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"QUALITY BY DESIGN BASED RP-HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION OF MEMANTINE HCL AND DONEPEZIL HCL IN PHARMACEUTICAL DOSAGE FORM"

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ABSTRACT

A Novel simple, Accurate, precise, specific, sensitive and robust Reverse Phase High performance liquid chromatography (RP-HPLC) method was developed and validated. Design Expert® (11.0.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for response surface methodology (RSM). The selected CMPs (critical method parameter) were systematically optimized using Central-composite design (CCD). Chromatographic separation was accomplished on Agilent C18 (50 x 2.1 mm, 1.7µm) analytical column and UV detection was set at 230 nm. The optimized and predicted data from Design Expert software consisted of mobile phase 0.01N Potassium dihydrogen phosphate: acetonitrile in 55.2:44.8 v/v proportion, as it is pumped at a flow rate of 1 ml/min. The developed chromatographic approach was validated according to ICH Q2 (R1) guidelines.

Key Words: Memantine HCl, Donepezil, RP-HPLC, QbD Method.

INTRODUCTION

Memantine HCl chemically is 3, 5-dimethyladamantan-1-amine hydrochloride and having Molecular formula $C_{12}H_{22}ClN$. Donepezil HCl is 2[(1-benzylpiperidin-4-yl) methyl]-5, 6-dimethoxy-2,3 having molecular formula $C_{24}H_{30}ClNO_3$. Memantine is an antagonist of the glutamate receptor subtype known as the NMDA (N-Methyl-D-Aspartate)-receptor. It is used to lessen neurotoxicity that is suspected to contribute to Alzheimer's disease and other neurodegenerative diseases [1]. Memantine blocks the NMDA-receptor subtype of glutamate receptors preventing over-activation of glutamine receptors while allowing the normal activity. Its blocking effects antagonize an overactive glutaminergic system in the central nervous system (CNS) which is thought to be involved in the neurotoxicity seen in Alzheimer disease [2-3]. Acetylcholinesterase is inhibited quickly, centrally, and irreversibly by donepezil hydrochloride, a piperidine derivative. Donepezil inhibits acetylcholine hydrolysis

by binding reversibly to acetylcholinesterase. This increases the amount of acetylcholine available at synapses, improving cholinergic transmission. Some *in vitro* data has suggested that the anticholinesterase activity of donepezil is relatively specific for acetylcholinesterase in the brain. It is structurally unrelated to other anticholinesterase agents like tacrine and physostigmine [4]. The literature review reveals that few analytical methods have been reported for its quantitative estimation in pharmaceutical formulation, which includes UV-Spectrophotometry, HPLC, Stability indicating HPLC [5-15].

The concept of "Quality by Design" (QbD) was described as an approach that includes improving scientific understanding of critical process and product qualities, designing controls and tests based on the limits of scientific understanding during the development phase, and using the knowledge gained during the life-cycle of the product to work on an environment of continuous improvement.

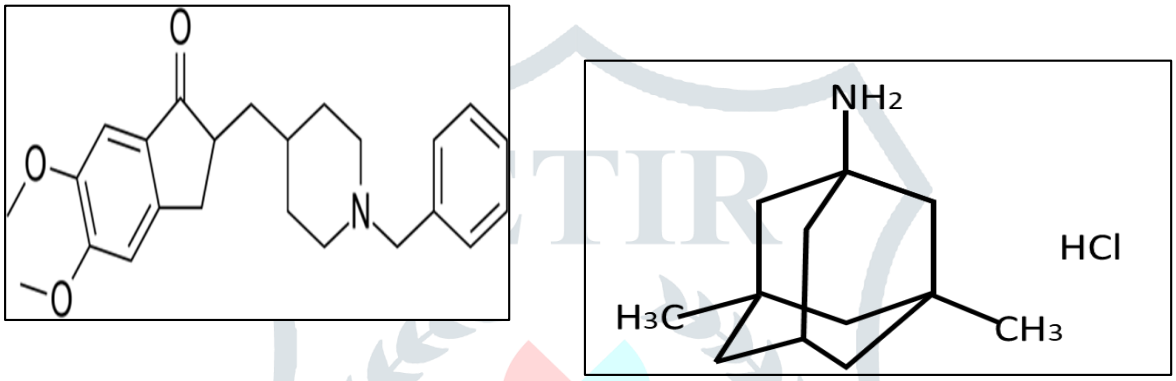

Figure 1: Chemical Structure of Donepezil

MATERIALS AND METHOD

Chemicals and Reagents :

