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R E S E A R C H A R T I C L E

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Donepezil and Memantine in Pure and Pharmaceutical Dosage Form

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A B S T R A C T

On the basis of experimental results, the proposed method is suitable for the quantitative determination of memantine and donepezil in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability. The estimation of Memantine 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, Make XTerra) 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 Memantine and donepezil were found to be from 25-125 µg/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 Memantine and donepezil LOD and LOQ was found to be within limit. The proposed method is precise, simple and accurate to determine the amount of Memantine 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.

Keywords: Memantine and donepezil was done by RP-HPLC

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Memantine is used to treat moderate to severe Alzheimer's disease. It acts on the glutamatergic system by blocking NMDA receptors. It was first synthesized by Eli Lilly and Company in 1968 as a potential agent to treat diabetes; the NMDA activity was discovered in the 1980s.

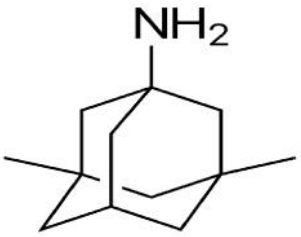

Figure 1: Structure of Memantine

Donepezil, marketed under the trade name Aricept, is a medication used in the palliative treatment of Alzheimer's disease. Donepezil is used to improve cognition and behavior of people with Alzheimer's, but does not slow the progression of or cure the disease. Common side effects include loss of appetite, gastrointestinal upset, diarrhea, difficulty sleeping, vomiting, or muscle cramping. It was developed by Eisai and Pfizer and is sold as a generic by multiple suppliers. Donepezil acts as a centrally acting reversible acetyl cholinesterase inhibitor.

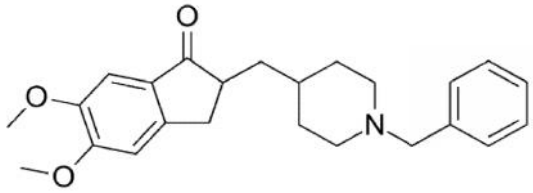

Figure 2: Structure of Donepezil

2. Materials and Methods

Instrumentation

HPLC Shimadzu Waters 996 LC 20 Software UV double beam UV 3000 UV Win 5 Lab India Digital weighing pH meter Ultra sonicator Suction pump.

Chemicals

Potassium dihydrogen, Acetonitrile, Methanol, Water

Chromatographic Conditions

Parameters	**:Description**
Column	**:**Symmetry C_{18} Column (4.6mm x 150mm)5µm.Make XTerra) or equivalent
Mobile Phase	**:**70% MEOH : 30% phosphate buffer pH-4.6
Buffer pH	**:**4.6
Flow rate	**:**1 ml/min
Column temperature	**:**Ambient
Wavelength	**:**273 nm
Run time	**:**7 min

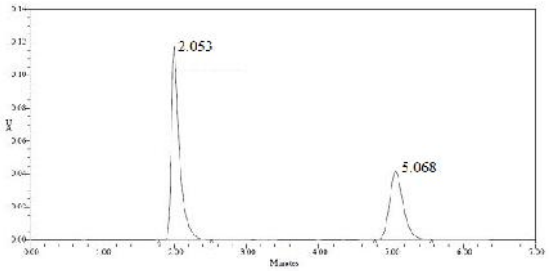

Figure 3: Optimized Chromatogram for domepezil and memantine

Observation: From the above chromatogram it was observed that the domepezil and memantine peaks are well separated.

Preparation of the individual Donepezil standard preparation:

10mg of Donepezil working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (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 diluant.

Preparation of the individual Memantine standard preparation:

10mg of Memantine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of diluent is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant. (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 diluant.

Preparation of Sample Solution :(Tablet)

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 in to a10ml volumetric flask and diluted upto the mark with diluant.

Method Validation

Accuracy:

Accurately weighed 10 mg of Memantine and 10mg of Donepezil working standard were transferred into a 10mL and 100ml of clean dry volumetric flasks.

About 7mL and 70ml of Diluents are added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Precision

Repeatability:

Preparation of standard stock solution:

Accurately 10 mg of Memantine and 10mg of Donepezil working standard were weighed and transferred into a 10mL and 100ml of clean dry volumetric flasks and about 7mL and 70ml of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Intermediate Precision (Ruggedness):
To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Specificity:
The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

LOD:
LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y-intercepts of regression lines.

LOQ:
LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines.

Linearity:
Accurately 10 tablets were weighed & crushed in mortar and pestle and weight equivalent to 10 mg of Memantine and Donepezil (marketed formulation) sample were transferred into a 10mL clean dry volumetric flask and about 7mL of Diluant was added and sonicated to dissolve it completely and made volume up to the mark with the same solvent.

Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 1µg-5µg and 100µg- 500µg of Donepezil and Memantine respectively.

Robustness: As part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method.

