International Journal of
Medicinal Chemistry & Analysis
e ISSN 2249 – 7587
Print ISSN 2249 - 7595
www.ijmca.com
Review Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD OF SIMULTANEOUS ESTIMATION FOR DONEPEZIL AND MEMANTINE TABLET DOSAGE FORMS

Sonja jose[1*], Ishwarya.P.J[2], Monisha.R[2], Sonu.S[2], Vignesh sharma.G[2]

Department of Pharmaceutical Analysis, Annai Veilankanni's Pharmacy College, Saidapet, Chennai-600015, Tamilnadu., India.

ABSTRACT

To develop a simple, cheap, accurate, and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method and validate as per ICH guidelines for estimation of donepezil and memantine in pharmaceutical dosage forms. The separation was conducted by using mobile phase consisting of phosphate buffer: methanol in the ratio (70:30). The wavelength was found at 273nm. HPLC alliance with empower software is used for chromatographic determination. The separation was conducted by using inertsil C-18 (4.6×250mm×5µm) at the flow rate of 1.0 ml/min using variable wavelength detector. The developed method resulted in donepezil and memantine eluting at 5.067 min and 2.003min. The method was found to be linear over the concentration range 25-125µg/ml with coefficient regression R2-0.999. Mean recovery was found to be in the range of 99.8%, during accuracy studies. The limit of detection (LOD) was found to be 2.75mg/ml and 3.14mg/ml. The limit of quantitation (LOQ) was found to be 9.96mg/ml and 10.05 mg/ml respectively. A cheap, accurate, precise, linear and rapid RP-HPLC method was developed and validated for the quantitative estimation of Donepezil and memantine as per ICH guidelines.

Keywords: RP-HPLC, Donepezil, Memantine, Validation.

Corresponding Author: - **Sonja Jose Email: -** sonjamanoj@gmail.com

INTRODUCTION

Donepezil chemically (*RS*)-2-[(1-Benzyl-4-piperidyl) methyl]-5, 6-dimethoxy-2, 3-dihydroinden-1-one is a centrally acting reversible acetyl cholisterase inhibitor used in the treatment of Alzheimer's disease. Donepezil binds and inactivate reversibly the cholinesterase, thus inhibiting hydrolysis of acetyl choline. This results in an increased acetyl choline concentrations at cholinergic synapses. Donepezil has also been studied in patients with mild cognitive impairment, schizophrenia, attention deficit disorder, post-coronary bypass cognitive impairment and Down syndrome.

Memantine chemically 3, 5 dimethyl adamantan-1- amine. NMDA receptor antagonist that binds preferentially to NMDA receptor operated cation channels. It is used to treat moderate to severe confusion (dementia) related to Alzheimer's disease. It improves memory, awareness, and ability to perform daily function.

Access this article online		
Home page: http://ijmca.com/ DOI: http://dx.doi.org/10.21276/ijmca.2020.10.2.3		Quick Response code
Received:25.05.20	**Revised**:12.06.20	**Accepted**:15.07.20

MATERIALS AND METHODS

Chemicals and Reagent

Methanol, water, acetonitrile of HPLC grade. Potassium dihydrogen orthophosphate of A.R grade, donepezil and memantine.

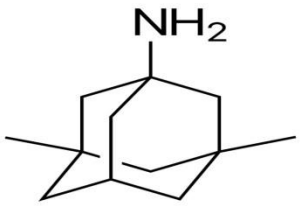
Instruments

HPLC alliance was performed with empower software with PDA detector. UV double beam spectrophotometer, digital weighing balance, pH meter, ultra sonicator and suction pump.

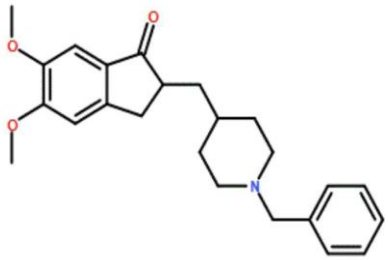
Methods

Selection of wavelength: suitable wavelength for HPLC was determined by recording UV spectrum in the range of 200-400 nm for donepezil and memantine. Suitable wavelength selected was shown in figure-1.

Structure of Memantine



Structure of Donepezil



Chromatographic Conditions

The developed method uses a reverse phase C18 column, phenomena inertsil C18 (4.6x 250mm, 5µm), mobile phase consisting of a mixture of pH 3 phosphate buffer: methanol (70:30% v/v). The mobile phase was set at a flow rate of 1ml/min and the volume injected was 10µl for every injection.

Preparation of Mobile Phase

A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of MEOH (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration. The detection wavelength was set as 273 nm.

Preparation of Donepezil Standard Stock Solution

10mg of Donepezil drug was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent(mobile phase) is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Memantine Standard Stock Solution

10mg of Memantine drug was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent (mobile phase) is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent (Stock solution). Further 1.0 ml from the above stock solution is pipette into a 10 ml volumetric flask and was diluted upto the mark with diluent.

