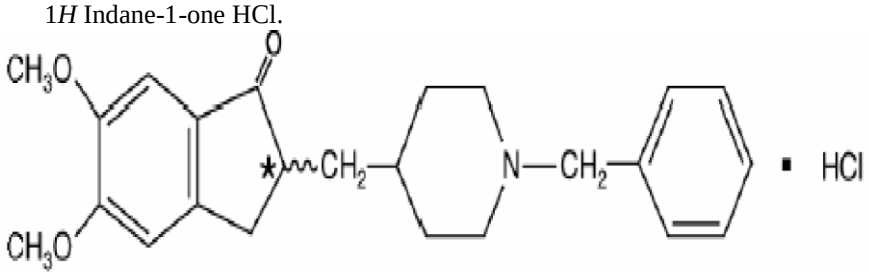
Development And Validation Of Donepezil HCL By Using RP-HPLC Method

Varanasi Prasanth[1], K.Abhinav[2], S.Seetaram Swamy[3], K.Sudheer Kumar[3]
Dept. of Pharmaceutical Chemistry, Venkateswara College of Pharmacy, Srikakulam, A.P
Dept. of Chemistry, Acharya Nagarjuna University, Guntur, Andrapradesh
Dept. of Pharmaceutical Analysis, Chilkur Balaji College of Pharmacy, Aziz Nagar, Telangana
E-mail: varanasi.prash@gmail.com

ABSTRACT:
A simple, specific and accurate reverse phase HPLC method was developed for the simultaneous determination of Donepezil Hcl .in table dosage forms. Phenomenex ($C_{18\ 4.6}$ X 150 mm, 3.5µm.)Column with mobile phase Methanol: buffer (60:40%v/v) was used. The flow rate was 1 ml/min and effluent was monitored at 230 nm. The retention time of Donepezil Hcl was 4.3 min, respectively. The method was validated for specificity, linearity, accuracy, and precision, robustness limit of detection and limit of quantitation. Linearity, accuracy, and precision were acceptable in the ranges the linearity range for donepezil was in the range of 100-500 µg/ml, respectively. The proposed method was also validated and successfully applied to the estimation of Donepezil Hcl tablet formulations. High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture
Keywords: Donepezil, validation, RP-HPLC

I.INTRODUCTION

DONEPEZIL HCl : ±2, 3-dihydro 5, 6, dimethoxy2-[1-(phenylmethyl) - piperidynyl]-1*H* Indane-1-one HCl.



Donepezil hcl

Molecular Formula – $C_{24}H_{29}NO_3HCl$
Molecular Weight – 461.91
It is a choline esterase inhibitor that prevents the breakdown of acetyl choline in the brain.
Category : Acetyl cholinesterase inhibitor.
Solubility : Freely soluble in chloroform, glacial acetic acid, ethanol and water.

Chromatographic Method:
A simple and sensitive reverse phase HPLC method has been developed for the analysis of donepezil Tablets. The method utilizes sample preparation followed by separation on a Column Phenomenex $C_{18\ 4.6}$ X 150 mm, 3.5µm or equivalent. Analytes were monitored by UV detection at 230nm using an isocratic mode with Mixture of Methanol: buffer(60:40%v/v) as mobile phase. The flow rate was set at 1ml/min and effluent was monitored at 230nm. The retention time was 4.3min. Calibration curves for donepezil was found respectively

Equipment and Apparatus used:
- Electronic balance
- HPLC Waters Separation Module LC-20AT Prominence Liquid Chromatography
- UV Detector
- Chromatographic data Software : EMPOWER
- Phenomenex $C_{18\ 4.6}$ X 150 mm, 3.5µm.
- Vacuum filter pump

- Mobile phase reservoir
- Ultra Sonicator , Membrane filter(0.45 and 0.2microns)

Reagents:

- Metanol HPLC grade
- Water (HPLC)
- Potassium Dihydrogen Phosphate
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II.METHOD DEVELOPMENT

Preparation of the Donepezil Hcl Standard & Sample Solution:

Standard Solution Preparation: Accurately weighed quantity of 10mg of Donepezil hydrochloride was transferred to 100 ml volumetric flask, dissolve and dilute to volume with mobile phase mixed.

