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Original Article

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF MEMANTINE HCl AND DONEPEZIL HCl USING RP-HPLC

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ABSTRACT

Objective: To develop and validate stability indicating method for the analysis of Memantine HCl and Donepezil HCl.

Methods: The chromatographic separation was performed on Hypersil BDS (4.6 x 150 mm, 5µ) using Sodium dihydrogen ortho phosphate: Acetonitrile (30:70v/v) at a flow rate of 1 ml/min and detection of both the eluents was carried out by UV Detector.

Results: The Retention time of Memantine HCl and Donepezil HCl were found to be 2.833 min. and 4.777 min respectively. Method was found to be linear over the range of 40-120µg/ml for Memantine HCl and 20-60µg/ml for Donepezil HCl. Percentage recovery of Memantine HCl and Donepezil HCl was found to be 99.62% and 99.45% respectively. The percentage purity thus found is 98.5% and 98.6% for Memantine HCl and Donepezil HCl. The Limit of Detection of Memantine HCl and Donepezil HCl is 3.69 µg/ml and 2.72 µg/ml. and Limit of Quantification of Memantine HCl and Donepezil HCl is 11.13 µg/ml and 8.25 µg/ml respectively. In the stress degradation studies, it was found that Memantine HCl and Donepezil HCl showed no degradation in acid (0.1M HCl), base (0.1M NaOH), peroxide, heat and sunlight.

Conclusion: A new sensitive, simple, and stability indicating high performance layer chromatographic (HPLC) method has been developed and validated for determination of Memantine HCl and Donepezil HCl. The proposed method can be used for routine determination of Memantine HCl and Donepezil HCl stability.

Keywords: Memantine Hydrochloride, Donepezil Hydrochloride, RP-HPLC, UV Detector.

INTRODUCTION

Memantine hydrochloride chemically is 1-amino-3,5-dimethyl-adamantane hydrochloride. It is an anti-Alzheimer's drug and exerts its action through uncompetitive N-Methyl D-Aspartate (NMDA) receptor antagonism, binding preferentially to the NMDA receptor-operated cation channels. Donepezil hydrochloride is chemically (±)-2, 3-dihydro-5, 6-dimethoxy-2-[1-(phenylmethyl)-4-piperidinyl] methyl]-1H-inden-1-onehydrochloride, a piperidine derivative that is a centrally active, reversible inhibitor of acetyl cholinesterase.

Its proposed mechanism of action involves the reversible inhibition of cholinesterase, which prevents the hydrolysis of acetylcholine, and leads to an increased concentration of acetylcholine at cholinergic synapses.

The literature survey revealed that a number of methods being reported for the estimation of Memantine HCl [11-20] and Donepezil HCl [21-32] individually but only two methods have been reported on the combination [33,34]. The present work involves a simple and reliable stability indicating RP-HPLC method for the estimation of Memantine HCl and Donepezil HCl in bulk and pharmaceutical dosage forms.

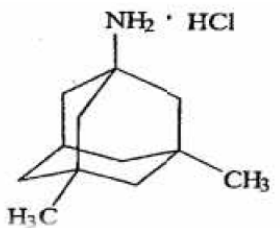


Fig. 1: Chemical structure of Memantine HCl

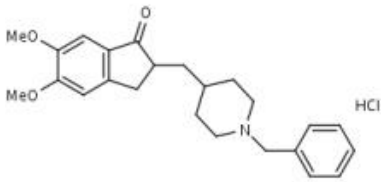


Fig. 2: Chemical structure of Donepezil HCl

MATERIALS AND METHODS

Instrumentation

HPLC (Agilent-1220 infinity series), pump–2690, UV Detector (Nicolet evolution 100), EZ Chrome software, Sonicator & degasser (PCI Analyte), pH meter (Lab India), Hot air oven (Lab India), Nylon filter.

Chemicals and reagents

Memantine HCl and Donepezil Hydrochloride were obtained from the Dr. Reddy Labs, Hyderabad, India, as a gift sample. Combined dose tablet formulation, Donamem 10 (Sun Pharmaceuticals Ltd) containing Memantine HCl 10 mg and Donepezil HCl 5 mg was obtained from local pharmacy store. Sodium dihydrogen phosphate, Ortho Phosphoric acid, Acetonitrile, Triethyl Amine are obtained from commercial sources (S. D. fine chemicals) and all the reagents are of Analytical grade.

