STABILITY INDICATING AND COMPARATIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF MEMANTINE HCl BY RP-HPLC AND UV SPECTROPHOTOMETRY

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ABSTRACT

Memantine HCl is an N-methyl-D-aspartate receptor antagonist used for the management of Alzheimer's disease. The present study deals with comparative development and validation of stability-indicating high-performance liquid chromatography and Ultra-violet spectrophotometric method for the quantitative determination of Memantine HCl without any prior derivatization. The separation in HPLC was carried on the C-18 column and a mobile phase of acetonitrile and phosphate buffer pH 3.2 in the ratio of 50:50%v/v with 0.8mL/min flow rate. The wavelength at 190nm shows the highest response in the UV spectrophotometric and HPLC with UV detection. The regression coefficient of the calibration curve of HPLC and UV method is 0.9934 and 0.995 respectively. The limit of detection and limit of quantification of the HPLC method are 0.871 and 2.639 µg/mL respectively and 0.818 and 2.639µg/mL respectively for the UV method. The percentage of purity was found to be 98.8% and 97.9% in HPLC and UV methods. There is no degradation peak observed for the degradant products indicating the stability of the memantine HCl under test. A rapid, simple, accurate, and precise stability-indicating HPLC and UV method is developed and validated, and compared for the determination of memantine HCl. The developed method can be used for routine analysis of memantine HCl.

Keywords: Memantine HCl, Alzheimer, HPLC, UV Spectrophotometry, Degradation Studies, Validation

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INTRODUCTION

Memantine HCl (1-amino-3,5- dimethyl adamantane)¹ as shown in Fig.-1 is a primary aliphatic amine and a member of adamantanes.² It's a non-competitive NMDA (N-methyl-D-aspartate) receptor antagonist³ used to manage moderate to severe Alzheimer' disease.⁴⁻⁶ The mechanism of the action differs from the acetylcholine esterase inhibitors medication that is usually used to treat Alzheimer's disease.⁷ It binds preferably to the NMDA receptor-operated cation channels⁸ and blocks the activation of the receptor by glutamate,⁹ a neurotransmitter that may lead to neuron excitability and excessive stimulation in Alzheimer's disease. It is also used as a neuroprotective agent, anti-depressant, and antiparkinson¹⁰ drug due to dopaminergic agent. It does not completely cure the disease enhances memory, awareness, and the ability to perform daily activities. After the acetylcholinesterase inhibitors such as donepezil, it is the most preferred drug for the management of Alzheimer's. It was analyzed by various methods like HPLC with UV detection¹¹, GC-FID¹², Mass spectroscopy¹³, LC/MS/MS.¹⁴ There are only a few spectrophotometric methods are reported as per the literature survey.¹¹

The Memantine HCl lacks chromophores^{16,17} in its chemical structure, thus showing poor absorbance in UV-VIS spectroscopy¹⁸, therefore many existing research papers had performed derivatization to impart UV activity to the memantine.¹⁹ However, some data indicate that memantine contains chromophores that absorb at a wavelength less than 290nm. The present study describes a simple, rapid, inexpensive, and derivatization-free UV-VIS spectrophotometric and HPLC method for analysis of Memantine HCl.

Fig.-1: Chemical Structure of Memantine HCl

EXPERIMENTAL

Instrumentation and Reagents

The HPLC analysis was performed out on the Shimadzu system consisting of a quaternary pump, autosampler, SPD-20A-UV detector, and LC-20AD prominence software. The chromatographic condition of the RP-HPLC is given in Table-1.

Table-1: Chromatographic Conditions of HPLC for estimation of Memantine HCl

S. No.	Parameters	Condition
1	Column	Luna Phenomenex C ₁₈
2	Mobile phase	Acetonitrile: sodium phosphate buffer (pH- 3.2) in ratio 50:50 (v/v)
3	Oven temperature	Ambient
4	Detector	UV
5	Wavelength	190nm
6	Flow rate	0.8mL/min
7	Injection volume	20μl
8	Retention time	2.6 min

UV-Visible spectrophotometer empowered with Vision Pro software was used to determine the absorption maxima of the memantine drug. Millipore water was used as a solvent for dissolving the drug. Memantine hydrochloride (Admenta) tablet formulation was purchased from the community pharmacy of our college.