Pure Memantine and donepezil was procured from Spectrum Pharma lab (Hyderabad). Hydrochloric acid AR grade (HCL) and Sodium hydroxide AR grade (NaOH) were obtained from Rankem, India. Acetic acid AR grade, Acetonitrile (ACN) and methanol (MeOH) HPLC grade were purchased from Fischer scientific.

Apparatus and equipment

HPLC studies were carried out on WATERS HPLC 2965 SYSTEM with a Photo diode array detector (PDA) set at 230 nm for UV detection. . Columns, Agilent C18 (150×4.6 mm, 5µm), were utilized in the study. Design Expert® (11.0.0) modeling software Minneapolis, Minnesota, USA-based Stat-Ease Inc. was used for generation of contour plots and 3D space. Additional equipment includes a pH metre, an analytical balance from Mettler Toledo, an IKAVortex vortex metre, and an ePEI ultrasonic generator sonicator (Eutech instruments pH tutor, pH meter, India).

Preparation of Standard stock solutions:

Accurately weighed 7mg of Memantine, 5mg of Donepezil and transferred to 50ml volumetric flask , 3/4th of diluents was added , and sonicated for 10 minutes. Flask was made up with diluents . (140µg/ml of Memantine and 100µg/ml of Donepezil)

Wavelength selection for detection

The detection wavelength was selected 230nm.

Preparation of Sample stock solutions:

After weighing 10 tablets and calculating the average weight of each tablet, the weight of each tablet was placed into a 100 ml volumetric flask. After adding 50 ml of diluents and sonicating it for 25 minutes, the volume was made up with diluent. and filtered by HPLC filters (140µg/ml of Memantine and 100µg/ml of Donepezil).

Buffer: Buffer Of concentration. 0.01N Potassium dihydrogen ortho phosphate and 0.1% ortho phosphoric acid buffer was used.

Optimization of method

Total Three factors viz; % Organic concentration, Flow rate and column temperature were needed to be optimized. So, CCD was applied to improve these parameters which were varied over three level (high, mid and low). Different ranges of Organic phase ranging from 31.59-48.41%, 0.01N potassium dihydrogen orthophosphate, column temperature 24.95 and 35.05 ℃ and 0.83-1.17ml/min flow rate respectively were taken and counter and 3D surface plot showing the effect of each parameter on Retention Time, Area, Theoretical plates and Asymmetry (CQA) were generated and desirability function applied to the optimized conditions.

Factor	Name	Level	Low Level	High Level
A	FR	0.9541	0.9000	1.10
B	% Organic phase	44.78	35.00	45.00
C	Temperature	30.17	27.00	33.00

Table 1: Factors

Table 2: Design summary of CCD

Design Summary					
File version: DX 11.0.0 Study Type: Response surface Design Type: central composite design Design Model: Quadratic			ATP: Robustness CQA: Retention time, Area, Theoretical plates and Asymmetry Runs: 20		
CMPs	Unit	Type	Subtype	Min.	Max.
column temperature	^{0}C	Numeric	Continuous	24.95	35.05
Flow rate	ml/min	Numeric	Continuous	0.83	1.17
% Org ratio	%	Numeric	Continuous	31.59	48.41

Method Validation

The final optimized chromatographic analytical method was validated as per the Q2 of the International Conference on Harmonization (ICH) (R1) guidelines for system suitability, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

