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A pre-column derivatization technique for the development and validation of a stability indicating HPLC-UV method for the determination of memantine in bulk and formulations by using (2-napthoxy) acetyl chloride

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ABSTRACT

A simple, specific, precise, and accurate stability indicating RP-HPLC method has been developed and validated by using (2-Napthoxy) Acetyl chloride as derivatization agent and Amantadine as an internal standard. The separation was achieved by Inertsil ODS-3V, 250 x 4.6, 5µm column using mobile phase consisting of 0.02 M ammonium acetate buffer and methanol in the ratio (12:88) at a flow rate of 1.5 mL/min and UV detection at 226 nm. The Method was developed in isocratic mode. The retention time for Memantine and Amantadine was around 8.62 and 6.23. The method showed linearity with correlation coefficient < 0.999 over the range of 3.1-18.57 µg/mL. The mean recoveries were found to be in the range of 98.5--99.9% for Memantine Hydrochloride.LOD and LOQ values were found to be 0.12 and 0.32µg/mL respectively. The method was validated as per the ICH guidelines for linearity, limit of detection, limit of quantification, accuracy, precision, robustness and solution stability. Stability indicating capability of the developed method was established by analyzing forced degradation of samples with separation of degradation products from analytes peak was achieved. The method can be successfully applied for routine analysis of quantitative determination of Memantine Hydrochloride in pharmaceutical dosage form.

Keywords: Memantine hydrochloride, HPLC, precolumnderivatization, (2-Napthoxy) Acetyl chloride, stability indicating.

INTRODUCTION

Memantine hydrochloride is designated chemically 3,5-dimethyladamantan-1-amine .It is an orally active N-methyl-D-aspartate (NMDA) receptor antagonist. Memantine hydrochloride occurs as a fine white to off-white powder and is soluble in water. Its molecular weight and molecular formula is 215.76 and $C_{12}H_{21}N.HCL$.Persistant activation of Central nervous system N-methyl-D-aspartate (NMDA) receptors by the excitory amino acid glutamate has been hypothesized to contribute to the symptomatology of Alzheimer'sdisease. Memantine is postulated to exert its therapeutic effect through its action as a low to moderate affinity uncompetitive NMDA receptor antagonist, which binds preferentially to the NMDA receptor-operated cation channels.Due to lack of required chromophores, Memantine cannotbe readily assayed by HPLC-UV techniques. It's highly basic (pKa 10.42) and lipophilic (log P

3.28) nature suggests that it may show binding with (2-Napthoxy) Acetyl chloride,9-fluorenylmethyl chloroformate, dansyl chloride etc due to interaction of its primary amine group.

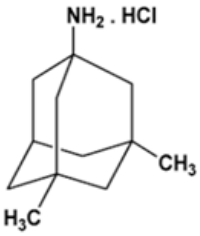


Fig.1 Structure of Memantine. HCL

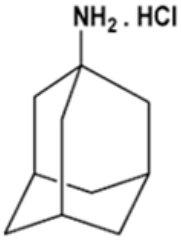


Fig.2 Structure of Amantadine.HCL

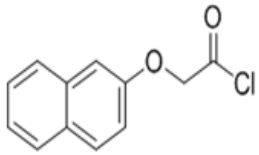


Fig.3 Structure of 2-Naphthoxy acetyl chloride

Literature survey states that various analytical methods have been reported such as Spectrophotometric [1-3], HPLC-UV [4-5]and HPLC-fluorescence detection [6-11] by using various Derivatizationagents. Also few LCMS and GC-FID/MS methods were reported [12-19] for the determination of Memantine hydrochloride in bulk and biological samples. However all these methods are time consuming, Non economical and not routine for commercial scale. So we felt to develop a simple, precise and accurate Stability indicating method for the determination of Memantine hydrochloride in bulk and formulation by using amantadine as an internal standard and (2-Napthoxy) Acetyl chloride[20] as a derivatization agent[21]. The developed method was validated as per ICH Guidelines [22, 23].

