An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DONEPEZIL HCL IN PHARMACEUTICAL DOSAGE FORMS

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ARTICLE INFO

Key Words
Donepezil HCl, RP-HPLC, Estimation and Tablets

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

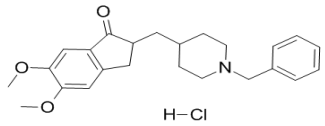
ABSTRACT

Simple and accurate methods to determine donepezil, in tablet dosage form, were developed and validated using liquid chromatography (LC). The LC separation was achieved on a Inertsil C8-3, 25 cm x 4.6-mm, 5 µ in the isocratic mode using Methanol: HPLC water (70:30)v/v, adjusted to pH 2.50±0.05 with orthophosphoric acid, as the mobile phase at a flow rate of 1.0 mL/min. The methods were performed at 271 nm. In the LC method, quantification was achieved with PDA detection over the concentration range of 10, 15,20,25,30 µg/mL with mean recovery of 98%±102%. The method was validated by determining its sensitivity, accuracy and precision. The proposed method is simple, fast, accurate and precise and hence can be applied for routine quality control of donepezil HCl in bulk and tablet dosage form.

INTRODUCTION

Donepezil HCl ARICEPT® (donepezil hydrochloride) is a known chemically as (±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one hydrochloride (also known as E2020 or Aricept®. It has an empirical formula of $C_{24}H_{29}NO_3HCl$ and molecular weight of 415.96. The registered trademark of Eisai Co. Ltd, Tokyo, Japan) is a piperidine based inhibitor of the enzyme acetylcholinesterase (AChE). It has recently been approved for marketing in the USA, Canada and several EU member states, including the UK, for the symptomatic treatment of mild to moderate Alzheimer's disease. *In vitro* studies have demonstrated that donepezil has a significantly greater degree of selectivity for AChE in the central nervous system (CNS) than for butyrylcholinesterase (BuChE) in the periphery. Alzheimer's disease is a progressive disease that destroys memory and other important mental functions. It is a neurological disorder in which the brain cells die and causes memory loss and cognitive decline. It is a neurodegenerative type of dementia, where the disease starts mild and gets progressively worsens as the disease prolongs. This disease can be for life long .The most common symptom seen in AD is difficulty in remembering recent events which is called short-term memory loss. As the disease prolongs, symptoms can include problems with speaking and language, , mood swings, loss of motivation, not managing self care, behavioural changes and issues and easily getting lost. Although the disease progression can vary, the average life span may decrease from three to nine years following diagnosis. Donepezil hydrochloride, a piperidine derivative, is a reversible and specific inhibitor of acetylcholinesterase with actions similar to those of neostigmine. It is highly selective for the central nervous system and is used for the symptomatic treatment of mild to moderately

severe dementia in Alzheimer's disease. donepezil hydrochloride is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetylcholinesterase.



Structure of Donepezil

Literature survey revealed the availability of only a few analytical methods such as capillary electrophoresis[6,7], HPLC[8-12], LC-MS[13-15] and HPTLC[16] for estimation of donepezil hydrochloride in pharmaceutical formulations and in biological fluids. In the present investigation, a simple and accurate RP-HPLC method has been developed using RP-C_{18} column and a mixture of acetonitrile and 0.025 M phosphate buffer (50 : 50 v/v) as a mobile phase.

2. MATERIALS AND METHODS: In the stage of optimization the preliminary conditions of sets that have come from the development first stages are improved in terms of resolution and peak shape, theoretical plates, asymmetry factor, capacity factor, time of the elution, limit of detection, limit of Quantitation and all the parameters.

Method optimisation is follows by generally two approaches: 1. Manual and 2. Computer driven. The various parameters that include being optimized during method development 1) Selection of Mode of separation 2) Selection of stationary phase (Column), 3) Selection of mobile phase (Solvent), 4) Selection of detector

2.1. Method Development of Donepezil: The system of HPLC employed HPLC WATERS with EZChrom Software using PDA Detector.

Chemicals used: Donepezil hydrochloride was obtained as a gift sample from Sun Pharma India Limited. HPLC grade methanol and Hplc water were procured from Merck, Mumbai, India.. Water for HPLC was produced from Millipore apparatus.

Mobile phase preparation: The mobile phase is used in this method development consists of a mixture of Methanol & HPLC Water in the ratio of 70:30.

Chromatographic condition: The HPLC system consisting of Inertsil C8-3, 25 cm x 4.6-mm, 5 µ was stabilized with the mobile phase at a flow rate of 1.0 mL/min. The test solutions were injected into the system by filling a 20 µl fixed volume loop manual injector. The chromatographic run time of 18 min. was maintained for the elution of the drug from the column. The eluates were monitored with a PDA detector at 271 nm.

Standard preparation: About 10mg of pure donepezil hydrochloride was accurately weighed and dissolved in mobile phase in 10mL volumetric flask to get 1 mg/ mL stock solution. A series of standard solutions in the concentration range of 10, 15, 20, 25, 30µ,g/mL were prepared followed by a suitable dilution of stock solution with the mobile phase.

Sample preparation: 10 ml takes from the above solution and transferred to a clean and dry 10ml of volumetric flask. Then make up the volume with the mobile phase.

Procedure for estimation of the drug: Each standard solution of 2, 4, 6, 8, 10 g/mL was injected thrice into the system followed by one blank injection and a calibration curve was constructed by plotting concentration of donepezil hydrochloride on X-axis and corresponding mean peak area on Y-axis. The sample prepared above was injected twice into the system and average peak area from chromatograms was determined. The concentration of the drug was computed from the calibration curve.

2.2. Method Validation: Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use. The method validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures.