Preparation of Standard Solution

100 mg of pure memantine HCl was weighed accurately into a 100 mL standard volumetric flask that contains distilled water. Again 10 mL was pipette out and diluted to 100 mL with distilled water. From that solution, serial dilutions in the range of 25-125 ug/mL concentration were prepared with the same solvent.

Preparation of Sample Solution

The average weight of 20 tablets is calculated and the tablets were crushed into a fine powder with the help of a motor and pestle. An accurately weighed amount of powder equivalent to 10 mg of Memantine drug was taken and dissolved in a 100 mL volumetric flask containing water. From the stock solution, the required dilutions were done to get the final concentration of 100 μg/mL.

Validation

Linearity

The linearity is the ability to indicate that the response of the test is proportional to the linear concentration of the analyte in the sample in a given range. Linearity was performed as per the ICH Q2 (R1) between the concentration range of 25 to 125ug/mL as shown in Table-2. A linear response is observed in both HPLC and UV methods as presented in Figs.-2 and 3 respectively.

Table-2: Linearity Data for Memantine HCl in HPLC and UV

Conc (μg/mL)	Peak area	Absorbance
5	5516	-
25	48038	0.201

50	160199	0.312
75	240368	0.459
100	301548	0.599
125	404229	0.735

Fig.-2: Linearity Graph of Memantine HCl in HPLC

Fig.-3: Linearity Graph of Memantine HCl in UV Spectrophotometry

Precision

Precision on an analytical method is the degree of closeness among the measured value obtained when the method is applied to multiple repeated sampling of the same sample. The precision of the proposed method was performed by analyzing 6 samples of 75 ug/mL concentrations for intra-day and inter-day precision as presented in Table-3.

Table-3: Intra-day and Inter-day Precision Studies of Memantine HCl

Conc (ug/mL)	HPLC		UV	
	Intra-day	Inter day	Intra-day	Inter day
75	248487	254730	0.475	0.413
75	248487	252720	0.471	0.419
75	250662	251197	0.473	0.415
75	249956	253288	0.474	0.414
75	249816	249574	0.472	0.416
75	249936	255479	0.473	0.4154
Avg	249557.3	252831.3	0.001414	0.0023022
SD	880.9935	2196.237	0.298988	0.5542063
RSD	0.353022	0.868657	0.475	0.413

Accuracy

The accuracy of the method is the closeness of the measured value to the actual value. The accuracy of the proposed method is assessed by the percentage recovery of three different levels of 50%, 100%, and 150%. Three different concentrations of a standard solution of 25, 50, and 75 µg/mL were spiked in each 5 µg/mL sample memantine solution, and the response of each spiked solution is measured in HPLC and UV spectroscopy. Table-4 shows that the mean percentage recovery at each level in both HPLC and UV is close to 100 which indicates the proposed method has adequate accuracy.

Table-4: Accuracy Studies of Memantine HCl in HPLC and UV Method

Recovery level %	Conc. sample µg/mL	Conc. standard µg/mL	%Recovery (HPLC)	%Recovery (UV)
50	5	25	99.02365	99.00498
	5	25		100.995
	5	25	99.21855	97.9602
			100.2611	
Mean	-	-	99.5011	99.32007
100	5	50	97.92993	100.3205
	5	50		100.641
	5	50	98.29599	99.67949
			98.30085	
Mean	-	-	98.17559	100.2137
150	5	75		98.03922
	5	75	98.5617	98.69281
	5	75	97.82685	103.0501
			98.7444	
Mean	-	-	98.37765	99.92738

Robustness

Robustness is the ability of the method to remain steady to small intended alteration in the method parameters. The robustness of the proposed method is assessed by the small deliberate change in the method parameters such as the wavelength and flow rate. Table-5 shows the robustness studies performed by the change in flow rate and wavelength in HPLC and UV methods.