Preparation of Sample Solution

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Memantine and Donepezil (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7ml of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

Calibration Curve

Appropriate aliquots of 1ml of stock solution was taken in 10ml of volumetric flask and diluted up to the mark with diluent(mobile phase) to obtain a final concentration 2,3,4 and 5µg/ml. Each level was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated and it is shown in figure-2&3.

RESULTS AND DISCUSSION

Method Development

A reverse phase HPLC method was developed considering the system suitability parameters i.e. tailing factor (T), the number of theoretical plates (N), run time and the cost effectiveness. System suitability tests are an integral part of method development and validation system suitability parameters for donepezil and memantine. The results are given in table (1).

Table 1. System Suitability Parameters For Donepezil And Memantine

S. No	Name	Retention time(min)	Area(µV sec)	Height(µV)	USP resolution	USP tailing	USP plate count
1	Memantine	2.003	920101	116666		1.6	2711.8
2	donepezil	5.067	552058	41531	11.0	1.3	3428.2

Table 2. Analytical Performance Parameter of Donepezil and Memantine

Parameters	Memantine	Donepezil
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R2)	0.999	0.999

Table 3. Results of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		ARTE	PIPE	ARTE	PIPE	ARTE	PIPE
5O%	**1**	460064	276931	324.9	25.0	99.8	100
	2	460124	276694	24.6	24.9	99.6	99.6
	3	460216	276891	24.8	24.9	99.8	99.6
	Average recovery					**99.7%**	**99.7%**
100%	**1**	923429	554156	49.9	50.0	99.8	100
	2	923654	554897	49.8	49.9	99.6	99.8
	3	923742	556371	49.8	49.9	99.6	99.8
	Average recovery					**99.6%**	**99.8%**
150%	**1**	1387901	828113	74.8	75.0	99.8	100
	2	1385360	828794	74.9	74.9	99.8	99.8
	3	1386984	828349	74.6	74.8	99.6	99.8
	Average recovery					**99.7%**	**99.8%**

Table 4. Results of Intermediate Precision for Donepezil and Memantine

S. No	Sample area	Standard area	Percentage purity
1	979556	984395	99.30
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average			**99.63**
%RSD			**0.54**

Table 5. Results of Intermediate Precision For Donepezil

S. No	Sample area	Standard area	Percentage purity
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average			**99.27**
%RSD			**0.27**

Table 6. Results for Effect of Variation In Flow

S. No	Peak area for less flow (0.7ml/min)		Peak area for more flow (0.9ml/min)	
	Memantine	Donepezil	Memantine	Donepezil
1	983465	575351	971563	592641
2	985134	580381	973021	592352
3	983467	587724	975674	595471
4	985217	583190	978974	594416
5	994245	584468	984542	583453
Mean	**986306**	**582223**	**976755**	**591667**
%RSD	**0.45**	**0.80**	**0.53**	**0.80**

Fig: 1. Spectrum of Donepezil and Memantine

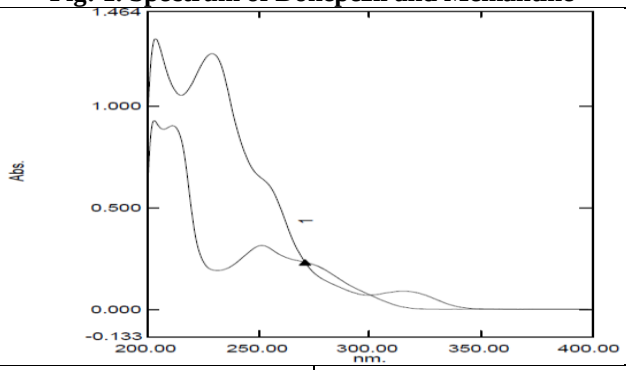


Fig: 2 Caliberation Graph For Memantine at 273 nm

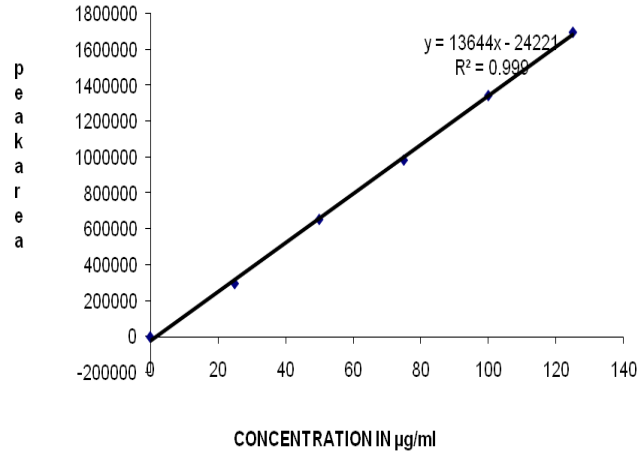
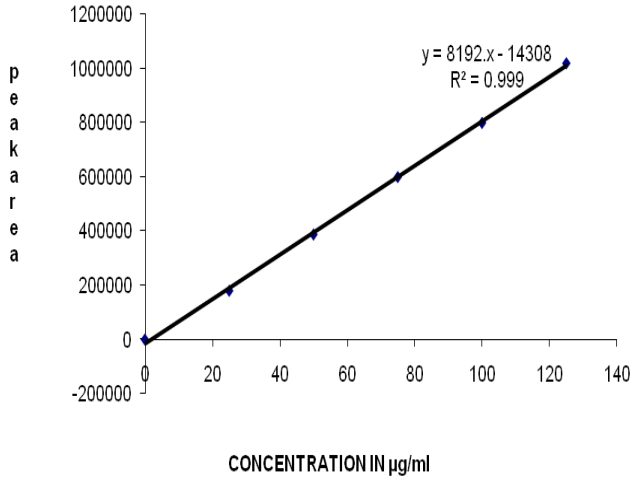