RESULTS AND DISCUSSION

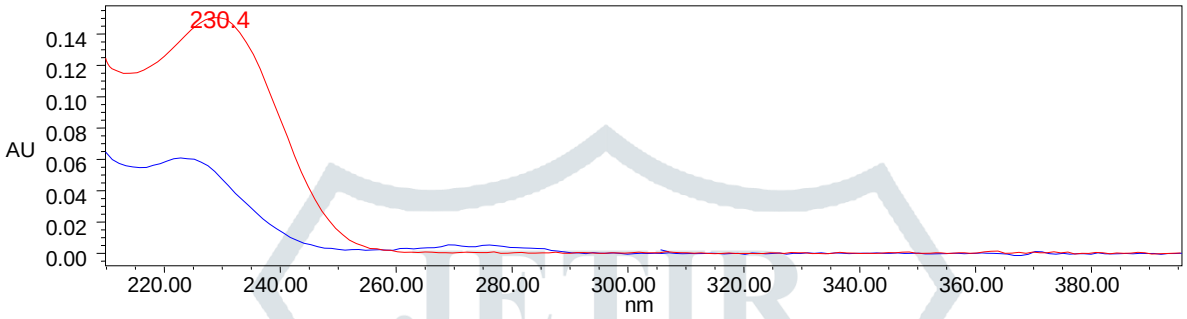

Figure 1: UV overlay spectra of memantine HCl and donepezil HCl

Simple and Robust RP-HPLC method development by DOE approach

Optimization of method

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
Std	Run	A:FR	B:% organicphase	C: Temperature	RT1	RT2	RS	NTP	TF
		ml/min	%	0 C	min	min	num	num	num
13	20	1	40	24.9546	----	4.024	8.7	7608	1.3
3	6	0.9	45	27	2.807	3.673	6.3	7877	1.2
4	8	1.1	45	27	1.835	2.405	5.9	6386	1.2
2	10	1.1	35	27	1.888	3.398	12.3	6248	1.2
1	16	0.9	35	27	2.9	5.178	12.6	7669	1.3
12	17	1	48.409	30	2.191	2.698	4.2	6517	1.2
19	4	1	40	30	2.235	3.326	8.2	7258	1.3
16	7	1	40	30	2.234	3.322	8.2	7079	1.3
20	9	1	40	30	2.235	3.318	8.2	7133	1.3

18	12	1	40	30	2.245	3.326	8.2	7083	1.3
17	14	1	40	30	2.235	3.32	8.2	7252	1.3
10	15	1.16818	40	30	1.889	2.804	8.1	6448	1.2
15	18	1	40	30	2.235	3.322	8.2	7050	1.3
9	19	0.831821	40	30	2.739	4.047	8.3	7761	1.3
11	2	1	31.591	30	2.308	5.043	16.9	7041	1.3
7	3	0.9	45	33	2.823	3.686	6	6152	1.2
8	13	1.1	45	33	1.819	2.389	5.8	6490	1.2
5	1	0.9	35	33	2.888	5.176	12.7	6819	1.3
6	11	1.1	35	33	1.872	3.382	12.1	6570	1.2
14	5	1	40	35.0454	1.913	2.82	8.2	6421	1.2

Table 3: Box-Behnken experimental design matrix with response

Response	Name	Units	Observant	Minimum	Maximum	Mean	Std. Dev.	Ratio	Model
R1	RT1	min	20	1.819	2.9	2.30	0.3818	1.59	Linear
R2		min	20	2.389	5.178	3.53	0.8227	2.17	Linear
R3	RS	num	20	4.2	16.9	8.86	3.03	4.02	Quadratic
R4	NTP	num	20	6152	7877	6943.10	527.08	1.28	Quadratic
R5	TF	num	20	1.2	1.3	1.26	0.0510	1.08	Quadratic

Table 4: Factor

Response	**Type of Model**	**R- Square**	**Model P- Value**	**% CV**	**Adequate precision**
RT1	Linear	0.8381	< 0.0001	7.28	18.9490
RT2	Linear	0.8630	< 0.0001	9.39	17.8710
RS	Quadratic	0.9932	< 0.0001	3.89	18.9490

NTP	Quadratic	0.9502	< 0.0001	2.34	47.5322
TF	Quadratic	0.9067	0.005	1.71	8.7355

Table 5: Anova for CCD

In order to understand the results, contour plots and 3D plot were generated after processing all data using the Design Expert® software. It shows the two- d i m e n s i o n a l contour plot on account of Column temperature, Flow rate and organic ratio. Based on the color code, the working region can be easily identified. Retention time maps represent the value of the retention time, with warm "red" colors indicating larger retention time, cold "blue" colors lower and light green to yellow color represent intermediate retention time.