Sample Solution Preparation:

Preparation of mobile phase:

3.5gm of potassium dihydrogen phosphate solution was dissolved in 500 ml of water and adjust the PH to 6.9 with orthophosphoric acid filtered and degassed. Mixed above the buffer: methonal 40:60%v/v

Chromatographic Parameters

Equipment : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector

Column : Phenomenex $C_{18\ 4.6}$ X 150 mm, 3.5µm

Flow rate : 1ml per min

Wavelength : 230 nm

Injection volume : 20 □l

Column oven : Ambient

Run time : 15min

Assay

Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and percentage purity was found out by following formulae.

Procedure

Preparation of phosphate buffer (0.01%):

3.5gm of KH_2PO_4 solution was dissolved in 500 ml of water and adjusted with orthophosphoric acid at pH 6. Mixed the above buffer and mobile phase in the ratio of 50: 50 and degassed in sonicated for about 5 min.

Standard preparation:

Transferred about 10 mg of Donepezil hydrochloride working standard in to 100 ml volumetric flask, dissolved and diluted to volume with mobile phase and mixed. Pipetted out 1.2 ml of the above solution into 10 ml of volumetric flask, diluted to volume with mobile phase.

Test preparation:

For estimating the tablet dosage form, 20 tablets from a batch were randomly selected and powdered, Weigh accurately 0.7gm of ground tablet powder (equivalent to 10mg of Donepezil hydrochloride) transfer it in 100 ml of volumetric flask, and add 100ml of mobile phase, shake the flask on a rotator shaker for 30 min and sonicate for 15 min with intermediate shaking. Keep the solution on a rotatory shaker for 30 min at 200 rpm. Centrifuge the portion of above solution at 4000 rpm for 5 min. pipette out 3 ml of above clear solution and transfer it to25ml volumetric flask and make up the volume with mobile phase.

III.RESULTS AND DISCUSSION

Method Development

Drug quality control, stability, metabolism, pharmacokinetics, and toxicity studies all necessitate the determination of drugs in pharmaceutical formulations and biological samples.

Correspondingly, efficient and validated analytical methods are very critical requirements for all these investigations. Chromatographic parameters were preliminary optimized to develop a LC method for validation report for assay of Donepezril HCL with short analyses time and acceptable resolution (Rs> 2).

In order to identify a suitable organic modifier, various compositions of acetonitrile andmethanol were tested. Methanol produced a high retention time for Donepezril HCL highcolumn pressures due to the high viscosity. Acetonitrile was found to display advantageous separations. Change of percentage of acetonitrile in the mobile phase brought about a great influence on retention time.

The system suitability parameters prove that the proposed method is equally suitable for validation of, the Donepezril HCL chromatogram were found to be satisfactory Phenomenex $C_{18\ 4.6}$ X 150 mm, 3.5µm or equivalent, using mobilephase

composition of 6.9 PH Potassium Dihydrogen Phosphate and metanol in the ratio (400:600) with flow rate of 1ml/min. The above method is suitable routine pharmaceutical applications involving the validation of Donepezil.

Method Validation:

Validation of analytical method for determination of assay of Donepezil 10 mg tablets was performed for the parameters including – Specificity, Linearity and Range, Precision (System precision, Method precision), Intermediate precision (Ruggedness), Accuracy and Robustness values are in given below tables.

