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Development and Validation of A Reverse Phase HPLC Method for Determination of Memantine In Pure and Pharmaceutical Formulations

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ABSTRACT

A simple, accurate and precise HPLC method for the estimation of memantine in bulk and pharmaceutical dosage form has been reported. Chromatography was performed with Shimadzu HPLC equipment comprising an LC-10A VP quaternary pump, a variable-wavelength programmable UV–visible detector, an SPD-10AVP column oven, and an SCL 10AVP system controller. A Rheodyne injector fitted with a 20µL loop was also used and data were recorded and evaluated using Class-VP 5.032 software. The Compound was separated, at ambient temperature (25 ± 2°C), on a BDS C_{18}, (4.6 mm i.d x 250 mm, 5µm reversed phase column with 100% methanol as mobile phase at a flow rate of 1.0mL.min^{-1}. Before use, the mobile phase was filtered through a 0.22-µm Nylon filter. UV detection was performed at 274nm. A linear response was observed in the concentration ranges of 5-25µg/ml with a regression coefficient of 0.9999. The method was then validated for different parameters as per the ICH guidelines. This method can be used for the determination of memantine in quality control of formulation without interference of the excipients.

Keywords: Memantine, RP-HPLC, ICH.

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INTRODUCTION

Memantine [1.2] (1-amino-3,5- di methyladamantane) (Figure.1) is an NMDA (N-methyl-D-aspartate) receptor antagonist used for the treatment of dementia[3], severe Alzheimer's disease[4] and in the treatment of other neurological disorders including Parkinson's disease, pervasive developmental disorders[5], schizophrenia, alcohol abuse and withdrawal[6]. Several HPLC methods [7,9] and three LC/MS[10-12] have been reported for analysis of Memantine in plasma that suffer from either undesirably long chromatographic run times and requirement for gradient analysis or use of an internal standard. Two spectrophotometric methods [13,14] have also been reported. The objective of the present research is, therefore, to develop and validate a simpler, economic, rapid, precise and accurate RP-HPLC method with good sensitivity for quantitative analysis of Memantine in pure and pharmaceutical formulations in accordance with International Conference on Harmonization (ICH) The direct use of the mobile phase for dilution of the formulations for quantitative analysis would minimize errors that might occur during tedious extraction procedures.

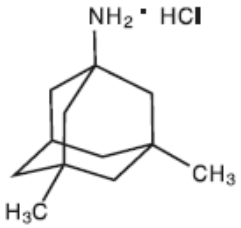

Figure 1: Chemical structure of Memantine

MATERIALS AND METHOD

Chemicals

Memantine was obtained as a gift from Hetro Drugs Ltd, Hyderabad. All the chemicals and reagents used were of AR grade and purchased from S.D. Fine Chemicals (Mumbai, India).HPLC-grade methanol was purchased from E. Merck (Mumbai, India). HPLC grade water was used to prepare all solutions.

Preparation of stock standard solution

A stock solution of Memantine (1.0mg.mL^{-1}, calculated as the free base) was prepared in mobile phase methanol.

Calibration Solutions

Calibration solutions for Memantine were prepared by diluting the stock solution to furnish concentrations in the range 5–25mcg mL^{-1}.

Preparation of sample solutions

Ten tablets were finely powdered and weighed. A portion of the powder equivalent to about 100mg of memantine hydrochloride was weighed accurately and transferred into100mL volumetric flask and mixed thoroughly for 20minutes for complete dissolution of memantine hydrochloride and then the sample solution was filtered and diluted to 100mL with mobile phase methanol to get concentration of 100mcg/mL and used for analysis. The samples were filtered through 0.45 µm nylon membrane before injection in the HPLC.

HPLC instrumentation and chromatographic conditions:

Chromatography was performed with Shimadzu HPLC equipment comprising an LC-10A VP quaternary pump, a variable-wavelength programmable UV–visible detector, an SPD-10AVP column oven, and an SCL 10AVP system controller. A Rheodyne injector fitted with a 20µL loop was also used and data were recorded and evaluated using Class-VP 5.032 software. The Compound was separated, at ambient temperature (25 ± 2°C), on a BDS C_{18}, (4.6 mm i.d x 250 mm, 5µm reversed phase column with 100% methanol as mobile phase at a flow rate of 1.0mL.min^{-1}. Before use the mobile phase was filtered through a 0.22-µm Nylon filter. UV detection was performed at 274nm.