Methodology

Preparation of 0.02M Sodium dihydrogen phosphate

3.12 gm of Sodium dihydrogen phosphate (NaH_2PO_4) was accurately weighed and dissolved in 1000 ml water. This prepared buffer was

filtered through the nylon membrane filter having a pore size of 0.45 µm and degassed with the help of a sonicator.

Standard solution of Memantine HCl

Standard stock solution of Memantine HCl was prepared by dissolving 10 mg of drug in 0.02M Sodium dihydrogen phosphate solution to get concentration of 400 µg/ml.

Standard stock solution of Donepezil HCl

Standard stock solution of Donepezil HCl was prepared by dissolving 10 mg of drug in 0.02M Sodium dihydrogen phosphate solution to get concentration of 200 µg/ml.

Selection of analytical wavelength

The standard solution of mixed solution of Memantine HCl & Donepezil HCl in methanol was scanned over wavelength range 200 to 400 nm by using UV-Visible spectrophotometer. Wavelength 271 nm was selected for analysis where the combined drug solution showed higher absorbance.

Chromatographic conditions

Chromatographic separation was carried out on Hypersil BDS (4.6 x 150 mm, 5µ) using mobile phase Sodium dihydrogen ortho phosphate: Acetonitrile (30:70v/v) at a flow rate 1 ml/min for 6 min. Detection of both the eluents was carried out by UV Detector.

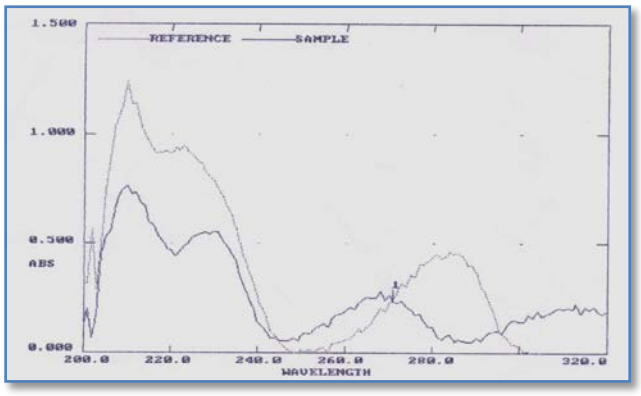


Fig. 3: UV spectrum of mixed drug solution between 200-400 nm

Assay

To carry out the assay of the method, %purity was calculated. 5 injections of standard and sample (formulation) having 100% concentration (80µg/ml of Memantine HCl and 40µg/ml of Donepezil HCl) of drugs were injected simultaneously and the peak area were recorded.

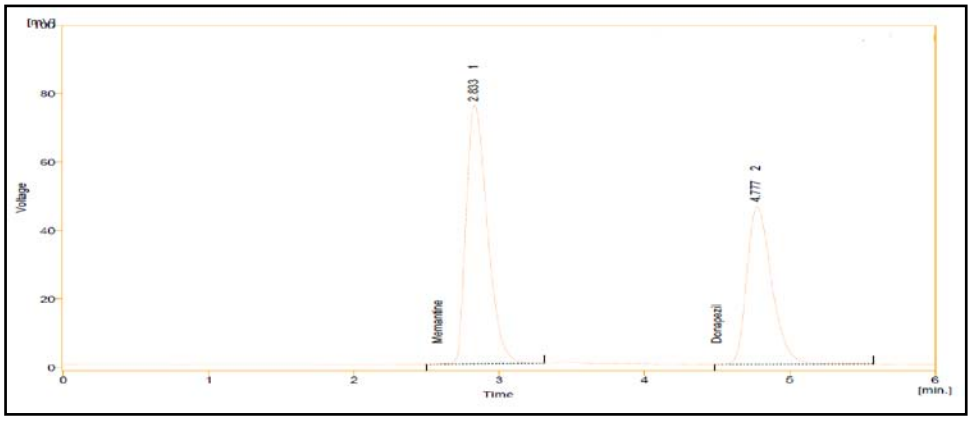


Fig. 4: Chromatogram showing retention time of Memantine HCl and Donepezil HCl

Memantine			Donepezil	
	Standard area	**Sample area**	**Standard area**	**Sample area**
Injection-1	768.820	761.374	546.817	538.641
Injection-2	767.589	767.612	543.171	541.972
Injection-3	764.499	764.499	537.068	537.068
Injection-4	767.339	767.339	542.561	542.561
Injection-5	761.946	761.946	546.817	546.817
Average area	766.0386	764.554	543.2868	541.4118
Tablet average weight	195.6		195.6	
Standard weight	100		50	
Equivalent weight	195.6		195.6	
Label amount	10 mg		5 mg	
std. purity	99.8		99.8	
Amount found in mg	9.85		4.93	
Assay(%purity)	98.5		98.6	