Fig: 3 Caliberation Graph for Donepezil at 273nm



METHOD VALIDATION

Authentication of the investigative method is the process that starts by laboratory studies in which the requirements of the performance properties of method are met for the intended analytical application. To the validation of analytical procedures RP-HPLC method developed was validated according to International conference on harmonization (ICH) and USP guidelines. Various parameters or criteria are used for the method of validification, such as linearity, accuracy, precision, system suitability, ruggedness, limit of quantification (LOQ) and limit of detection (LOD).

System Suitability Parameters

The system suitability of the method was checked by injecting five different preparations of the donepezil and memantine standard. The parameters of system suitability were checked.

Linearity

The linearity range was found to lie from 25% to 125% and chromatograms.

Accuracy

Sample solution at different concentrations (50%, 100% & 150%) were prepared and the % recovery was calculated. The results are shown in table (3).

Precision

Precision of the method was carried out for both sample and standard solutions. The corresponding chromatograms are resulted. The results are shown in table (4).

Intermediate Precision (Ruggedness)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system variation.

Robustness

The standard and samples of donepezil and memantine were injected by changing the conditions of chromatography.

Limit Of Detection for Donepezil and Memantine

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal noise ratio.

Limit of Quantitation for Donepezil and Memantine

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal noise ratio.

CONCLUSION

On the basis of experimental results, the proposed method is suitable for the quantitative determination of memantine and donepezil in pharmaceutical dosage form. The estimation of Memantin and donepezil was done by RP-HPLC. The Phosphate buffer was pH 4.6 and the mobile phase was optimized which consists of MeOH: Phosphate buffer mixed in the ratio of 70:30 % v/v. A Symmetry C18 (4.6 x 150mm, 5µm) column used as stationary phase. The detection was carried out using UV detector at 273 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Memantin and donepezil were found to be from 25-125µg/ml. Linear regression coefficient was not more than 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Memantin and donepezil LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Memantin and donepezil in formulation. High percentage of recovery shows that the method is free from the interference of excipients used in the formulation. So the method can be useful in the routine quality control of these drugs.

REFERENCES

1. A.Suneetha et al, A Validated Rp-Hplc Method for Simultaneous Estimation of Darunavir in Combined Dosage Form. International Journal of Pharmacy and Pharmaceutical Sciences. ISSN- 0975-1491 Vol 3, Issue 1, 2011.
2. B.K. Sharma, Textbook of Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 23rd Ed, p.288-289.
3. D.A.Skoog, F.J.Holler, S.R.Crouch, Textbook of Instrumental Analysis, Brooks/Cole, Cengage Learning India Private Limited, 2007, p.900-906.
4. G.R.Chatwal, S.K.Anand, Text book of Instrumental Methods of Chemicals Analysis, Himalaya Publishing House,5th Ed, 2002, p.2.566-2.570.
5. G.Vidyasagar, Textbook of Instrumental Methods of Drug Analysis, Pharmamed Press, 2009, p.106-120.
6. G.W.Ewing, Text book of Instrumental Methods of Chemical Analysis, Mc Graw-Hill Book Company, 5th Ed, p.375-385.
7. H.H.Tackett, J.A.Cripe, G.Dyson, Positive displacement reciprocating pump fundamentals-power and direct acting types, Proceedings of the twenty-fourth international pump user's symposium,2008, p.45-58.
8. H. H Willard, L. L Merritt, J. A Dean, and F. A Settle, Textbook of Instrumental Methods of Analysis, CBS publishers and distributors, New Delhi, 7th Ed,1986, p.592-596.
9. ICH: Q2B, Analytical Validation – Methodology (1996).
10. K.Gowtham et al, Sensitive Analytical Method Development and Validation of Ritonavir Bulk drugs by RP-HPLC. Journal of Scientific Research in Pharmacy.2013; 2(1); 125-127.

Attribution-NonCommercial-NoDerivatives 4.0 International