Table-5: Robustness Studies of Memantine HCl in HPLC and UV Method

Conc (µg/mL)	Response at λ _{max} 192nm		Peak area at Flow Rate	
	Peak area	Absorbance	0.7mL/min	0.9mL/min
75	211954	0.413	239512	186363
75	215781	0.419	242177	188437
75	215508	0.415	239948	185856
Avg	214414.3	0.414	240545.7	186885.3
SD	2135.079	0.416	1429.497	1367.485
%RSD	0.995773	0.4154	0.594272	0.731724

Ruggedness

The ruggedness of the analytical method is the measure of reproducibility of the test result achieved by variation in the condition such as different laboratories, different analysts, different instruments, etc. Ruggedness studies by the different analysts are shown in Table-6.

Table-6: Ruggedness Studies of Memantine HCL by the Different Analysts in HPLC and UV Method

Conc. (µg/mL)	Peak area		Absorbance	
	1 st analyst	2 nd analyst	1 st analyst	2 nd analyst
75	247387	249730	0.473	0.474

75	249487	252632	0.47	0.471
75	251662	249197	0.469	0.473
75	248956	252288	0.472	0.474
75	250816	249574	0.474	0.472
75	252936	252672	0.472	0.469
Avg	250207.3	251015.5	0.471667	0.472167
SD	1998.557	1674.138	0.001862	0.001941
RSD	0.79876	0.666946	0.394749	0.411039

LOD and LOQ

The limit of detection and limit of quantification is the lowest amount of the analyte in the sample that can be detected or quantified. The LOD and LOQ of the proposed method in chromatography and UV spectroscopy are calculated and presented in Table-7.

Table-7: LOD and LOQ of Memantine HCl in HPLC and UV Method

	HPLC	UV
LOD ($\mu\text{g/mL}$)	0.871	0.818
LOQ ($\mu\text{g/mL}$)	2.639	2.480

Stress Degradation Studies

Stress degradation studies are conducted to assess the intrinsic stability of the molecule by exerting forced degradation of the analyte under different stress conditions such as acidic, basic, oxidation, dry heat, light, and pH. For each study, both control and sample solution containing the analyte is subjected to the same stress conditions.

Acid Degradation

The acid degradation is carried out by adding 1mL 0.1N HCl to the 1mL of working standard solution and heated on a water bath at 60°C for 1 hour. The sample solution is then cooled to room temperature and neutralized by adding 1mL 0.1N NaOH and diluted to obtain 100 $\mu\text{g/mL}$. The solution is filtered using a 0.45-micron syringe filter and injected into the HPLC column.

Basic Degradation

1mL of 0.1N NaOH is added to the 1 mL of working standard solutions and heated on a water bath at 60°C for 1 hour. The solution is then cooled at room temperature and neutralized by adding 1mL of 0.1N HCl followed by dilution using water to get 100 $\mu\text{g/mL}$. The solution is filtered using a 0.45-micron syringe filter and injected into the HPLC column.

Oxidative Degradation

1mL of the 3% H_2O_2 is mixed with 1mL of working standard solution and heated on a water bath at 60°C for 1 hour. The solution is then cooled at room temperature and then diluted to obtain 100 $\mu\text{g/mL}$. The final resulting solution is filtered using a 0.45-micron syringe filter and injected into the HPLC column.

Thermal Degradation

1mL of the working standard solution is kept in the oven at 105°C for 1 hour. The solution is then cooled at room temperature and diluted to get the 100 $\mu\text{g/mL}$ and analyzed in HPLC.

Photolytic Degradation

1mL of the working standard solution is exposed under direct sunlight for 24 hours. The solution is made up using the same solvent to get a final concentration of 100 $\mu\text{g/mL}$ which is analyzed in the HPLC.