All analytical methods that are intended to be used for analyzing any clinical samples will need to be validated. The validation of analytical methods is done as per ICH guidelines.

Validation parameters: The following are typical analytical performance characteristics which may be tested during methods validation:

1. System suitability determination.
2. Accuracy
3. Precision
4. Repeatability
5. Intermediate precision
6. Linearity
7. Detection limit
8. Quantitation limit
9. Specificity
10. Range
11. Robustness

3. RESULTS AND DISCUSSION

HPLC method development: A reverse phase gradient liquid chromatographic technique was developed, optimised and validated for the determination of bulk form with UV detection at 271 nm by using Inertsil C8-3, 25 cm x 4.6-mm, column with mobile phase composition contains Methanol and HPLC Water in the ratio 70:30 and in optimised isocratic program.

Table- 1 System Suitability Testing of Donepezil IN HPLC

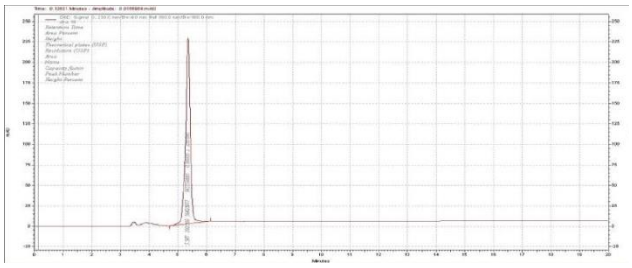
Injection number	Peak area	Theoretical plates	Tailing factor	Retention time
1	221025200	9723	1.06	4.46
2	410595400	9714	1.08	4.51
3	611485400	9752	1.04	4.47
4	811237400	9712	1.02	4.58
5	982215200	9710	1.09	4.49
Mean	21039612	9726	1.058	4.46
%RSD	1.34	0.15	0.78	1.11
Acceptance criteria	NMT 2.0	NLT 2000	NMT 2.0	-

Inference: The obtained experimental values in system suitability trials (n=5) were found to be within the limits proposed by ICH guidelines.

Table:- 2 Results For Accuracy In HPLC

S.NO	% Level	Standard amount	Spiked amount	Amount found	% Recovery	Mean Recovery
1	50%	20	10	29.80	99.67	Mean=99.26 SD = 0.5006 %RSD=0.50
		20	10	29.64	99.66	
		20	10	29.22	98.70	
2	100%	20	20	38.96	98.70	Mean =99. 20 SD = 0.436 %RSD=0. 44
		20	20	39.54	99.37	
		20	20	39.62	99.52	
3	150%	20	30	49.12	99.12	Mean =99. 03 SD =0. 087 %RSD=0. 09
		20	30	48.95	98.97	
		20	30	49.02	99.02	

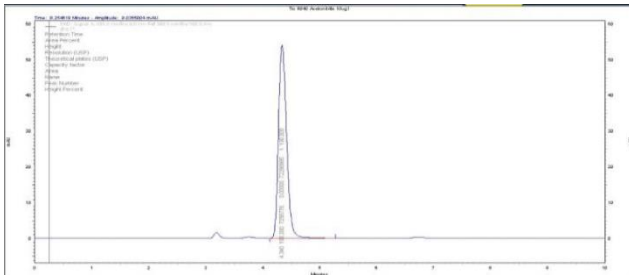
TRAIL:1



Discussion: Peak was eluted with moderate retention time but presence of high intensity peaks was observed even after many equilibrations.base line distributions are observed,it was not satisfactory.

Fig: 1 Chromatogram Of Trail -1

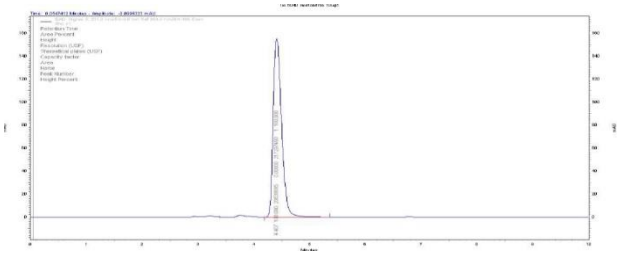
TRAIL:2



Discussion:When mobile phase composition was also used for the preparation of diluent, excellent results were observed. But baseline disturbance was observed. Flow rate was changed to make the method more rapid and retention time was satisfactory.

Fig :2 Chromatogram Of Trail 2

OPTIMIZED CHROMATOGRAM :



Discussion: Symmetric peak with asymmetry factor not more than 2 and theoretical plates was obtained. Reproducible peaks with excellent peak characteristics were obtained with rapid retention time of 4.40 minutes.

Fig :3 Optimized Chromatogram

Table:-3 Summary for RP-HPLC method

S.No	Parameter	Acceptance criteria	Results obtained
1	System suitability	Theoretical Plates-NLT 2000	9724.8
2	Specificity	No interference of blank	Passed
3	Linearity	Correlation coefficient NLT 0.996	0.998
4	Precision Intra Day Inter Day	%RSD NMT 2 %RSD NMT 2	1.3 0.01
5	Accuracy	Percentage Recovery 98-102%	99.68

4. CONCLUSION:

A validated stability-indicating HPLC analytical method has been developed for the determination of DONEPEZIL in bulk and in tablet dosage form. The results of stress testing undertaken according to the ICH guidelines revealed that the method is selective and stability-indicating. The proposed method is simple, accurate, precise, and specific, and it has the ability to separate the drug from degradation products and excipients found in the dosage form but from all stability conditions the DONEPEZIL was found to be a highly stable molecule. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability

experiments to predict expiration dates of pharmaceuticals.

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