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ANALYTICAL METHOD DEVELOPEMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MEMANTINE HCL AND DONEPEZIL HCL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract: A simple, accurate and specific RP-HPLC method has been developed and validated for the simultaneous estimation of Memantine HCl and Donepezil HCl in bulk and pharmaceutical dosage form. Chromatographic separation was achieved on Amino, Column 250 X 4.8 Mm (5 µm) having mobile phase HPLC Grade Water (100%) at a flow rate of 1 mL/min and detection of both the eluents carried out by Waters Refractive Index Detector (RI Detectors). The retention time of MEM and DONE was found to be 3.561 min. and 4.212 min respectively. Method was found to be linear over the range of 50-90 µg/mL for Memantine and 50-90 µg/mL for Donepezil. Assay results of the marketed formulation were shown that %label claim of Memantine HCl and Donepezil HCl was found to be 100.96 ± 0.48 and 100.35±0.76 respectively. % recoveries of Memantine HCl and Donepezil HCl were obtained in the range of 98.56%–99.76% and 98.38%– 99.25% respectively. The developed method was found to be simple, precise and accurate and can be utilize as a quality control tool for the simultaneous estimation of both drugs from their pharmaceutical dosage form.

Keywords: Memantine HCl, Donepezil HCl, RP-HPLC, RI Detectors, Amino Column

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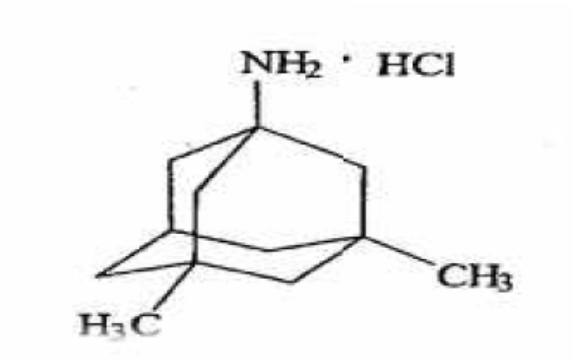
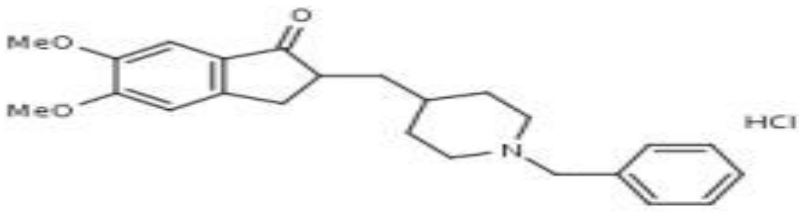
INTRODUCTION

Memantine HCl Chemically is 1-amino-3,5-dimethyladamantane hydrochloride. It is an anti-alzheimer's, Uncompetitive NMDA Receptor Antagonist. Donepezil HCl is chemically (±)-2,3-dihydro-5,6-dimethoxy-2-[1-(phenylmethyl) -4 piperidinyl]methyl]-1H-inden-1-one hydrochloride, Cholinesterase Inhibitors. Memantine HCl is not official in any pharmacopoeia. Donepezil HCl is official in IP-2010. Literature survey revealed that a number of methods have been reported for estimation of MEM and DONE individually. For this combination One Chromatographic (RP-HPLC) method has been reported. Objective of this study is to develop a Simple, fast, accurate and precise method for simultaneous estimation of MEM and DONE by RP-HPLC method.



Structure of Memenatine HCl



Structure of Donepezil HCl

MATERIALS AND METHODS

Instrumentation: Waters410 Differential Refrectometer, Waters Refrective Index Detectors (RI detectors), Amino Column (250mm x 4.6mm, 5 µm), Shimadzu – AUX 220 balance was used.

Chemicals & Reagents: Memantine Hydrochloride and Donepezil Hydrochloride were obtained from the Allembic Pharmaceuticals Ltd., Baroda, Gujarat, India., as a gift sample. Combined dose tablet formulation, Donemem 5 (Sun Pharmaceuticals Ltd) containing MEM 5mg and DONE 5mg was obtained from local pharmacy store. HPLC Grade Water was obtained from Lichrosoly -E. Merck (India) Ltd. Mumbai.