Validation

Working standard solution of Memantine HCl and Donepezil HCl

20 tablets (each tablet contains 5 mg of Donepezil HCl and 10 mg of Memantine HCl) were weighed and taken into a mortar and crushed to fine powder and mixed uniformly. Tablet stock solution was prepared by dissolving powder weight equivalent to 50 mg of Donepezil HCl and 100 mg of Memantine HCl in sufficient mobile phase. The solution was filtered through the nylon membrane filter having a pore size of 0.45 µm and degassed with the help of a sonicator.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample (dosage form). The specificity of the method was

confirmed by comparing the peaks of the sample and standard injected having the same concentration of drugs.

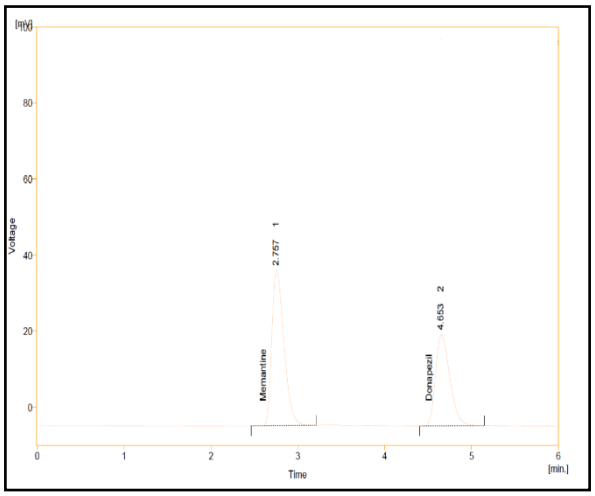
Linearity

Appropriate volumes were injected from standard stock solution containing 40-120 µg/ml of Memantine HCl and 20-60µg/ml of Donepezil HCl respectively. Each standard was analyzed in five replicates, and peak area was noted. The relationship between peak area and concentration was established by the simple regression equation method.

Accuracy

To check accuracy in the method, recovery studies were carried out at three different levels 75%, 100%, 125%. 0.8 ml of working solution was pipette out and dissolved in 10 ml mobile phase which gave a 100% solution.

Similarly 0.6 ml and 1.0 ml of working solution were pipette out and dissolved in 10 ml mobile phase to give 75% and 125% solutions respectively. The solutions were prepared in triplicates and the accuracy was indicated by the percentage recovery.



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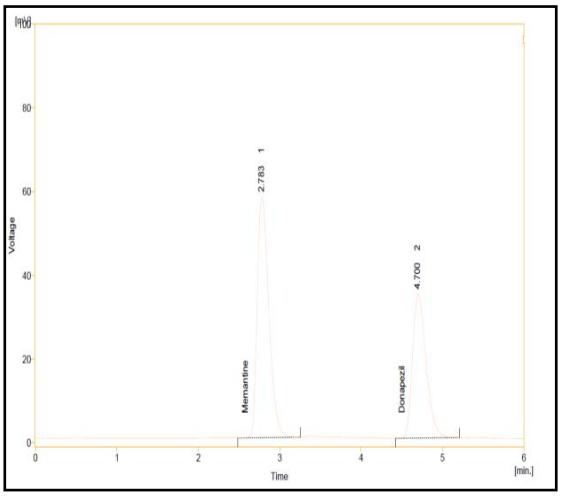

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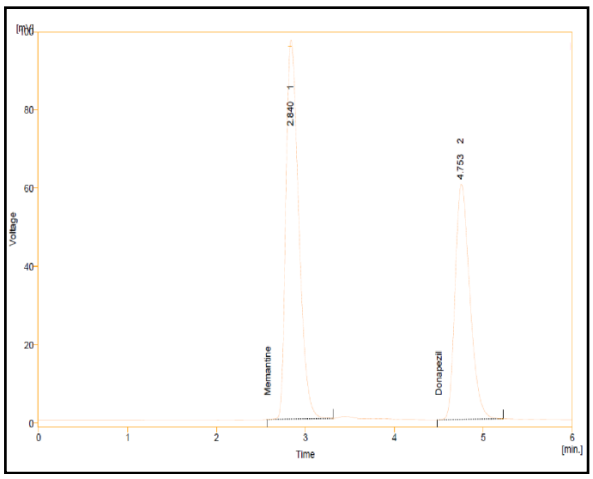