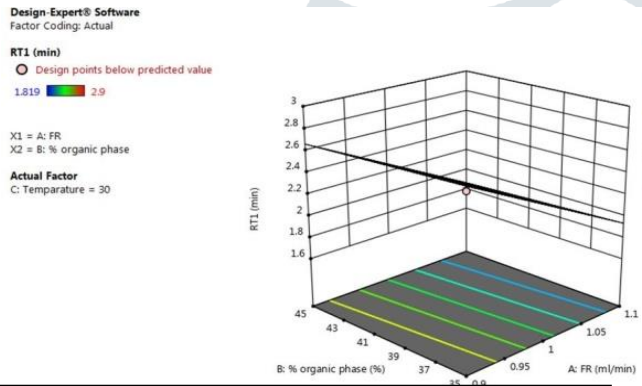

Figure 4: D contour plot of retention time 1 as a function of organic ratio

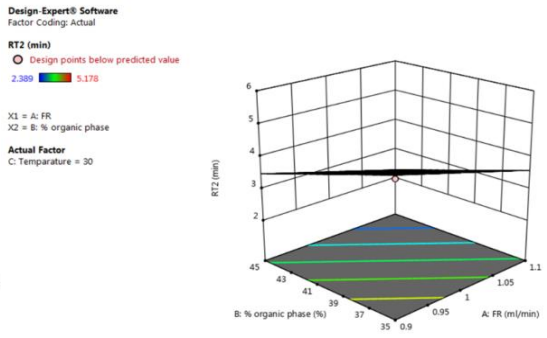

Figure 5: D contour plot of retention time 2 as a function of organic ratio

Table 6: Response

	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	FR	ml/min	Numeric	0.8318	1.17	-1 ↔ 0.90	+1 ↔ 1.10	1.0000	0.0848
B	% Organic phase	%	Numeric	31.59	48.41	-1 ↔ 35.00	+1 ↔ 45.00	40.00	4.24
C	Temperature	0 C	Numeric	24.95	35.05	-1 ↔ 27.00	+1 ↔ 33.00	30.00	2.54

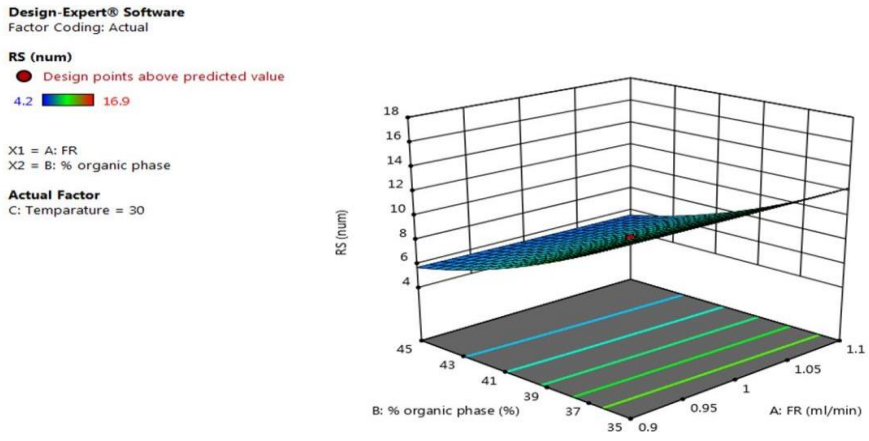

Figure 6: D contour plot of resolution as a function of organic ratio.