System suitability:
5 mg of Donepezil and 500 mg of memantine in working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and add about 20ml of diluant and sonicated to dissolve it completely and make volume up to the mark with the same solvent

3. Results and Discussion

Linearity:

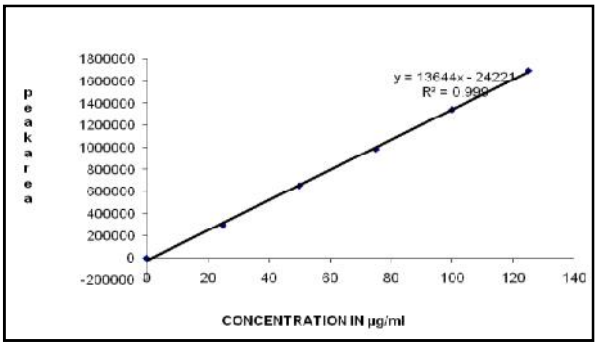

Figure 4: Calibration graph for memantine at 273 nm

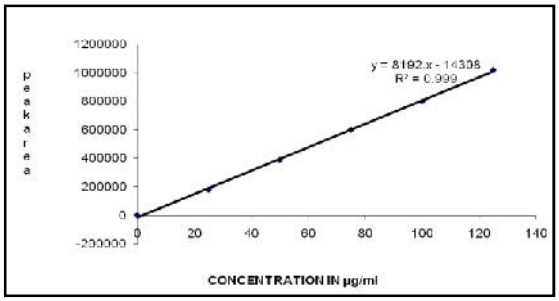

Figure 5: Calibration graph for donepezil at 273 nm

Robustness:

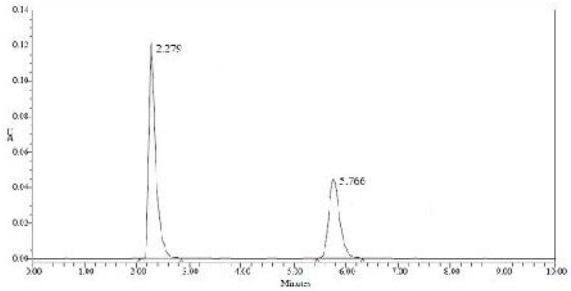

Figure 6: Chromatogram showing less flow of 0.7ml/min

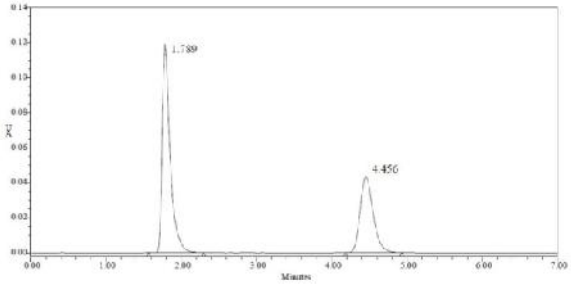

Figure 7: Chromatogram showing more flow of 0.9ml/min

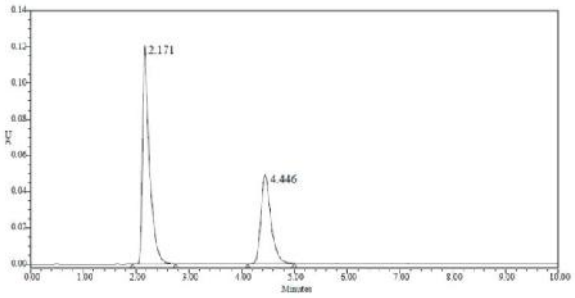

Figure 8: Chromatogram showing less organic composition

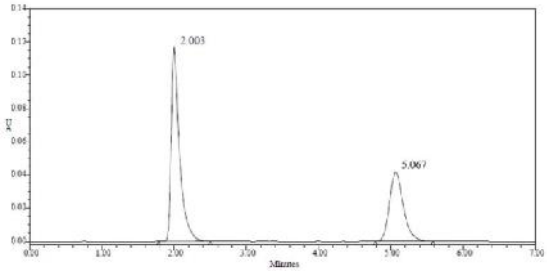

Figure 9: Chromatogram showing more organic composition

4. Conclusion

On the basis of experimental results, the proposed method is suitable for the quantitative determination of memantin and donepezil in pharmaceutical dosage form. The method provides great sensitivity, adequate linearity and repeatability.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, Make XTerra) 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 25-125 µg/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 Memantine 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.

Table 1: Results of system suitability parameters for donepezil and memantine

S.No	Name	Retention time(min)	Area (µVsec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Memantine	2.053	920101	116666		1.6	2711.8
2	Donepezil	5.068	552058	41531	11.0	1.3	3428.2

Table 2: Results of method precision for memantine

S. No	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average			**100.84**
%RSD			**0.304**

Table 3: Results of method precision for donepezil

S. No	Sample area	Standard area	Percentage purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09
Average			**101.24**
%RSD			**0.46**

Table 4: Results of Intermediate precision for 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 of Accuracy

Sample concentration	Sample set no	Sample area		Assay		% Recovery	
		DONEP	**MEM**	**DONEP**	**MEM**	**DONEP**	**MEM**
50%	**1**	460064	276931	24.9	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 7: Area of different concentration of memantine and donepezil

Concentration (µg/ml)	Peak area of memantine	Peak area of donepezil
25	296800	179891
50	653819	387781
75	983775	599708
100	1342535	799619
125	1694286	1019614

Table 8: Analytical performance parameters of donepezil and memantin

Parameters	Memantin	Donepezil
Slope (m)	13644	8192
Intercept (c)	24221	14308
Correlation coefficient (R^2)	0.999	0.999

Table 9: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Memantine	56	176	3.14
Donepezil	56	154	2.75

Table 10: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Memantine	56	563	10.05
Donepezil	56	558	9.96

Table 11: Results for effect of variation in flow

S. No	peak area for Less flow (0.7 ml/min)		peak area for More flow (0.9 ml/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**

Table 12: Results for effect of variation in mobile phase composition

S. No	peak area for Less organic(70%)		Peak area for More organic (90%)	
	Memantine	**Donepezil**	**Memantine**	**Donepezil**
1	984565	574371	981565	593761
2	986134	585481	983527	592462
3	984268	587627	985489	594491
4	986216	585362	987954	596316

5	995247	585448	994672	587353
Mean	**987286**	**583658**	**986641**	**592877**
%RSD	**0.45**	**0.90**	**0.51**	**0.57**

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