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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF DONEPEZIL HCL FROM BULK DRUG

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ABSTRACT

Stability of Donepezil Hydrochloride(DONE) was investigated using stability indicating Reverse phase high performance liquid chromatography (RP-HPLC) utilizing C-18 column and mobile phase containing Acetonitrile:Water (pH 3.5) in ratio of 40:60 at flow rate of 1 ml min-1. Peaks of donepezil and degradation products were well resolved at retention times < 7 min. Stability was performed in 0.1N hydrochloric acid, 0.1N sodium hydroxide, 3 % hydrogen peroxide, neutral, photolytic and dry heat conditions. Fast hydrolysis was seen in alkaline condition as compared to oxidative and neutral conditions. Methods was validated with respect to linearity, precision, accuracy, specificity and robustness LOQ and LOD. It was also found to be stability indicating, and therefore suitable for the routine analysis of Donepezil hydrochloride in the pharmaceutical formulation.

Keywords: Donepezil hydrochloride, RP- HPLC, Method Development, Stability studies.

INTRODUCTION

Donepezil is a reversible inhibitor of the enzyme acetylcholinesterase (AchE) approved for use in Alzheimer's disease.[1] The pathogenesis of Alzheimer's disease attributed some of them to a deficiency of cholinergic neurotransmission. Therefore, AChE inhibitors, which prevent the hydrolysis of acetylcholine, may exert their therapeutic effect by enhancing cholinergic function. The first AChE inhibitor (tacrine) has been used, however, associated with a high incidence of gastrointestinal (GI) side effects and hepatotoxicity.[2] Donepezil is a potent and more selective AChE inhibitor in the central nervous system with little effect on peripheral tissue, therefore, has a lower incidence of GI and cardiovascular adverse effects. The drug produces modest improvements in cognitive scores and has a long half-life allowing once daily dosing. Donepezil is slowly absorbed from the GI tract.[3] The present work describes the utility of RP- HPLC in forced degradation-stability study under different chemical conditions.

EXPERIMENTAL

Chemical

Donepezil HCl was gifted by Sun Pharmaceutical Pvt. Ltd. Broda, Gujarat

Methanol HPLC Grade(FINAR)

HPLC Water

HCl AR Grade (MERCK)

NaOH AR Grade (MERCK)

H_2O_2 AR Grade (RANKEM)

Potassium dihydrogen Phosphate LRGrade(RENKEM)

Acetonitrile HPLC Grade(FINAR)

Instrumentation

Company : Shimadzu

Model No : SPD 10 A-LC

Softwer : WINCHROME SOFTWER

Operation : Semi Automatic

Semi micro analytical balance (Sartorius CD2250, Germany) was used for weighing purpose.

HPLC water was obtained using Arium®611VF(Sartorius).

Magnetic stirrer (1 MLH, Remi) was used for mixing purpose.

pH tutor (313927, Eutech Instruments) was used for pH measurement.

Sonication of solutions were done using Ultrasonic cleaner (D 120/1H, Trans-O-Sonic).

Nylon membrane filters (0.22 µm, 47 mm D)

All volumetric glasswares used were calibrated.

DEGRADATION STUDIES

All degradation studies were done at a drug concentration of 50µg ml-1. For acid decomposition studies, drug was dissolved in 0.1N HCl and solution was boiled under reflux for 1 hr. The studies in alkaline conditions were done in 0.1N NaOH boiling under reflux for 1 hr. For study in neutral conditions, drug in water was boiled under reflux for 1 hr. For oxidative conditions, initial studies were done in 3% H_2O_2

solution. The solution was kept at room temperature for 1 hr. Photolytic studies were done by exposing solid drug directly to uv light for 2 hr. Thermal decomposition studies were performed by exposing solid sample of drug to dry heat at 70ºC for 2 hr in hot air oven.

HPLC ANALYSIS[4]

CHROMATOGRAPHIC SEPERATION

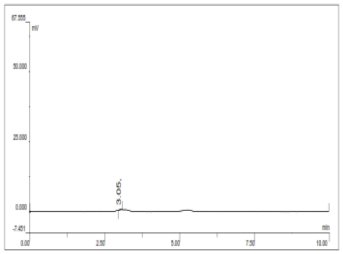
Standard and sample solutions were injected in column. The chromatogram was run for appropriate time duration with degassed mobile phase, mixture of Acetonitrile:Water pH 3.5 (40:60 v/v), using UV detector **(SPD-20AV)** at wavelength 230 nm. The chromatogram was stopped after separation was achieved completely. Data related to peak like area, height, retention time, resolution etc was recorded using CLASS-VP software **(**version 2.31).