Procedure

Preparation of standard stock solution

Preparation of MEM stock solution: Accurately weighed MEM 25 mg was transferred to 25 mL volumetric flask and dissolved in mobile phase and diluted up to the mark with mobile phase to give 1000 µg/mL.

Preparation of DONE stock solution: Accurately weighed DONE 25 mg was transferred into 25 mL volumetric flask and dissolved in mobile phase and diluted up to the mark with mobile phase to give 1000 µg/mL.

Preparation of binary mixtures of MEM and DONE: Accurately weighed 25 mg MEM and 25 mg of DONE were transferred to 25 mL volumetric flask. It was dissolved with sufficient mobile phase and diluted up to mark with mobile phase to give concentration of 1000 µg/mL of MEM and 1000 µg/mL of DONE.. Above solution was diluted further to get the concentration range of 50, 60, 70, 80, 90 µg/mL of MEM and 50, 60, 70, 80, 90 µg/mL of DONE.

Chromatographic conditions: Chromatographic separation was carried out on Amino, Column 250 X 4.8 Mm (5 µm) having mobile phase HPLC Grade Water (100%) at a flow rate of 1 mL/min and total run time of 6 min. Detection of both the eluents carried out by Waters Refractive Index Detector.

VALIDATION

Linearity

The linearity is expressed in term of correlation co-efficient of linear regression analysis. The linearity of response for MEM and DONE was assessed by analysis of five independent levels of calibration curve in range of 50-90 µg/mL for MEM and 50-90 µg/mL for DONE.

Precision

Result should be expressed as relative standard deviation (RSD) or co-efficient of variance.

A. Repeatability

2.5 mL of combined working standard solutions (1000 µg/mL of MEM and 1000 µg/mL of DONE) were transferred into separate 50 mL volumetric flasks and diluted up to mark with mobile phase to get 50 µg/mL of MEM and DONE. Each concentration was prepared and analyzed. The peak area obtained with each solution was measured and % R.S.D was calculated.

B. Intra-day precision

Mixed solutions containing 50, 60, 70 µg/mL MEM and DONE were analyzed on the same day and % R.S.D was calculated.

C. Inter-day precision

Mixed solutions containing 50, 60, 70 µg/mL MEM and DONE were analyzed on different days and % R.S.D was calculated.

Accuracy

Twenty tablets were weighed and powdered.

Powder equivalent to 100 mg of MEM and 100 mg of DONE was weighed and transferred into a 100 mL of volumetric flask, dissolved and diluted up to mark with mobile phase. (1000 µg/mL of MEM and 1000 µg/mL of DONE).

To the 50 µg/mL solution , 40,50 and 60 µg/mL (80%,100%,120%) solutions were spiked .Area of peak obtained with each solution was measured for MEM and DONE.

The amount of MEM and DONE was calculated at each level and % Recoveries were computed.(Table 1.4)

LOD and LOQ

The LOD was estimated from the 5 calibration curves. The LOD may be calculated as

LOD = 3.3 × (SD / Slope)

Where, SD = Standard deviation of the Y- intercepts of the 5 calibration curves

Slope = Mean slope of the 5 calibration curves.

The LOQ was estimated from the calibration curves used to determine method linearity. The LOQ may be calculated as **LOQ = 10 × (SD/Slope)**

Where, SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves

Robustness: The solution containing concentration (70 µg/mL) of Memantine HCl and Donepezil HCl was analyzed in different temperature and Flow rate and the peak area obtained with each solution was measured and % R.S.D was calculated.(Table 1.6)