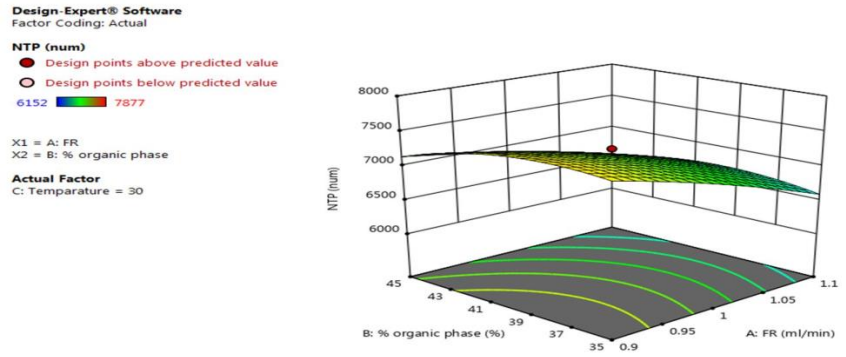

Figure 7: 3D contour plot of NTP as a function of organic ratio

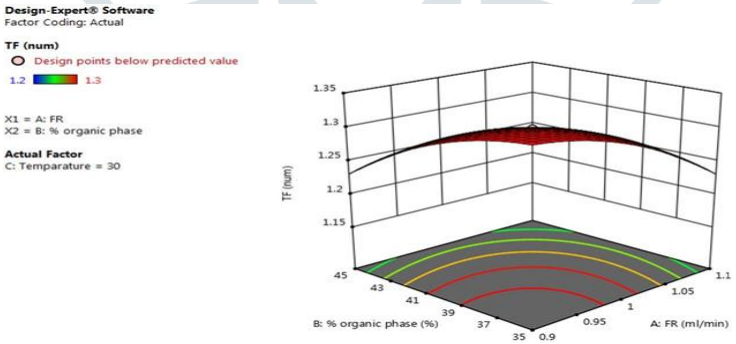

Figure 8: D contour plots of asymmetry as a function of organic ratio

Property	Value
Mobile phase	0.01N KH_2PO_4 (55.2%): Acetonitrile (44.8%)
Flow Rate	1 ml/min
Column Temp.	30 ^{0}C

Table 5: Final developed and optimized HPLC method parameter

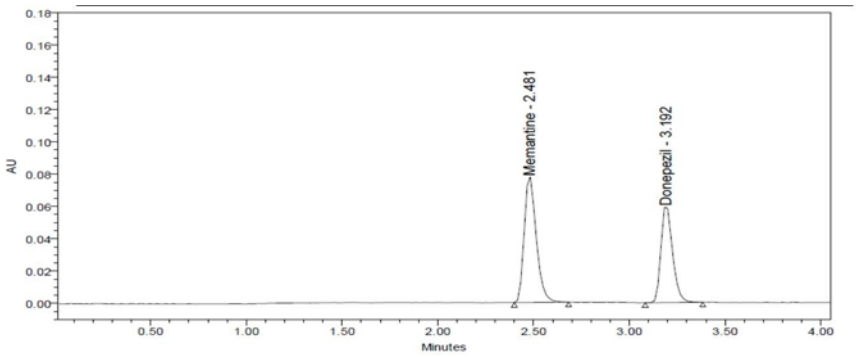

Figure 9: Chromatogram of final optimized method

Memantine and Donepezil were eluted at 2.481 min and 3.192 min respectively with good resolution. Plate count and tailing factor was very satisfactory.