Table1:Chromatographic conditions of developed method

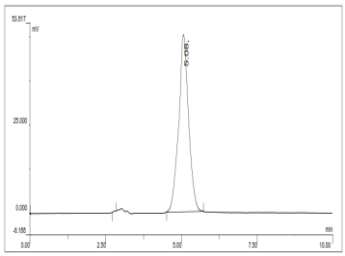
Sr no	HPLC Conditions	Results
1	Elution	Isocratic
2	Mobile Phase	Acetonitrile:Water pH 3.5 (40:60 v/v)
3	Diluent	Acetonitrile
4	Flow Rate	1 ml/min
5	Detector	UV Visible
6	Injection volume	20 µL
7	Wavelength	230 nm
8	Column Temperature	Room temperature
9	Run time	10 min
10	Column	InertSustainSwift™ C_{18} (250mm×4.6mm i.d.) 5µm

STANDARD CHROMATOGRAM

Blank



Donepezil HCl



CONDITION

Table 2:Mobile Phase: Acetonitrile:Water pH 3.5(40:60 v/v)

Name	Retention time(min)	Theoretical Plates	Tailing Factor
Donepezil HCl	5.08	8528.92	0.51

Table 3:Observed value for system suitability test

Sr !	System SuitabilityParameter	Donepezil HCl	IP'2007 specification
1	Number of Theoretical Plates (N)	8528.92	>2000
2	Resolution	-	>2
3	Tailing Factor(T_f)	0.51	<2

VALIDATION OF DEVELOPED RP-HPLC METHOD[5]

Specificity

Specificity was measured by injecting a blank solution and another blank solution which was previously spiked with common excipients. No any interference by excipients was observed. The entire base line was found stable.

Linearity Range

The linearity range for Donepezil HCl was found to be 10-100 µg/ml. Correlation co-efficient for calibration curve of Donepezil HCl(DONE) was found to be 0.998.

The regression line equation for Donepezil HCl is as follows:

y = 25514x + 41169

Where, y= corresponding peak area from CLASS VP software(v2.31)

x= Concentration of Donepezil HCl in µg/ml.

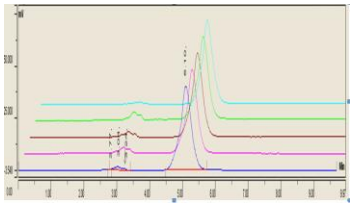


Fig 1:Chromatogram of standard Donepezil HCl

Table 4:calibration data and calibration curve

Sr No.	Donepezil HCl (µg/ml)	Mean Area ±SD (n=6)
1.	10	317958 ±620
2.	20	533316 ±3349
3.	30	787252 ±3677
4.	40	1068169 ±4172
5.	50	1326223 ±2028

PRECISION

Intraday Precision

Table 5:The results for intraday precision for donepezil hcl are presented in table.

Conc. (µg/ml)	Peak Area ± SD (n=3)	%RSD	Mean % RSD
10	318860 ± 992	0.31	
30	787616 ± 405	0.05	0.21
50	1329368 ± 3805	0.28	

Table 6:Interday precision

Conc. (µg/ml)	Peak Area ± SD (n=3)	%RSD	Mean % RSD
10	326043 ± 1340	0.41	
30	801079 ± 3947	0.19	0.50
50	1414963 ± 13058	0.92	

ACCURACY

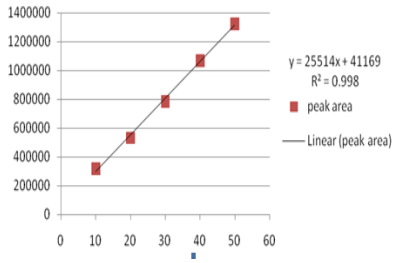
Table 7:The data for accuracy study for donepezil hcl are presented in table. % recovery for donepezil hcl was found to be in range of 99.0-100.53%.

