International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI: 10.7439/ijpc
CODEN: IJPCH3 (American Chemical Society)
Research Article

Quantitative estimation of Donepenzil hydrochloride tablet by HPLC

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Abstract

A simple, rapid, reliable and accurate HPLC method has been developed and validated as per the ICH guidelines for the quantitative determination of Donepezil HCl in bulk and tablets. The water HLC-10, high performance liquid chromatography system were used. The LOQ is detected at concentration 0.05mg/ml and the linearity range is 50-150µg/ml. An optimum concentration scanned in the range 200-350 nm and λ max of the drug is 230 nm. No any interference at developed (R_t = 8.22 min) and also the λ_{max} peak is sharp. The Accuracy of the method was found to be in the range of 99.70% to 100.26%. The mean Inter and Intraday assay Relative Standard deviation (%RSD) were less than 0.69%. The Proposed method was found to be Linear, precise and accurate for the quantitative estimation of Donepezil in tablet formulations and can be used for commercial purposes.

Keywords: Validation, Donepezil, HPLC, Tablet, Formulation.

1. Introduction

Donepezil HCl (DH) is chemically 2, 3-Dihydro-5, 6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1H-inden-1-one hydrochloride [2]. This drug is used in the treatment of Alzheimer's disease disease where it is used to increase cortical acetylcholine. The action of the drug is centrally acting reversible acetyl cholinesterase inhibitor to increase cortical cetylcholine [1,3]. Literature review of DH reveals that *ultraviolet* (UV)-visible spectrophotometry method in tablet dosage form, HPLC methods in tablets, HPLC methods in human plasma, HPLC method in human and rat plasma, blood and microdialysates,8 liquid chromatography mass spectrometry (LC-MS)-MS method, HPTLC method in tablet dosage form and enantiomers separation by HPLC method and its application in pharmacokinetics methods were being reported for the estimation of DH in biological samples and in tablet dosage form[2]. High-performance liquid chromatography [3, 4], UV and Spectro-fluorimetric method [5] has been reported for Donepezil HCl. No specific method has so far been reported for the estimation of Donepezil HCl by HPLC.

2. Material & Equipments

Donepezil HCl (Jubilant Life Science, Roorkee), Tri ethylamine (HPLC grade), Ortho-phosphoric acid (AR grade), Nylon membrane (filter), Potassium dihydrogen phoshphate, Phosphoric acid, Buffer (pH-3.5), Solvent (Acetonitrile); all the chemicals were used of standard grades purchased from the local market. U.V.-Systronics Model No. 2202, HPLC water HLC 10, Column HypersilBDSC-18(250 mm x4.6 mm), pH meter Systronics Model No. MK6.

2.1 Experimental method & preprations

2.1.1 Instrumentation

The HPLC system used was system water HLC 10 HPLC, Member degasser DGU-14, Column Hypersil BDS column C18 (250 mm x 4.6 mm, 5µm). Advanced type of UV detector SPD- M10AVP UV/VIS photodiode

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array detector. Digisun electronics digital pH meter 7007, RC Systems sonicator and vacuum pump were used throughout the experiment.

2.1.2 Chromatographic Conditions

The composition of the mobile phase used was use of mixture of buffer (To 1000ml of water was added 1ml triethylamine), and acetonitrile in the ratio 70:30with pH adjusted to 3.5 using dilute orthophosphoric acid was finalized as mobile phase The mobile phase was filtered through a membrane filter of 0.45micron. Flow rate of 1.0 ml/min employed for analysis. After equilibration of column with the mobile phase indicated by a stable baseline, aliquots of sample (20 µL) were injected and the total run time was kept 10 min. The absorbance of the eluents was monitored at 230 nm at a detection sensitivity of 0.1000 aufs.

2.1.3 Standards and Sample Solutions Preparation

The standard stock solution of DH was prepared by dissolving 50 mg of Donepezil hydrochloride working standard to a 50 ml volumetric flask. Add about 20 ml of diluents and sonicate to dissolve. Make up the volume with diluents and mix. Dilute 5 ml of this solution to 100 ml with diluents and mix (50 µg/ml).Sample solution in the range (50-150 µg/ml) with dilution of 50, 70, 90,100,110,130,150 µg/ml. Filter through 0.45 µm nylon membrane filter. After equilibration of column with the mobile phase indicated by a stable baseline, aliquots of sample (20µL) were injected. The chromatograms were observed for the peak area (Fig 1, 2, 3, 4).