Analysis Of Tablet Formulation

- Twenty tablets were weighed and powdered.
- The tablet powder equivalent to 100 mg of MEM and 100 mg of DONE was transferred in 100 mL volumetric flask, dissolved and diluted up to mark with mobile phase.
- The solution was filtered the solution through Whattman filter paper no.41 and first few drops of filtrate were discarded.
- 2.5 mL of the filtrate was diluted to 50 mL with mobile phase to get 50 µg/mL solution.
- 20 µL of this solution was injected in HPLC and area of the peak was measured.(Table 1.5)

RP-HPLC METHOD

The method discussed in the present work provide a convenient and accurate way for simultaneous analysis of MEM and DONE. The mobile phase consisting of Water (HPLC Grade). The optimized method was applied for marketed formulation and the % label claim for MEM and DONE was found to be 100.96 ±0.48 % and 100.35±0.76%. The method is accurate and precise and can be used for routine pharmaceutical analysis. The data for linearity, precision, accuracy, LOD, LOQ is represented in the table 1.2 and 1.3 . Recovery studies were carried out by standard addition method to check the accuracy of the developed methods and to study the interference of formulation additives (Table 1.4).The validated method was successfully applied for the determination of tablet mixture of MEM and DONE. The results are given in Table 1.5, indicate that the amount of drug in tablet samples met with requirements. Robustness Evaluation of Method for MEM and DONE is given in table 1.6

CONCLUSION

The developed RP-HPLC method provides simple, specific, precise, accurate, economical and reproducible quantitative analysis for simultaneous determination of MEM and DONE in combined tablet dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ).The method can be used for routine analysis of MEM and DONE in combined dosage form.