Conc µg/m	%Spiking	Total Conc µg/ml	Peak Area	Recovered Amt µg/ml	%Recovery	Mean % Recove
10	-	10	253049	9.91	99.18	
10	80%(8)	18	462472	18.12	100.70	
10	100%(10)	20	517568	20.28	101.42	
10	120%(12)	22	560704	21.97	99.89	100.29%

LOD AND LOQ

Table 8:The data for lod and loq for donepezil hcl are presented in table. lod for donepezil hcl was 1.3527µg/ml and loq was 4.0993µg/ml.

Sr No.	Parameter	Donepezil HCl
1.	S.D of the Y-intercepts of 6 calibration curve	41169
2.	Mean slope of the 6 calibration curves.	25514
3.	LOD = 3.3 × (SD/Slope) (µg/ml)	0.85 µg/ml
4.	LOQ = 10 × (SD/Slope) (µg/ml)	2.59 µg/ml



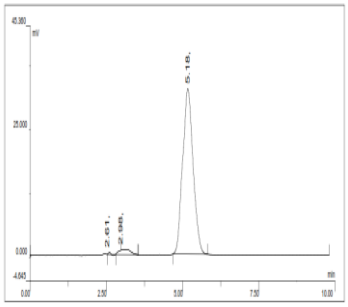
ASSAY

Twenty 'AERICEP' Tablets (containing 10 mg of Donepezil HCl) were accurately weighed and ground to fine powder.
An accurately weighed quantity equivalent to 100 mg of Donepezil HCl (DONE) from the formulation fine powder was transferred to 100 mL volumetric flask and 40-50 ml Acetonitrile is added to dissolve the drug, Sonicate for 15 mins.Volume is made up to the mark with the Acetonitrile.
Filter Stock solution(100 µg/mL).

Table9:Assay

Sample Label	Claim (mg)	Concentration taken (µg/ml)	Concentration found (µg/ml)	% Assay
Donepezil HCl	10 mg	30	31.92	103.40%

STANDARD



TEST

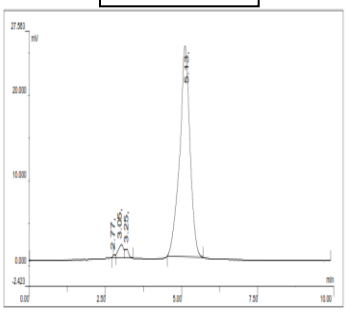


Table 10: Robustness

Sr No.	Factor	Conc	Level	Mean(n=3) ±SD	%RSD
1	Change in flow rate	50µg/ml		1532054 **±** 6104	0.39
			1.2	1332303 ±7842	0.53
2	Change in	50µg/ml	232	1485170**±**4023	0.27
	wavelength		228	1337647±8685	0.64

Table 11:Summary of validation parameters:

Sr No.	Parameters	Result
1.	Wavelength (nm)	230 nm
2.	Linearity range (µg/ml)	10-50 µg/ml
3.	Standard Regression equation	Y=25514x + 41169
4.	Correlation coefficient (R^2)	0.998
5.	Precision (%RSD)	
	Intraday	0.21
	Interday	0.50
6.	% Recovery (Accuracy, n = 3)	100.29%
7.	LOD (µg/ml)	0.85
8.	LOQ (µg/ml)	2.59
9.	Robustness	
	Flow rate change	0.46
	Temperature change	0.45
10.	Assay (% Label claim)	103.46%

FORCE DEGRADATION STUDIES

Acid degradation

100 mg drug dissolve in 1 ml Acetonitrile and dilute up 100 ml of 0.1N HCl. Pipette out 0.5 ml and solution neutralize with equivalent amount of 0.1 N NaOH and dilute with acetonitrile up to 10 ml .Then this solution is kept for 1 hour at , 70˚C and peak is recorded . Sample withdrawal at 0 min, after 10 min, 30 min, 1hr at 70˚C.

Alkali degradation

100 mg drug dissolve in 1 ml Acetonitrile and dilute up 100 ml of 0.1N NaOH. Pipette out 0.5 ml and solution neutralize with equivalent amount of 0.1 N HCl and dilute with acetonitrile up to 10 ml .Then this solution is kept for 1 hour at 70˚C and peak is recorded. Sample withdrawal at 0 min, after 10 min, 30 min, 1hr at 70˚C.