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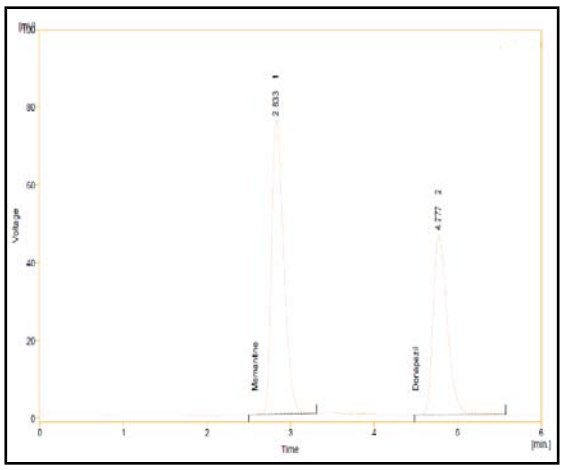

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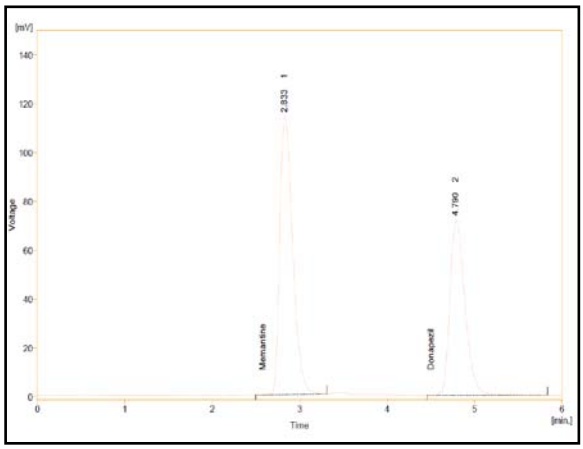

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Fig. 5: (a) (b) (c) (d) (e)–Chromatograms of Linearity

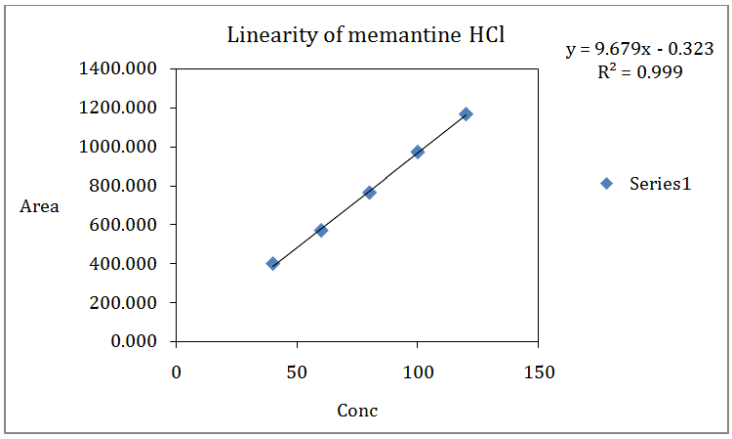

Fig. 6: Calibration curve of memantine HCl

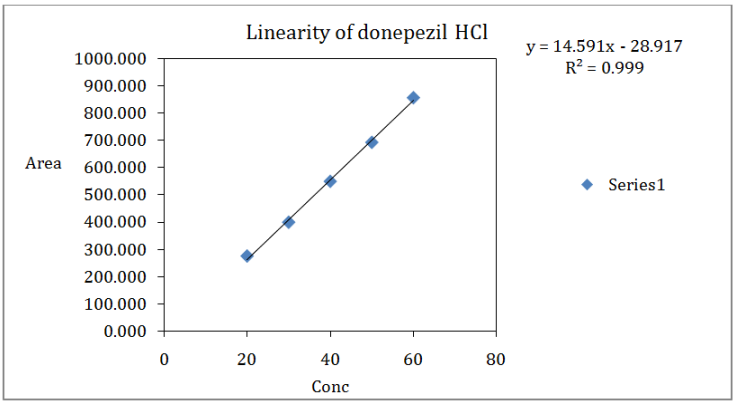

Fig. 7: Calibration curve of Donepezil HCl

Table 1: Linearity data for Memantine HCl and Donepezil HCl

Regression Analysis	Memantine HCl	Donepezil HCl
Regression equation	y = 9.6796x-0.323	y = 14.591x-28.917
Correlation co-efficient	0.9991	0.999
Slope	9.6796	14.591
Intercept	0.323	28.917