Method validation

1. **SPECIFICITY:**

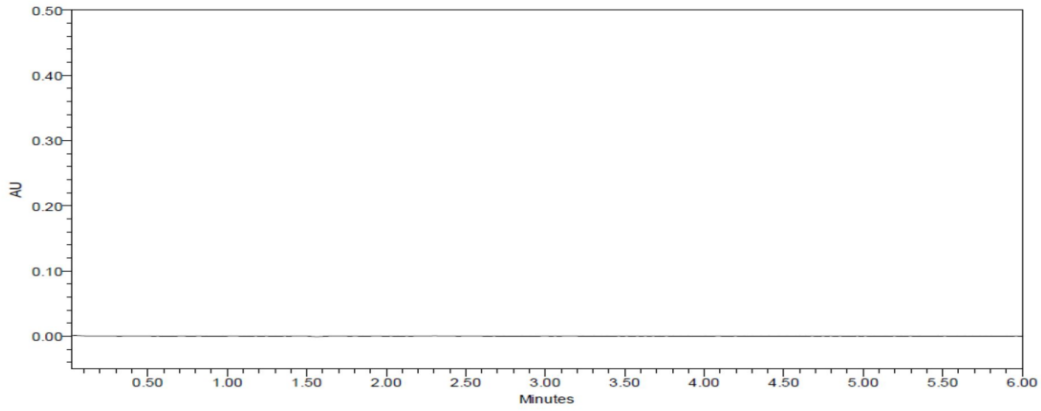

Figure 10: Blank Chromatogram

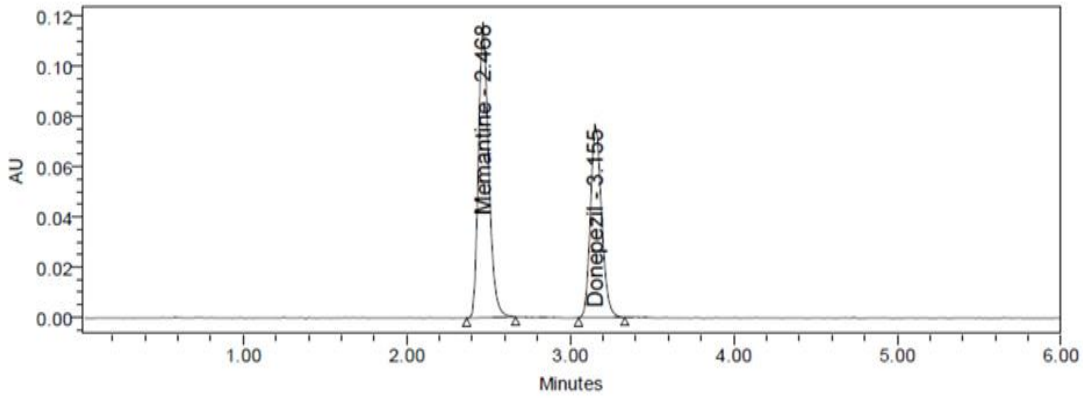

Figure 11: Sample Chromatogram

Retention times of Memantine and revealed to be 2.468 min and 3. 155 min. At the retention durations of these medications using this approach, we did not detect any interfering peaks in the blank or placebo samples.

PRECISION:

Repeatability: Six Standard solutions of 14 µg/ml of Memantine and 10 µg/ml of donepezil are injected and the % amount found was calculated and % RSD was revealed to be 0.3 and 0.7 respectively.

Sr. No.	Memantine Peak Area	Donepezil Peak Area
1	509146	370964
2	512283	368731
3	509080	369163
4	511817	364964
5	514960	366130
6	513795	365079
AVG	511847	367505
S.D	2392.8	2467.8
% RSD	0.5	0.7

Table 6: Repeatability data

Reproducibility: Six sample solutions of 14µg/ml and 10µg/ml of Memantine and donepezil are injected and the % amount wasrevealed to be calculated and %RSD was revealed to be 0.8 and 0.9 respectively.

Sr. No.	Memantine Peak Area	Donepezil Peak Area
1	511084	365052
2	506540	370104
3	519188	368583
4	512147	365061
5	509207	361285
6	509606	368067

AVG	511295	366359
S. D	4310.3	3194.6
%RSD	0.8	0.9

Table 7: Reproducibility data

Intermediate precision: Five working sample solutions of 14µg/ml and 10µg/ml are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.6 and 1.1.

Sr. No.	Memantine Peak Area	Donepezil Peak Area
1	507356	366532
2	505606	368212
3	512444	364691
4	510135	359210
5	514226	359015
6	511408	362076
AVG	510196	363289
S.D.	3221.8	3823.5
%RSD	0.6	1.1

Table 8: Intermediate precision data

3. LINEARITY:

Sr.no	Concentration Memantine (µg/ml)	Response	Concentration Donepezil (µg/ml)	Response
1	0	0	0	0
2	3.5	134422	2.5	92406
3	7	265133	5	196568
4	10.5	394740	7.5	285877
5	14	518311	10	371998
6	17.5	641401	12.5	464967
7	21	766562	15	557040

Table 9: Linearity Concentration and Response

Six linear concentration of Memantine (3.5ppm-21ppm) and Donepezil (2.5ppm to 15 ppm) were injected. Linearity equations for Memantine and Donepezil found to be, y = 36396x + 6493.1 and y = 37024x + 3585.7. Regression co-efficient value was 0.999 for both Memantine and Donepezil.

4. ACCURACY:

Three different concentrations: 50%, 100%, and 150% are Injected in a triplicate manner and % recovery was calculated as 99.29% and 100.05% for memantine and Donepezil respectively.