RESULTS AND DISCUSSION:

HPLC method development and optimization:

To optimize the chromatographic conditions, different combinations of methanol (50%,60%,70%,80%,90% and 100%) were tested. Methanol with 100% was preferred because it resulted in a greater response to Memantine after several preliminary investigatory runs compared with the different mobile phase combinations.

The effect of the flow rate was studied in the range 0.8 to 1.2 mL.min^{-1}. Lower methanol content and flow rate resulted, in prolonged analysis time. Mobile phase (Methanol) with 100% concentration was therefore used at a flow rate of 1.0mL min^{-1}, was used for further studies. Under these conditions, the analyte peak obtained was well-defined and free from tailing (Figure.2). The retention time (RT) was 2.642 min. Other advantages of this mobile phase included its low cost and simplicity. The short retention time achieved implied that many samples can be run using a small quantity of mobile phase, thus minimizing analysis time and cost per analysis. The optimized chromatographic conditions for the determination of Memantine are represented in Table 1.

Table 1.:Optimized chromatographic conditions

Chromatographic Parameters	
Elution	Isocratic
Mobile phase	Methanol(100%)
Column	BDS C_{18} RP (4.6 mm i.d x 250 mm)
Flow rate	1.0mL/ min
Detection	UV at 274 nm
Injection volume	20 micro liters
Temperature	Ambient
Retention time	2.642 minutes
Run time	10 minutes
Area	45004817 mAU
Concentration	10.0 mcg/mL
Pressure	20-25 Mpa

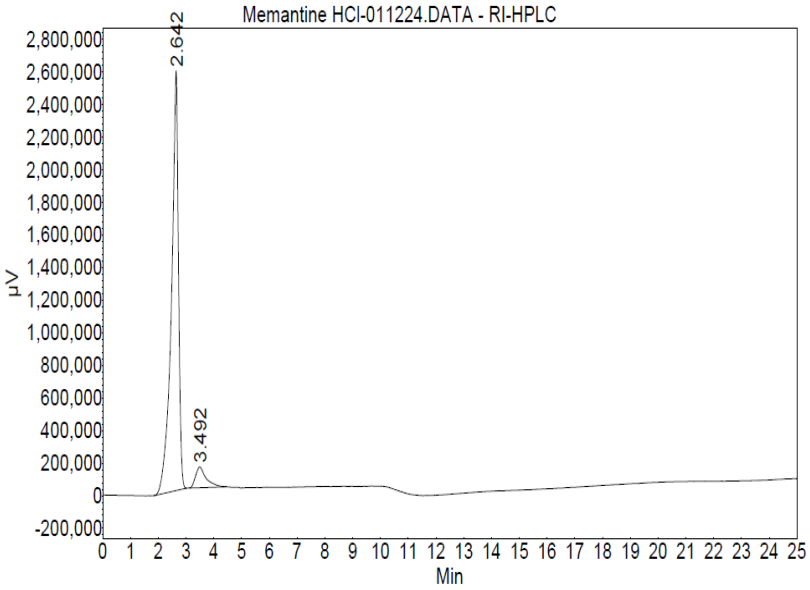

Figure 2: HPLC Chromatogram of Memantine

Validation of the method:

When method development and optimization are complete, it is necessary to accomplish method validation. The validation studies include linear range (correlation coefficient), sensitivity studies (LOD & LOQ), method precision (RSD, %), method accuracy (% recovery and RSD, %) and method selectivity.

Linearity:

The linearity of the method was evaluated by preparing five series of standard solutions of Memantine in the range of 5.0-25.0mcg/mL in mobile phase and injecting the solutions into the HPLC system. Excellent correlation between Memantine peak area and concentration was observed with R^2 > 0.9999(Figure.3). The regression equation was Y =4E+06x-28889 and the

mean values of the intercept and slope were 4E+06 and -28889, respectively. Statistical data are presented in Table 2.

