

## A Validated HPTLC Method for the Estimation of Donepezil HCL in Bulk and Its Tablet Dosage Form

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### Abstract

A simple, rapid, reliable and accurate HPTLC method has been developed for the quantitative determination of Donepezil HCl in bulk and tablets. Various aliquots of the sample solution were spotted automatically by means of camag Linomat 5 applicator on pre-coated silica gel 60 F<sub>254</sub> on aluminium sheet as stationary phase pre washed with methanol using Methanol: Chloroform (8:2 v/v) as mobile phase. The spots were scanned at 254 nm. The R<sub>f</sub> value of Donepezil HCl was 0.54 ± 0.02. Calibration curves were linear in the range of 200-1000 ng spot<sup>-1</sup>. The limit of detection and limit of quantification were found to be 120 ng spot<sup>-1</sup> and 165 ng spot<sup>-1</sup> respectively. The suitability of this method for the quantitative determination of compound was proved by validation in accordance with requirements of pharmaceutical regulatory standards.

### Keywords:

Donepezil HCl; HPTLC; Acetyl cholinesterase inhibitor

## 1. 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. No specific method has so far been reported for the estimation of Donepezil HCl by HPTLC in pharmaceutical dosage forms. The aim of present study is to develop a HPTLC method for the estimation of Donepezil HCl in bulk and in tablets.

## 2. Experimental and methods

### 2.1. Materials

An analytically pure gift sample of Donepezil HCl from Dr.Reddy's Laboratories Ltd, Hyderabad, India was used as working standard. Methanol and Chloroform of HPLC grade (Merck Chem.) were used to prepare the mobile phase.

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## 2.2. Instrumentation

Camag (Muttentz, Switzerland) Linomat 5 applicator, a Camag Twin trough TLC Chamber. Camag TLC scanner 3, Camag Wincats Software. Hamilton (Reno, Nevada, USA) syringe (100  $\mu$ L). HPTLC conditions are given in Table 1.

**Table 1.** HPTLC Conditions

Stationary Phase	TLC aluminium sheets Silica gel 60 F <sub>254</sub>
Mobile Phase	Methanol:Chloroform (8:2 v/v)
Migration distance	75 mm
Slit Dimensions	5.00 x 0.45 mm, micro
Wavelength scanning	254 nm
R <sub>f</sub> value of Donepezil HCl	0.54 $\pm$ 0.02

## 2.3. Standard preparation

Standard stock solution containing 1 mg mL<sup>-1</sup> of Donepezil HCl was prepared by dissolving 10 mg standard drug in 10 mL chloroform and used as working standard solution.

## 2.4. Sample preparation

Twenty tablets were weighed and powdered. An amount of powder equivalent to 10 mg of Donepezil HCl transferred to 10 mL calibrated volumetric flask. 5 mL of chloroform is added and sonicated for 10 min, the solution was made up of volume with the same solvent and filtered. A sample solution was spotted for the assay of Donepezil HCl.

## 2.5. Chromatography

Chromatography was performed on 10 cm x 20 cm aluminium packed silica gel 60 F<sub>254</sub> HPTLC plates. Before use, the plates were washed with methanol and dried in an oven at 50°C for 5 min. Samples were applied as 5 mm bands by spraying at a rate of 15  $\mu$ L S<sup>-1</sup> by means of a Camag Linomat 5 applicator equipped with a 100  $\mu$ L syringe, the distance between the bands was 10 mm. Ascending development of the plate, migration distance 75 mm, was performed at 25  $\pm$  2°C with Methanol: Chloroform, 8:2 (v/v), as mobile phase in a Camag twin-trough chamber previously saturated for 20 min. The average development time was 20 min. Densitometric scanning was performed with Camag TLC scanner 3 equipped with Wincats software Version 1.3.0 at  $\lambda_{\text{max}}$  254 nm using Deuterium light source, the slit dimensions were 5.00 x 0.45 mm, micro.

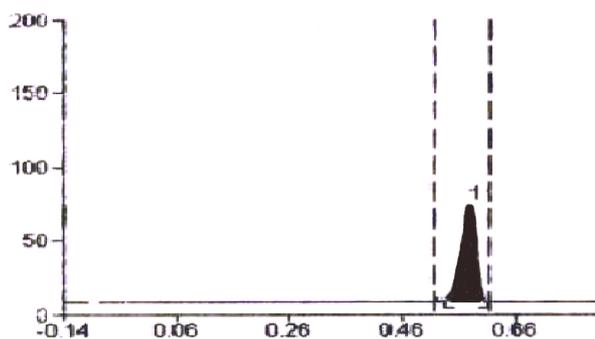
## 3. Results and Discussion

### 3.1. Validation of the HPTLC method

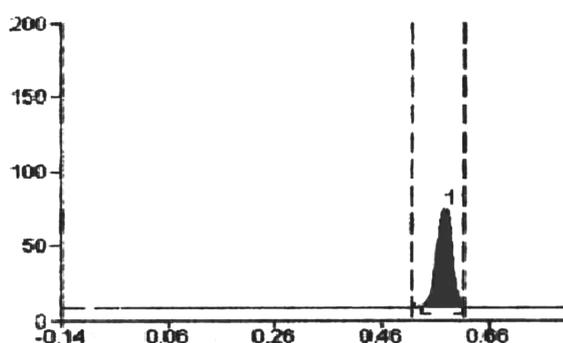
#### 3.1.1. Linearity

Amount of standard solutions equivalent to 200-1000 ng spot<sup>-1</sup> was spotted on the prewashed HPTLC plates. The plates were developed, dried and scanned as described above. The calibration curve was constructed by plotting the peak areas against the corresponding concentrations in ng spot<sup>-1</sup>. The linearity response for Donepezil HCl assessed in the concentration range is 200-1000 ng spot<sup>-1</sup>. The statistical analysis of data where the slope, intercept and correlation coefficient are found to be 2.9804, 3.9095 0.9995 respectively, over

