specificity of the method was ascertained by analyzing standard drug and the samples. The spot for Donepezil HCL in the sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. The peak purity of Donepezil HCL was assessed by comparing the spectra of standard at peak start, peak apex and peak end positions of spot. Good correlation was also found between standards and sample spectra (Figure 3). None of the formulation excipients were interferes in the quantification of Donepezil HCL at this Rf value.

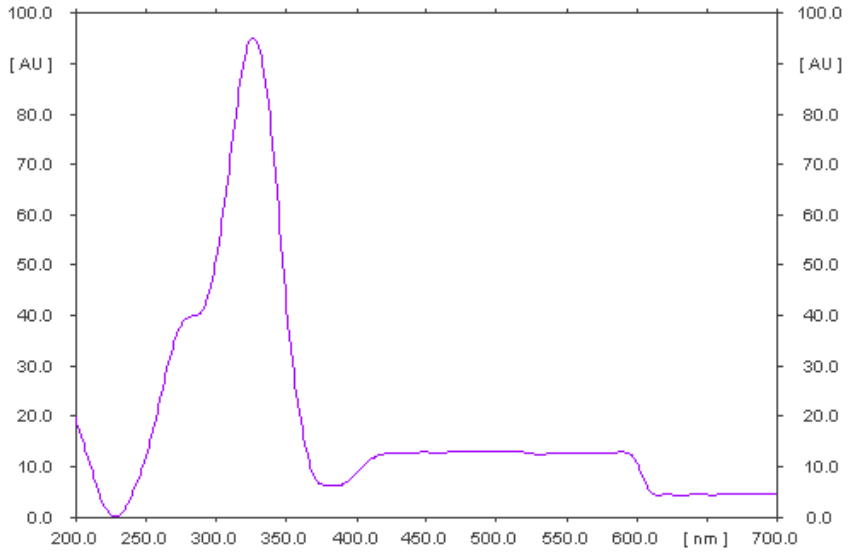

Figure 3: Overlain UV Absorption Spectrum of Standard and Sample Donepezil HCL

This method was applied to determine the content of Donepezil HCL in market sample of component Donepezil HCL tablet. The average percentage of Donepezil HCL in market sample was found to be 99.98 ± 0.75 (n = 6). The results are in agreement with the labeled value of Donepezil HCL in tablet dosage form (Table. No. 3). The results indicate that the proposed HPTLC method was found to be simple, sensitive, specific, precise and accurate for the estimation of Donepezil HCL in bulk and tablet formulations.

FORCED DEGRADATION STUDIES:

For Tablet :

Amount of tablet powder equivalent to about 105 mg of Donepezil HCL Tablet was separately transferred to five different 25.0 ml volumetric flasks (Flask no. 1, 2, 3, 4 and 5), added 5.0 ml of methanol as co-solvant. To flask no. 1, 2 and 3, added 3 ml 0.1 N HCl, 0.1 N NaOH and 3 % H_2O_2, respectively. The flask no. 1, 2, and 3 were kept in water bath at 80^0C for 2 hr. Flask no. 4 containing tablet powder was kept at 80^0C for 24 hrs in hot air oven to study the effect of heat on tablet sample (heat degradation). The forced degradation studies were performed in dark to

exclude the possible degradative effect of light. Flask no. 5 containing tablet powder was kept under UV chamber for 24 hr. to study the photolytic degradation of tablet sample. All the flasks were removed after stipulated time interval; the tablet samples were treated and analyzed in similar manner as described under analysis of tablet formulation.

For Standard:

Amount of pure powder equivalent to about 5 mg of Donepezil HCL Tablet was separately transferred to five different 10 ml volumetric flasks (Flask no. 1, 2, 3, 4 and 5), added 5.0 ml of methanol as co-solvant. To flask no. 1, 2 and 3, added 3 ml 0.1 N HCl, 0.1 N NaOH and 3 % H_2O_2, respectively. The flask no. 1, 2, and 3 were kept in water bath at 80^0C for 2 hr. Flask no. 4 containing pure powder was kept at 80^0C for 24 hrs in hot air oven to study the effect of heat on powder sample (heat degradation). The forced degradation studies were performed in dark to exclude the possible degradative effect of light. Flask no. 5 containing pure powder was kept under UV chamber for 24 hr. to study the photolytic degradation of powder sample. All the flasks were removed after stipulated time interval; the powder samples were treated and analyzed in similar manner as described under analysis of pure powder.

Table 5: Result of Forced Degradation Studies for Tablet

Sr. No.	StressCondition	Temperature and Time	% Assay of Active Substance	Rf Values of Degraded Peaks
1.	Acid(0.1 N HCl)	80^0C for 2 hr	79.95%	0.44
2.	Alkali(0.1 N NaOH)	80^0C for 2 hr	80.27%	0.49
3.	Oxide(3 % H_2O_2)	80^0C for 2 hr	75.25%	0.43
4.	ThermalDegradation	80^0C for 24 hr	70.02%	0.38
5.	PhotoDegradation	24 hr	71.49%	0.38

Table 6: Result of Forced Degradation Studies for Standard

Sr. No.	Stress Condition	Temperature and Time	% Assay of Active Substance	Rf Values of Degraded Peaks
1.	Acid(0.1 N HCl)	80^0C for 2 hr	83.84%	0.48
2.	Alkali(0.1 N NaOH)	80^0C for 2 hr	84.31%	0.49
3	Oxide(3 % H_2O_2)	80^0C for 2 hr	81.83%	0.48
4.	ThermalDegradation	80^0C for 24 hr	70.04%	0.38
5.	PhotoDegradation	24 hr	71.49%	0.38

CONCLUSION

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Donepezil HCL. The observations and results obtained from this study, including

specificity, linearity and range, accuracy, precision (method precision as repeatability and intermediate precision) are lie well within acceptable results. From the experimental studies it can be concluded that proposed method can be adopted for the routine analysis of Donepezil HCL in bulk and tablets without interference of excipients.

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