Table 2:Calibration of the RP HPLC for the Estimation of Memantine

Parameters	Results
Concentration in µg.mL^{-1}	Peak Area (mAU)
5	22501409
10	45004817
15	67408718
20	90009526
25	112510234
Regression equation Y = b X + a	4.00E+06
Slope (b)	
Intercept (a)	-28889
Correlation coefficient	0.999
Standard deviation on intercept (Sa)	500864
Standard deviation on slope (Sb)	30203
Standard error on estimation (Se)	477555

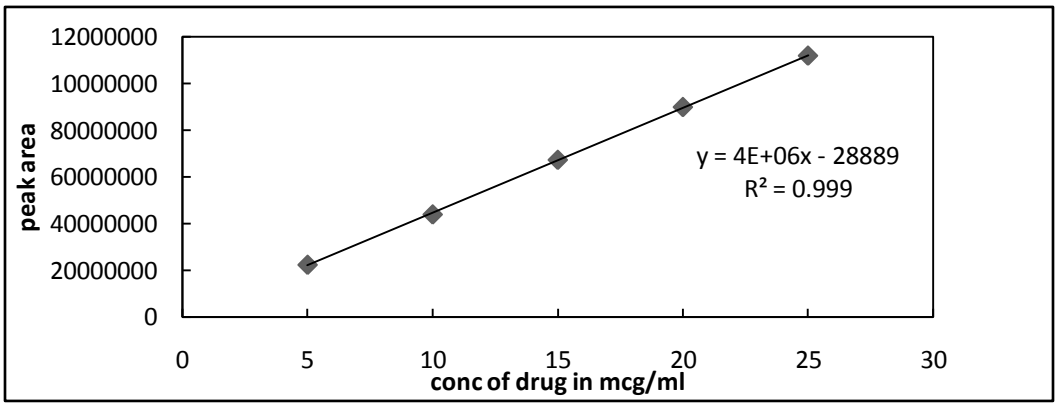

Figure 3: Linearity Graph Of Memantine

Sensitivity:

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for Memantine was found to be 0.3339mcg.mL^{-1} and 1.1132mcg.mL^{-1} respectively.

Precision:

The precision of the method was demonstrated by inter day and intraday variation studies. The author had carried out interday studies, by injecting six repeated injections of standard solution (10mcg/mL) of Memantine and the response factor of peaks obtained were recorded . From the

peaks obtained the %RSD was calculated and presented in Table 3**.** From the data obtained, the developed RP-HPLC method was found to be precise.

Table 3.:Precision data

Day	**Precession Area Mean***
Day-1	45671234
Day-2	45004817
Day-3	44998745
Day-4	45124567
Day-5	45982345
Day-6	44578901
Average	45226768
% RSD	1.1273

All The values are averages of six determinations

Accuracy [Recovery studies]:

Recovery studies were carried out for memantine by spiking the known standard drug in powdered formulations. This assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drug was calculated. The percentage of individual drug found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis(**Table 4)** were found to be 99.50% to 99.60% respectively. The results of analysis **(Table 5)** shows that the amounts of drug were in good agreement with the label claim of the formulation.

Table 4.:Recovery studies of the proposed RP-HPLC method

Labeled amount mcg/ml	**Amount added mcg/ml**	**Total amount mcg/ml**	**Amount found mcg/ml**	**% of Recovery**	**Mean**
10	5	15	14.94	99.60%	99.55%
10	10	20	19.90	99.50%	
10	15	25	24.89	99.56%	

All the values are the averages of three determinations

Table 5.:Results of recovery studies of tablet containing memantine

Pharmaceutical formulation	**Amount of Memantine**		**% of recovery**
	labeled	**found**	
Namenda	5.0 mg	4.97	99.40 %

All the values are the averages of three determinations

CONCLUSION:

A simple, rapid, accurate, and precise RP-HPLC method with UV detection for analysis of Memantine in pure and in tablet dosage forms of one market brand had been developed and validated in accordance with ICH guidelines. The RP-HPLC method developed by the author is

cost-effective due to short retention time which enabled analysis of memantine samples with a small amount of mobile phase. The low detection and quantification limits achieved indicate the method is very sensitive. The robustness data gathered during method validation showed the method is not susceptible to small changes in chromatographic conditions. The proposed RP-HPLC method developed by the author is suitable for routine analysis and quality assessment of Memantine in pharmaceutical product

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