Oxidation

3% H_2O_2 was taken in a 100ml volumetric flask then accurately weighed 100mg bulk drug was dissolved in it and then volume is made by 3 % H_2O_2.Then this solution is kept for 1 hr and peak is recorded .Sample withdrawal at 0 min, after 10 min, 30 min, 1hr .

Neutral Condition

First Distilled water was taken in a 100 ml volumetric flask then accurately weighed 100 mg bulk drug was dissolved in it., volume is made by Water(1000 ppm). Then this solution is kept for 1 hour at 70˚C and peak is recorded .Sample withdrawal at 0 min, after 10 min, 30 min, 1hr at 70˚C.

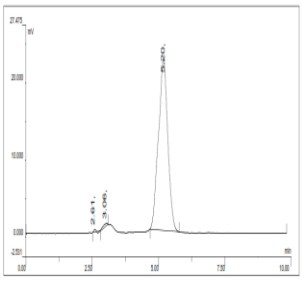
Photolytic Condition

100mg of bulk drug was put into the petridish and placed under direct UV Light for 1 hr.10mg is weighed and make upto 10 ml, using diluent Acetonitrile and chromatogram was recorded. Similar Procedure is followed for 2 hr.

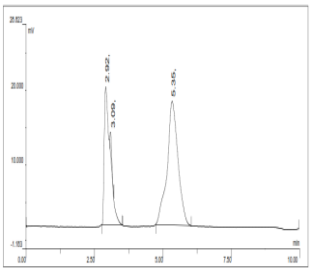
Thermal Condition

100 mg of bulk drug was taken in a cleaned Petridish and was put it into the oven at 70˚C for 1 hour. 10mg of bulk drug from the Petridish

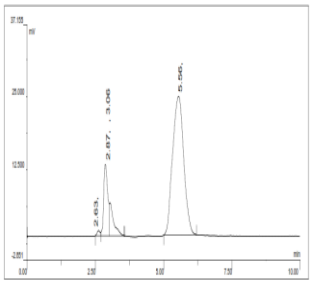
was weighed and dissolved in 10 ml of diluent Acetonitrile and chtomatogram was recorded. Similar Procedure is followed for 2hr.



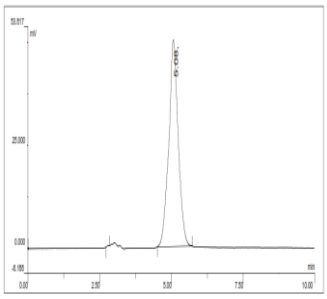
ACID DEGRADATION



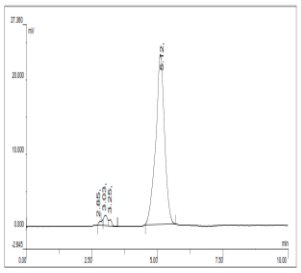
OXIDATIVE DEGRADATION



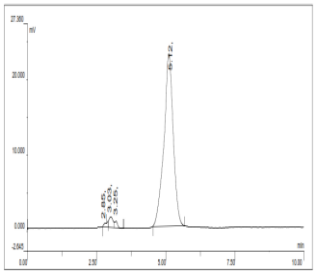
BASE DEGRADATION



PHOTOLYTIC DEGRADATION



NEUTRAL DEGRADATION



THERMAL DEGRADATION

Table 12: Summary of overall degradation study

CONDITION	% DEGRADATION AFTER 1 HR	AFTER 2 HR
ACIDIC	60.05%	-
BASIC	92.31%	-
NEUTRAL	13.58%	-
OXIDATION	78.05%	-
PHOTOLYTIC	1.62%	3.23%
THERMAL	10.76%	12.48%

DISCUSSIONS

The stability of the new donepezil hydrochloride is investigated using stability indicating RP-HPLC procedure. The method permits detection and quantitation of donepezilhydrochloride in the presence of its degradation products. It was subjected to stress conditions as per ICH guidelines. The drug was found to degrade in alkaline, oxidative and neutral conditions while it was found to be stable under photolytic and dry heat conditions. The drug can be analyzed specifically in the presence of different chromophoric degradation products by using isocratic conditions and mobile phase containing Acetonitrile :Water (pH 3.5)in the ratio of 40 :60. The method was validated for parameters like linearity, precision, accuracy, specificity and robustness.

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