Tables and Figures

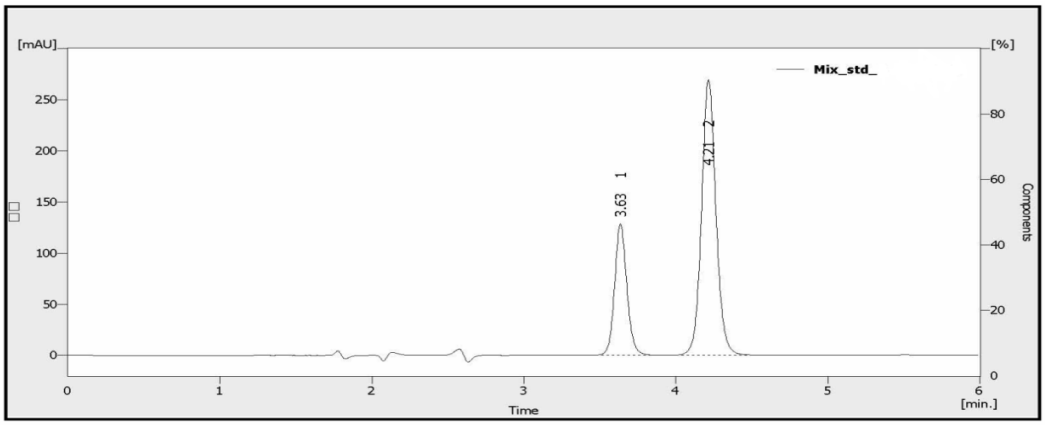


Fig 1: Chromatogram showing retention time of MEM and DONE

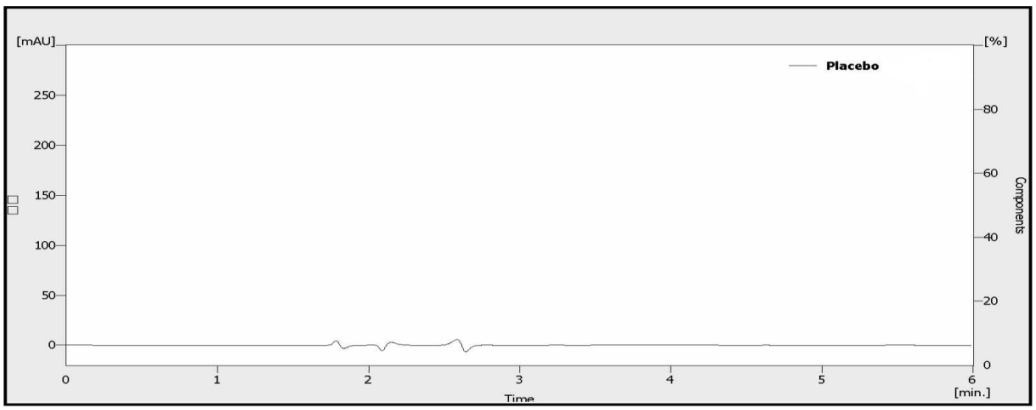


Fig 2: Chromatogram of placebo

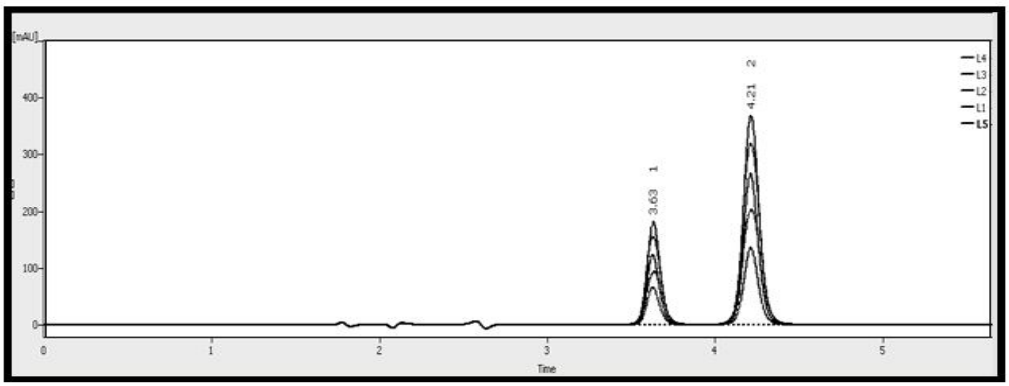


Fig 3: Chromatogram of different concentrations of binary mixtures of MEM & DONE

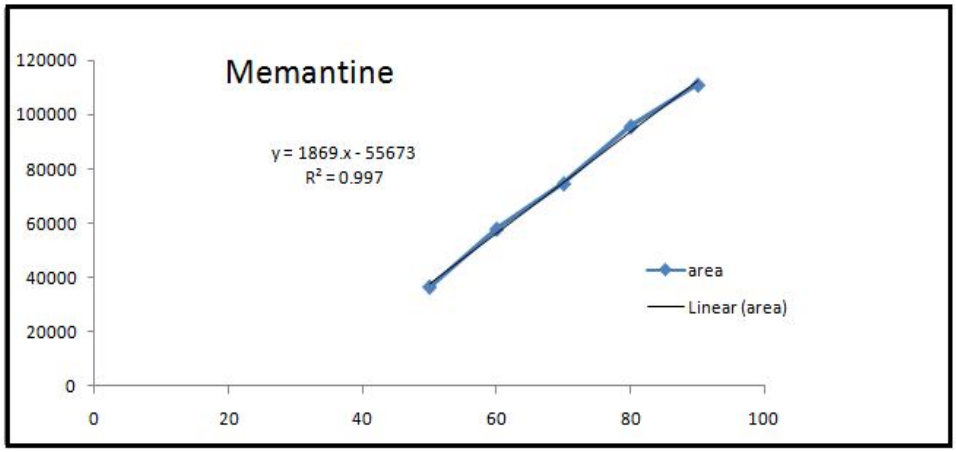


Fig 4: Calibration curve of MEM

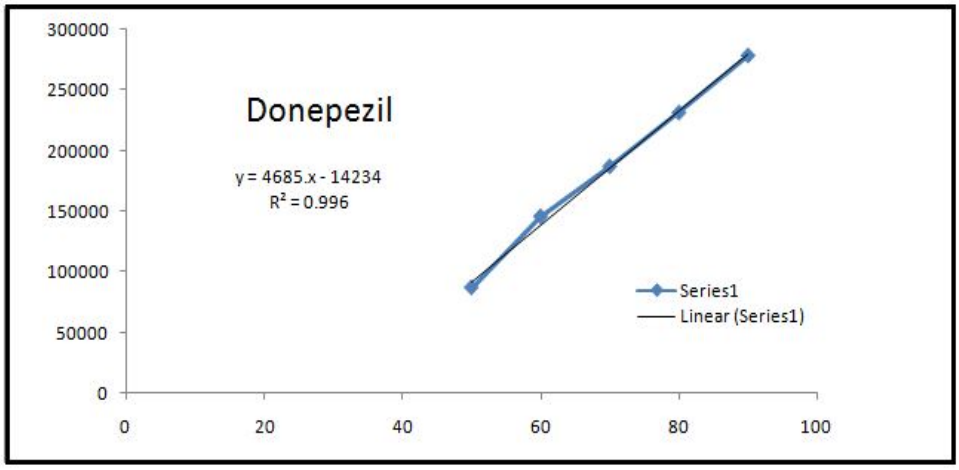


Fig 5: Calibration curve of DONE

Table 1: Results of system suitability parameters

Parameter	Memantine Mean ± S.D, (n=6)	Donepezil Mean ± S.D, (n=6)
Retention time (min)	3.567±0.01	4.256±0.03
Capacity factor	2.27±0.03	3.08±0.02
Theoretical plates	9773.11±21.52	9850.11±54.79
Tailing factor	1.115±0.03	1.078±0.06
Resolution	4.894±0.04	3.645 ±0.03

Table 2: Linearity data for MEM and DONE (n=5)

Regression Analysis	Memantine	Donepezil
Regression equation	y = 1869.9x – 55673	y = 4685.9x - 142346
Correlation co-efficient	0.997	0.996
Slope	1869.9	4685.9
Intercept	55673	142346

Table 3: Summary Of The Validation Parameters Of Proposed Method.

Sr. No.	Parameter		Memantine	Donepezil
2	Linearity Range		50-90 µg/ml	50-90 µg/ml
3	Regression equation		y = 1869.9x - 55673	y=4685.9x-14234
4	Correlation co-efficient		0.997	0.996