RESULTS AND DISCUSSION

The various chromatographic parameters such as flow rate, oven temperature, wavelength, the composition of the mobile phase ratio are optimized to get a high-resolution chromatogram. The composition of the mobile phase consisting of acetonitrile and sodium phosphate was altered with various ratios to obtain the

final ratio as 50:50% v/v and the pH of the buffer 3.2 was found to be the optimum for the chromatographic separation. The response of the analyte in the sample is highest at the wavelength of 190nm and ambient temperature. The variation in flow rate is carried and the 0.8mL/min flow rate is found to be the optimum to provide a sharp peak with less retention time anticipating overall short run time (2.6 min). The overlay chromatogram of standard memantine HCl and memantine tablet (Admenta 10) is shown in Figs. 4 and 5 respectively.

Fig.-4: Overlay Chromatogram of the Memantine HCl Standard Solutions obtained from HPLC

Fig.-5: Chromatogram of Memantine HCl tablet (Admenta 10)

The analysis of an analyte in a sample in UV spectroscopy is optimized by scanning the sample in the UV-Visible spectrophotometer in the spectrum range of 190 to 800nm. The maximum wavelength ($\lambda_{\max}$) was found to be 190nm as shown in Fig.-6.

Fig.-6: Overlay Spectrum of the Memantine HCl Standard Solutions in UV Spectrophotometry

Analytical Method Validation

The validation of the proposed method is found to be satisfactory according to ICH guidelines. The linearity is observed between the different concentration ranges and the response obtained from both HPLC and UV methods. The regression data obtained from the linearity curve is presented in Table-8 and the 3D calibration graph of linearity studies in HPLC is shown in Fig.-7. The precision studies show the percentage RSD < 2 in both intra-day and inter-day studies of HPLC and UV. The proposed HPLC and UV method shows adequate accuracy with the mean % recovery between 98% to 102%. The LOD, LOQ precision, and accuracy studies data of the HPLC and UV method is presented in Table-9.

Fig-7: 3D Calibration Graph for Memantine HCl

Table-8: Regression Data Obtained from the Calibration Curve in HPLC and UV Method

Regression Parameter	HPLC	UV
Linearity Range (ug/mL)	5-125	25-125
Number of Points	6	5
Regression Equation	$y=3337.7x-18070$	$y = 0.0057x + 0.026$
Regression Coefficient (R^2)	0.9934	0.9952
Slope	3337.7	0.0057
Intercept	18070	0.026

Table-9: Result of Validation Parameters in HPLC and UV Method

Parameter	HPLC	UV
Intraday Precision	0.353022	0.475
Inter-day Precision	0.353022	0.413
Accuracy	98.68478	99.82037
LOD (ug/mL)	0.871042	0.81863
LOQ (ug/mL)	2.639523	2.48070

Assay of Memantine HCl

The validated chromatographic and spectrophotometric methods were used to analyze the percentage purity of the memantine tablet (Admenta 10). The percentage purity of memantine tablets obtained from HPLC and UV spectrophotometry is presented in Table-10.

Stress Degradation

The drug substance is exposed to various stress conditions to deliberately degrade the drug molecule. The concentration of the reagent, conditions, and time are varied to optimize the degradation. There is no

degradation peak found in any degradant products which is indicative of the stability of the molecule under test. The summary of forced degradation studies of Memantine HCl is shown in Table-11.

Table-10: Percentage Purity of Memantine HCl obtained from the HPLC and UV Method (n=3)

Sample	Memantine Content (% Purity)	
	HPLC	UV
Admenta 10	98.8%	97.9%

Table-11: Forced Degradation Studies of Memantine HCl

Conditions	Peak Area	% Degradation
Acidic degradation	190319	0.36
Basic degradation	203269	0.32
Oxidative degradation	163210	0.45
Thermal degradation	221487	0.26
Photolysis	268065	0.11

CONCLUSION

The developed method provides an accurate, precise, robust, reproducible quantitative analysis of Memantine HCl tablet in HPLC and UV spectrophotometry. The proposed method is validated according to ICH guidelines and can be used for routine analysis of memantine HCl in any dosage form.

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