% Level	Amount Spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean %Recovery
50%	7	6.892266	98.46	99.29%
	7	6.919549	98.85	
	7	6.956449	99.38	
100%	14	13.95595	99.69	99.29%
	14	13.82171	98.73	
	14	13.91647	99.40	
150%	21	20.95112	99.77	99.29%
	21	20.83877	99.23	
	21	21.03085	100.15	

Table 10: Accuracy data of Memantine

% Level	AmountSpiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.959089	99.18	100.05%
	5	4.992959	99.86	
	5	4.990042	99.80	
100%	10	9.950203	99.50	100.05%
	10	10.00584	100.06	
	10	9.909823	99.10	
150%	15	15.07923	100.53	100.05%
	15	15.12833	100.86	
	15	15.24158	101.61	

Table 11: Accuracy data of Donepezil

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner. %Recovery was calculated and it was revealed to be within limits.

5. LOD and LOQ:

Parameters	Memantine	Donepezil
LOD	0.23µg/ml	0.08µg/ml
LOQ	0.69µg/ml	0.25µg/ml

Table 14: LOD and LOQ values for memantine HCl

This method's detection limits for memantine and donepezil were determined to be 0.23 g/ml and 0.08 g/ml, respectively. Quantitation limit of the Memantine and Donepezil were determined to be 0.69 g/ml and 0.25 g/ml, respectively, in this method.

6. ROBUSTNESS : Small, Systematic variations in the method parameters are made such as Flow rate and Mobile phase composition, Temperature and %RSD calculated.

Sr. No	Parameter		% RSD of Memantine HCl	% RSD of Donepezil HCl
1	Flow rate (ml/min)	0.85	1.4	1.0
		1.05	0.4	1.0
2	Mobile phase (%)	57B:43A	0.6	0.6
		47B:53A	0.7	0.9
3	Temperature (°C)	27	0.3	0.5
		33	0.4	0.8

Table 15: Robustness values for memantine HCl and donepezil

ASSAY OF MARKETED FORMULATION

Separate injections of the sample solution and the standard solution were made into the system; chromatograms were then recorded, and the amount of drug in the sample was determined.

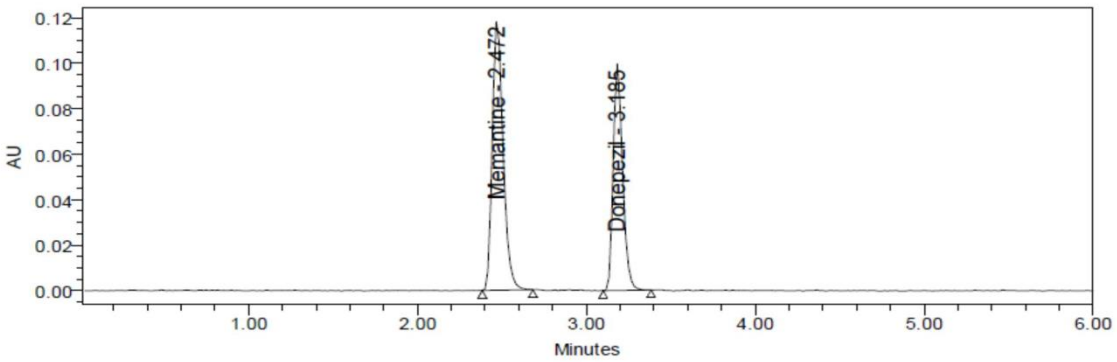

Figure 12: Sample Chromatogram

Sr. No.	Memantine %Assay	Donepezil %Assay
1	99.65	99.13
2	98.77	100.51
3.	101.23	100.09
4.	99.86	99.14
5.	99.29	98.11
6.	99.36	99.95

AVG	99.69	99.49
S. D	0.84	0.868
%RSD	0.84	0.9

Table 12: Assay of Formulation

CONCLUSION

A simple and robust HPLC analytical method was developed for the simultaneous determination of Memantine and donepezil by using Design Expert® software by QbD approach. In comparison to manual method creation, the automated QbD method development methodology using Design Expert software has produced a method that performs better and is more resilient in a shorter amount of time. The results of the statistical analysis show that the established method is reliable, accurate, and economical. The pharmaceutical sector will continue to employ this technology for routine analysis and quality monitoring.

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