5	Precision (% RSD)	Repeatability	0.45	0.52
		Interday	0.55-0.38	0.68-0.42
		Intraday	065-0.48	0.70-0.42
6	Accuracy (% recovery)		98.56 – 99.76	98.38 – 99.25
7	Limit of Detection		0.291	0.317
8	Limit of Quantification		0.882	0.962
9	Robustness		0.30	0.253
10	Solution Stability		Up to 24 hrs	Up to 24 hrs
11	Assay		100.96 ± 0.48	100.35 ± 0.76

Table 4: Recovery data for tablet formulation (DONEMEM 5)

Amt of Sample (µg/mL)		Amt. of std added (µg/mL)		Amt. found (µg/mL)		% Recovery± %RSD	
MEM	DONE	MEM	DONE	MEM	DONE	MEM	DONE
50	50	0	0	49.76	50.028	-	-
50	50	40	40	89.56	89.708	99.51±0.27	99.67±0.76
50	50	50	50	101.89	100.85	101.89±0.14	100.85±0.52
50	50	60	60	109.43	110.92	99.98±0.49	100.83±0.38

Table 5: Analysis of market formulation

Formulation	Label Claim		% Assay (Mean ± SD, n=6)	
	Memantine	Donpezil	Memeantine	Donepezil
DONEMEM 5	5	5	100.96 ± 0.48	100.35 ± 0.76

Table 6: Robustness data for Memantine HCl and Donepezil HCl

Condition		MEM			DONE		
		%Assay	% RSD	Total % RSD	%Assay	% RSD	Total % RSD
As such		100.50	0.27	0.30	100.25	0.48	0.253
Temperature (30°C)	25	99.84	0.25		99.74	0.10	
	35	100.09			99.88		
Flow rate	0.9	100.02	0.32		99.91	0.18	
(1 mL/min)	1.1	99.99			100.17		

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