1. **System Suitability Parameters**

Parameter	Donepezil
%RSD	0.13
Tailing Factor	1.43
Number of theoritical plate	2099

2. **Accuracy**

Sample No.	Spike Level	Amount (mg) added	Amount (mg) found	% Recovery	Mean % Recovery
1	50 %	5	5.04	100.89	100.26
	50 %	5	4.84	96.9	
	50 %	5	5.15	103	
3	100 %	10	9.80	98.05	98.05
	100 %	10	9.80	98.05	
	100 %	10	9.80	98.05	
5	150 %	15	15.23	101.5	101.5
	150 %	15	15.23	101.5	
	150 %	15	15.23	101.5	

3. **Linearity**

Concentration	Peak Area
100	307387
200	542671
300	807576
400	1103461
500	1323084

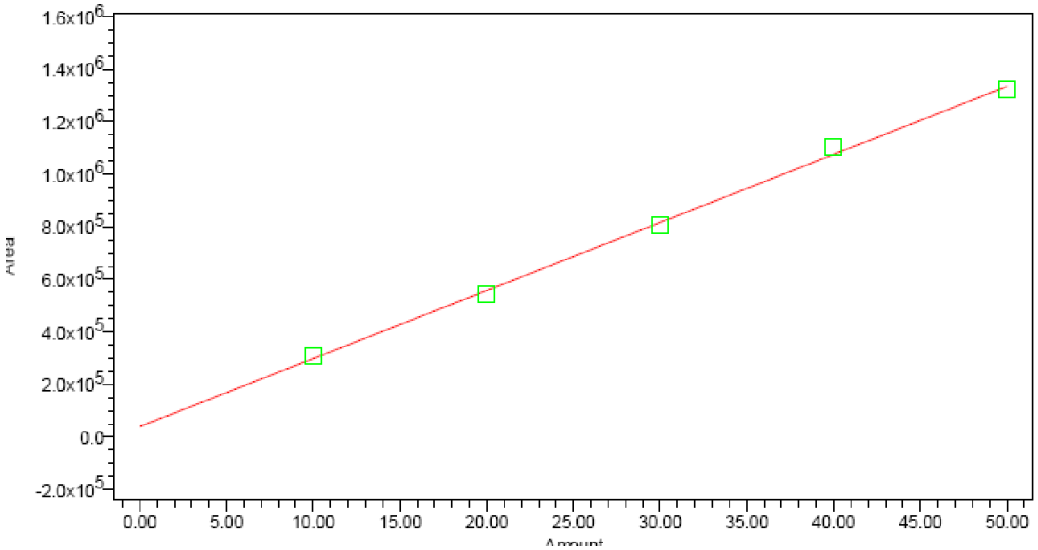


Fig.1.Linearity curve for Donepezil hydrochloride

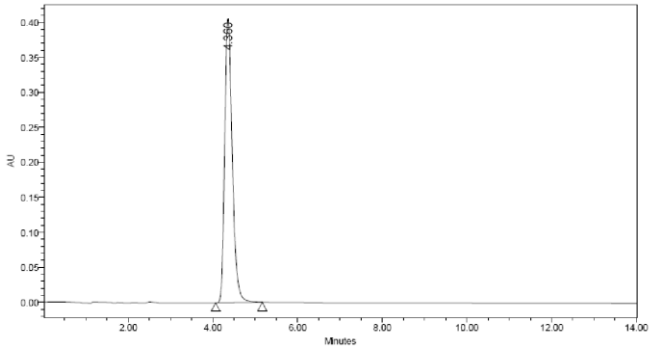
4.Precision

S.No	Peak Area	Rt
1	817118	4.335
2	816535	4.252
3	816758	4.201
4	816022	4.184
5	812152	4.165
6	809401	4.156
Mean	814664	4.215
Std.Deviation	3154.7	0.006
%RSD	0.39	1.5

5.Validation and system suitability parameters

S.NO	PARAMETERS	LIMIT	OBSERVATION
1	System suitability (%RSD of tailing factor)	Suitable	0.13
2.	Specificity	No interferences	Specific
3.	Precision: (a) System precision (b)Method precision	RSD NMT 2.0% RSD NMT 2.0%	0.25 0.34
4	Linearity	Correlation coefficient NLT 0.999	0.999
5	Accuracy	%Recovery range98-102 %	99.9
6	Robustness	RSD NMT 2%	Robustted
7	LOD	S:N Ratio should be more than 3:1	7.5
8	LOQ	S:N ratio should be more than 10:1	22.74

Chromatography of Donepezil Hcl



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