2.1.4 Method Validation

Method validation was carried in accordance to the International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The assay was validated with respect to linearity, precision, accuracy, sensitivity and robustness. Accuracy/Recovery Accuracy of the developed method was confirmed by performing a recovery study as per ICH norms at three different concentration levels (80%, 100%, 120%) by replicate analysis (n = 3). Standard drugs were added to a pre analyzed sample solution and chromatograms were recorded. The percent of standard drugs recovered were calculated.

A. Precision

The precision of the method was determined by repeatability, intermediate precision (intra- day, inter-day) and was expressed as % relative standard deviation (%R.S.D.). Intra- day precision was determined by performing analysis of triplicate injections of three different concentrations of combination on the same day at different time intervals and on three different days for inter-day precision. (Table- I -System precision SD 3529.29, % RSD 0.17, Table II- Method Precision SD 0.43, % RSD 0.43, Table-III. Intermediate Precision % RSD 0.21)

B. Linearity

Calibration curves were obtained from injecting the six out of seven dilution serial dilutions having result Slope-20272, Intercept- 9965, Regression Coefficient.0.999 by plotting a calibration graph of by producing a chromatogram of different dilution (Table – IV).

C. Sensitivity

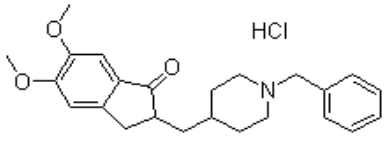
Sensitivity of the method was determined by means of the detection limit (LOD) and quantification limit (LOQ).Calculations for LOD and LOQ were based on the standard deviation of the Y-intercepts of the six calibration curves (σ) and the average slope of the six calibration curve (S), using the equation LOD= 3.3×σ/S and the equation LOQ=10×σ/S.

D. Robustness

Robustness of the method was evaluated by the analysis of solution under varying experimental conditions such as pH of the mobile phase and flow rate. The flow rate was varied ±0.03 mL/ min (3%) and pH of the mobile phase was changed ±0.15 units (2.5%). Their effects on the retention time (Rt), tailing factor (T) and resolution of the peaks (R) were studied.

Donepezil Hydrochloride[2]

2,3-Dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1H-inden-1-one hydrochloride;



3. Result and Observations

Table-I: System precision

Injections	Peak Area
1	2040565
2	2041065
3	2041846
4	2039658
5	2034722
6	2032403
Average	2038376
SD	3529.29
% RSD	0.17

Table-II: Method Precision

Samples	Area	Results
1	265550	99.3
2	266812	99.8
3	266652	99.6
4	265530	99.3
5	266939	99.9
6	266610	99.7
Average	266348	99.6
SD		0.43
% RSD		0.43

Observation: The Relative standard deviation found 0.17 %

Table-III Intermediate Precision

Details				
Variation	**Analysis Set-I**		**Analysis Set-II**	
Instrument	QC/HLC/015		QC/HLC/010	
Column	LC/11/110		LC/11/128	
Day	Monday		Wednesday	
Samples	**Area**	**Result**	**Area**	**Result**
1	265650	99.4	265550	99.3
2	265550	99.3	266812	99.8
3	263065	98.9	266652	99.6
4	265550	99.3	265530	99.3
5	263005	98.3	266939	99.9
6	265000	99.1	266610	99.7
Average	264636	99.15	266348	99.6
% RSD		0.21	0.21	
Overall Mean	99.28			
Overall RSD	0.21			

Observation: The Overall Relative standard deviation of results found 0.21 %.

Table- IV. Linearity

Conc.(µg/ml)	Area
50	1020841
70	1429177
90	1832513
100	2041682
110	2248850
130	2634186
150	3052583
Slope	**20272**
Intercept	**9965**
Regression Coefficient.	**0.999**