Table 2: Results for method precision of Memantine HCl and Donepezil HCl

	Memantine HCl	Donepezil HCl
Avg. Rt	2.7500	4.6393
Standard Deviation	0.0079	0.0113
%RSD	0.29	0.24

*Average of 6 injections

Table 3: Recovery data

Conc.	Amt of sample (µg/ml)		Amt. found (µg/ml)		% Recovery	
	Memantine HCl	Donepezil HCl	Memantine HCl	Donepezil HCl	Memantine HCl	Donepezil HCl
75%	60	30	59.91	29.91	99.84	99.71
100%	80	40	79.32	39.78	99.15	99.46
125%	100	50	99.89	49.59	99.89	99.18

* Average of 3 injections each

Precision

It was assessed by injecting six injections of a homogenous sample containing 80µg/ml of Memantine HCl and 40µg/ml of Donepezil HCl and % RSD was calculated.

Robustness

The robustness of the method was studied by small but deliberate variations from the optimized conditions in wavelength (±2 nm) and in Flow rate (±0.2 ml/min). One factor at a time was changed at a

concentration level of 80µg/ml of Memantine HCl and 40µg/ml of Donepezil HCl and the peak area obtained with each solution was measured and % R. S. D was calculated.

Ruggedness

Different Analysts performed the experiment keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded.

Limit of Detection and Limit of Quantification (LOD & LOQ)

The LOD and LOQ were estimated from the calibration curve. The LOD may be calculated as LOD = 3.3 × (SD/Slope)

The LOQ may be calculated as LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of the Y-intercept of the calibration curve.

Slope = Mean slope of the calibration curve.

Table 4: Robustness data

Condition		Memantine HCl (Tailing factor)	Donepezil HCl (Tailing factor)
Wavelength	269 nm	1.758	1.537
	273 nm	1.727	1.585
Flow rate	0.8 ml/min	1.773	1.630
	1.2 ml/min	1.714	1.556

Table 5: Ruggedness data

Memantine HCl	%Assay	Donepezil HCl	%Assay
Analyst1	99.36	Analyst1	99.67
Analyst2	98.67	Analyst2	98.45
%RSD	0.4%	%RSD	0.8%

Stress degradation studies

Stress degradation studies were carried under a condition of acid/base/neutral hydrolysis, oxidation, dry heat and photolysis. For each study, samples were prepared, the blank subjected to stress in the same manner for the drug solution, working standard solution of Memantine HCl & Donepezil HCl subjected to stress degradation. Dry heat and photolytic degradation were carried out in a solid state.

Acid degradation

One ml working standard solution was mixed with one ml of 0.1 N HCL and the solution was kept in the water bath at 60 °C for 1 hr. Then the solution was allowed to cool at room temperature. The solution was filtered using 0.45-micron syringe filter and sonicated for 5 min and diluted to 100 ml with the mobile phase. Further dilutions were made to prepare 40µg/ml of Donepezil HCl and 80µg/ml of Memantine HCl by adding 0.8 ml of the above solution to 10 ml of the mobile phase.

Base degradation

One ml working standard solution was mixed with one ml of 0.1N NaOH and the solution was kept in the water bath at 60 °C for 1 hr. Then the solution was allowed to cool at room temperature. The solution was filtered using 0.45-micron syringe filter and sonicated for 5 min and diluted to 100 ml with the mobile phase. Further dilutions were made to prepare 40µg/ml of Donepezil HCl and 80µg/ml of Memantine HCl by adding 0.8 ml of the above solution to 10 ml of mobile phase.

Oxidative degradation

One ml working standard solution was mixed with one ml of 1.0% H_2O_2 and the solution was kept in water bath at 60 °C for 1 hr. Then the solution was allowed to cool at room temperature. The solution was filtered using 0.45-micron syringe filter and sonicated for 5 min and diluted to 100 ml with mobile phase. Further dilutions were made to prepare 40µg/ml of Donepezil HCl and 80µg/ml of Memantine HCl by adding 0.8 ml of the above solution to 10 ml of mobile phase.

Dry Heat degradation

Sample was exposed at 105 °C in an oven for 1 hour.