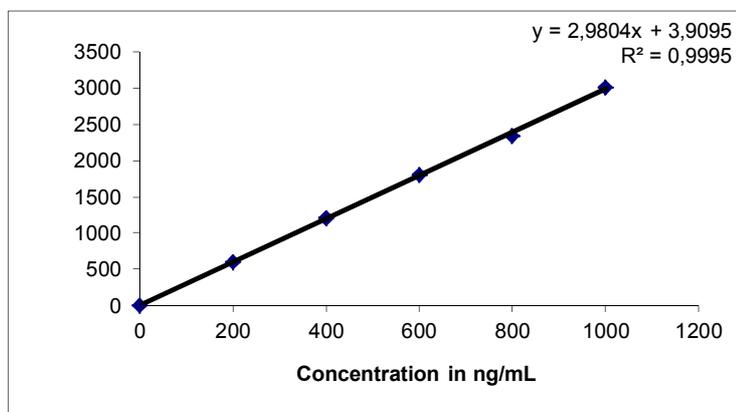
the concentration range studied with six replicate readings of each concentration. The chromatograms of Donepezil HCl showed in Fig. 1 (a), (b).



(a)



(b)



(c)

**Fig. 1** (a) Chromatogram of Donepezil HCl sample (b) Chromatogram of Donepezil HCl standard (c) Calibration curve of Donepezil HCl

### 3.1.2. Sensitivity

The sensitivity of proposed method is estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated by the use of equation  $LOD = 3 \times N/B$  and  $LOQ = 10 \times N/B$ , where N is the standard deviation of peak areas of the drug taken as a measure of noise, and B is the slope of the corresponding calibration curve. The limit of Quantification (LOQ) for Donepezil HCl is  $165 \text{ ng spot}^{-1}$  and the Limit of Detection (LOD) is  $120 \text{ ng spot}^{-1}$ .

### 3.1.3. Pharmaceutical preparation assay, precision evaluation

The amount of Donepezil HCl was found by number of replicates of the pharmaceutical preparations. The assay results were reported in Table 2. 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 intra-day variation is determined by analyzing Donepezil HCl standard drug solution in the calibration range, three times on the same day. Inter-day precision is assessed by analyzing the standard solution within the calibration range on three different days over a period of one week. The results of the precision studies are as showed in Table 3.

**Table 2.** Results of Donepezil HCl in Pharmaceutical formulation (n=5)

Brand	Sample No	Amount of Donepezil HCl (Label Claim 10 mg per tablet)
Donap	1	10.12
	2	10.55
	3	9.85
	4	10.05
	5	9.54
Mean assay		10.02 ± 0.60
(%) Mean assay		100.20 ± 1.52
(%) R.S.D		0.792

n is number of replications

**Table 3.** Results from precision evaluation

Donepezil HCl	Intra-day precision	Inter-day precision
Mean Peak area	604.15	604.06
S.D*	± 0.2459	± 0.0989
R.S.D (%)	0.0407	0.0163

\* Mean of six determinations

### 3.1.4. Accuracy

Accuracy of the developed method was tested by the use of standard addition at three different levels i.e. multiple level recovery studies. Sample stock solution of 0.6 µg spot<sup>-1</sup> of Donepezil HCl was prepared from tablet formulation and spiked amount is equivalent to 80, 100 and 120 % with respect to standard solution. The recovery data showed in Table 4.

**Table 4.** Results from accuracy evaluation

Type of Recovery, %	Label Claim (mg/tablet)	Amount added (mg)	Amount Recovered (mg)	Recovered*, %
80		5	4.98	99.60
100	10	10	10.11	101.10
120		15	15.10	100.66

\* Mean of six determinations

### 3.1.5. Specificity

The mobile phase designed for the method resolved the drug very effectively as shown in Fig.1. The  $R_f$  value of Donepezil HCl was found to be  $0.54 \pm 0.02$ . The peak purity of Donepezil HCl was tested by correlating the spectra's of Donepezil HCl at the peak start (S), peak apex (A) and at the peak end (E) positions. Finally the collected data concluded that no impurities or degradation products were found with the peaks of standard drug solution.

### 3.1.6. System suitability

To ascertain the effectiveness of the developed method in this study system, suitability tests were performed on freshly prepared standard stock solutions of Donepezil HCl. The tailing factor value and the No. of theoretical plates are found to be 1.089 and 2520 respectively. This result ensured that the symmetry of the peak and suitability of the method for the estimation of Donepezil HCl.

## 4. Conclusion

The developed HPTLC technique is precise, specific and accurate. The advantages lie in the simplicity of sample preparation and the low cost of reagents used. Statistical analysis proves that the method is suitable for the analysis of Donepezil HCl as bulk drug and in Pharmaceutical formulation without any interference from the excipient. Hence this HPTLC method can be used for routine drug analysis.

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