Figure 1: Standard Solution

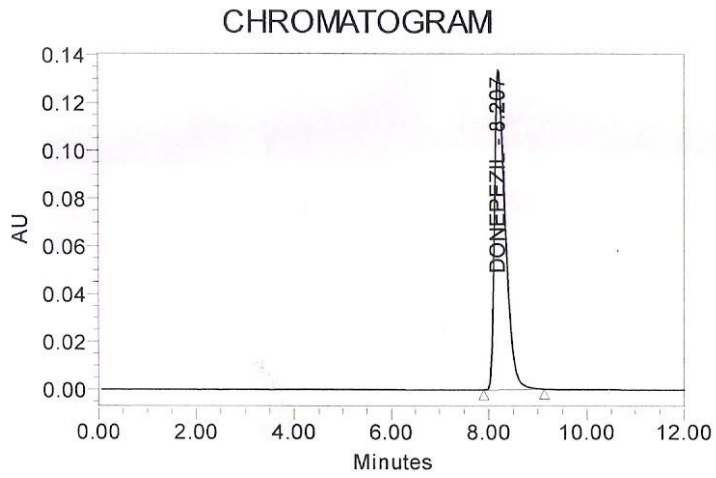


Figure 2: Standard Solution

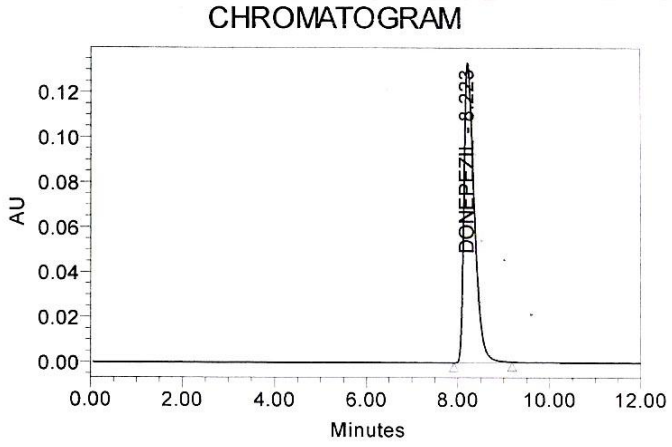


Figure 3: Standard Solution

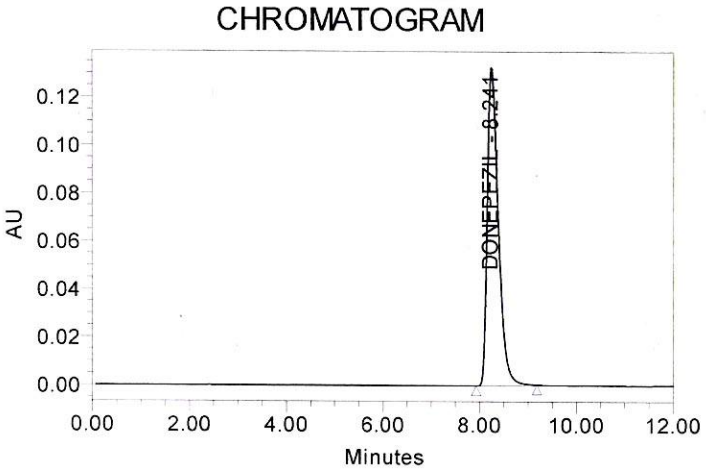
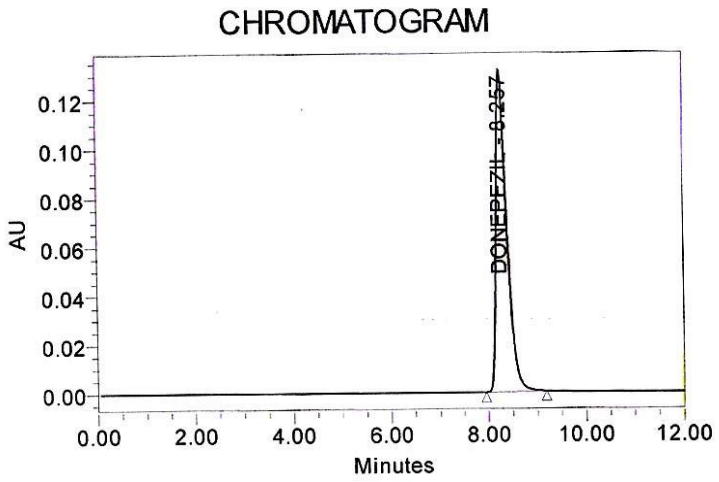


Figure 4: Standard Solution



4. Conclusion

The present work describes application of donepezil hydrochloride in tablet dosage form by HPLC methods. As comparative spectrophotometric methods because of low standard deviation and %COV, present work describe a new, reversed-phase HPLC method for analytical validation of donepezil in tablet formulation. It is found that, the developed method is accurate, precise, repeatable, linear, selective and specific; prove the reliability of the method. As the result shows that the method could find practical application hence, utilized as quality control tool for the analytical method validation of Donepezil in tablet formulation.

Acknowledgement

Author are thankful to Mr. Rahul Shakya. Asist manager, Quality Control, Jubilant Life Science, Pvt Ltd Roorkee, for providing necessary facilities.

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