Sample was withdrawn, dissolved in mobile phase and diluted to get 40µg/ml of Donepezil HCl and 80µg/ml of Memantine HCl by adding 0.8 ml of the above solution to 10 ml of mobile phase.

Photolytic degradation

Sample was exposed in sunlight for 24 h.

Sample was withdrawn, dissolved in mobile phase and diluted to get 40µg/ml of Donepezil HCl and 80µg/ml of Memantine HCl by adding 0.8 ml of the above solution to 10 ml of mobile phase.

Table 6: Results for Degradation studies of Memantine HCl

Conditions	Sample weight(mg)	Peak area	% claim	% Degradation
Sample Control	187.6	768.82	98.49	-
Acid Degradation	189.98	767.589	98.38	0.19
Alkali Degradation	189.56	756.523	97.19	1.3
Thermal Degradation	188.97	757.337	97.59	0.9
Peroxide Degradation	189.64	768.820	97.64	0.85
UV Degradation	189.32	763.374	98.19	0.3

Table 7: Results for Degradation studies of Donepezil HCl

Conditions	Sample weight(mg)	Peak Area	% claim	% Degradation
Sample Control	187.6	548.815	98.63	-
Acid Degradation	187.29	543.171	98.93	1.7
Alkali Degradation	187.23	523.198	95.67	2.96
Thermal Degradation	189.32	546	96.74	1.89
Peroxide Degradation	186.97	546.817	97.77	0.86
UV Degradation	186.89	533.408	97.36	1.27

RESULTS AND DISCUSSION

Optimization of mobile phase

Method development for Memantine HCl & Donepezil HCl was started with the development of chromatograms using neat solvents and combinations of Methanol, Sodium dihydrogen phosphate, Acetonitrile in different ratios. Sodium dihydrogen phosphate: Acetonitrile in the ratio of (30:70 v/v) was selected as the mobile phase for Memantine HCl & Donepezil HCl, which resulted in acceptable peak parameters. The retention time of Memantine HCl and Donepezil HCl was found to be 2.833 min. and 4.777 min respectively.

Method validation

The method validation results were satisfactory as per ICH Q_2R_1 guidelines. The peak areas were found to be linear over the concentration range 40-120µg/ml for Memantine HCl and 20-60µg/ml for Donepezil HCl with a correlation co-efficient of 0.9991 and 0.999 respectively. Method precision was less than 2%. Percent recovery in accuracy study was within the limit of 98 to 102%. The percentage purity thus found is 98.8% and 98.6% for Memantine HCl and Donepezil HCl. Thus, the optimized HPLC method for Memantine HCl & Donepezil HCl is based on use of simple ternary mixture of organic solvents. The method is found to be sensitive with LOD 3.69 µg/ml and 2.72 µg/ml and LOQ of Memantine HCl and Donepezil HCl is 11.13 µg/ml and 8.25 µg/ml respectively.

All the validated parameters are given in table 8.

Stress degradation

Drug was subjected to various forced degradation conditions. The conditions of stress were optimized with respect to the strength of the reagent and exposure period so as to achieve degradation in the range of 1 to 15%. There was no peak of degradation found in any of the degradation process.

Summary of stress degradation results is given in table 6 and 7.

The advantage of this method from the previous methods proposed for the drugs is that it gives a lesser retention time and also the detection method used is economical and easily available.

Table 8: Summary of the validation parameters of proposed method

S. No.	Parameter	Memantine HCl	Donepezil HCl
01	Linearity Range	40-120µg/ml	20-60 µg/ml
02	Regression equation	y = 9.6796x-0.323	y = 14.591x-28.917
03	Correlation co-efficient	0.9991	0.999
04	Specificity	No interference at the RT	No interference at the RT
05	Precision(% RSD)	0.29	0.24
06	Accuracy (% recovery)	99.62%	99.45%
07	Assay(%Purity)	98.8%	98.6%
08	Robustness	Tailing<2	Tailing<2
09	Ruggedness	Tailing<2	Tailing<2
10	Limit of Detection	3.69µg/ml	2.72µg/ml
11	Limit of Quantification	11.13µg/ml	8.25µg/ml

CONCLUSION

The developed RP-HPLC method provides simple, specific, precise, accurate, economical and reproducible quantitative analysis for simultaneous determination of Memantine HCl and Donepezil HCl 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 Memantine HCl and Donepezil HCl in combined dosage form.

CONFLICT OF INTERESTS

Declared None

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