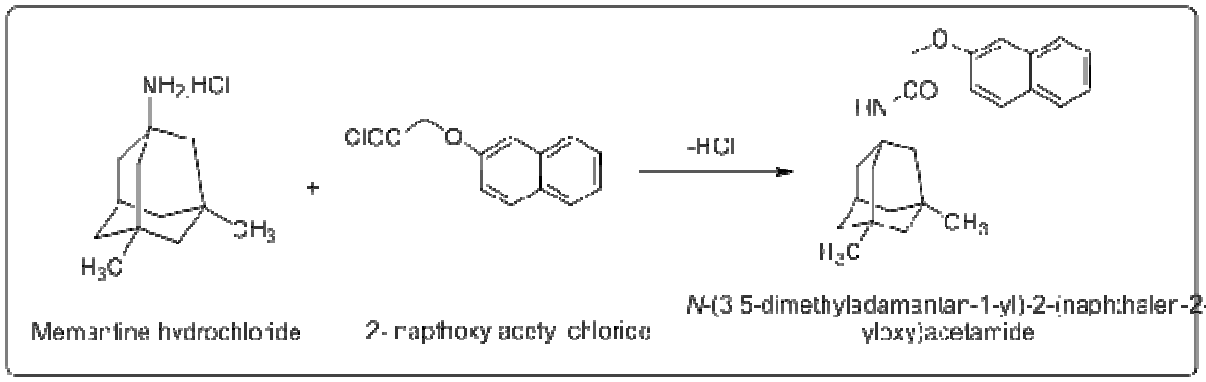


Fig.4 Reaction mechanism

MATERIALS AND METHODS

Chemicals and solvents:

Pure standard of Memantine Hydrochloride and Amantadine Hydrochloride were provided as a gift sample by Torrent Pharmaceuticals, Ahmadabad. Memantine tablets were procured from local market Hyderabad, India. Ammonium Acetate (AR grade), Sodium Hydroxide (GR grade) were procured from Merck India.(2-Naphthoxy) Acetyl chloride (GR grade) was procured from Aldrich, USA. Toluene (HPLC grade),Methanol(HPLC grade),Triethyl amine(GR grade) were procured from SDFCL Mumbai, India and High purity water was prepared by using Millipore Milli-Q Plus water purification system (Millipore, Milford, MA, USA).

Instrumentation and Apparatus:

The chromatography analysis was performed using Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2489 UV/visible detector or 2998 PDA detector ,degasser, quaternary pump, and auto sampler system. The output signals were monitored and processed using Empower 2 software. Cintex digital water

bath was used for hydrolysis studies. Photo-stability studies were carried out in the photo-stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (Cintex, Mumbai, India). The pH of the solutions was measured by a pH meter (Mettler-Toledo, Switzerland).Class A Volumetric flasks, pipettes, beakers, measuring cylinders and centrifuge tubes of Borosil glass were used.

Chromatographic conditions:
Separation of Memantine was achieved on Inertsil ODS-3V,250 × 4.6, 5 µcolumn and mixture of 0.02M ammonium acetate buffer and Methanol in the ratio of 22:88 (v/v) as mobile phase at flow rate of 1.5mL/minute with isocratic mode. Detection of Memantine was performed at 226nm. Column temperature was maintained at 40°C. Sample injection volume was 20 µL and total run time of the method was 12 minutes. Mixture of Toluene and Triethylamine was used as diluent.

Preparation of solutions:
Preparation of Derivatization Reagent:
Dissolved 300 mg of (2-naptoxy) acetyl chloride in 200 ml of toluene.

Preparation of Diluent:
2 ml of Triethylamine was added to 1000 ml of Toluene and mixed well.

Preparation of Mobile Phase:
120 ml of 0.02 M ammonium acetate buffer (1.5 g in 1000ml of water) was added to 880 ml of methanol .Degassed by sonication in an ultrasonic bath for 10 minutes.

Preparation of Standard stock solution:
65 mg of Memantine hydrochloride pure standard was added to 100 ml of volumetric flask and added about 70 ml of water and sonicated to dissolve and finally made up the volume with water.2 ml of the above solution is transferred to 50 ml volumetric flask and made up the volume with water.

Preparation of Internal standard stock solution:
65 mg of Amantadine hydrochloride pure standard was added to 50 ml of volumetric flask and added about 30 ml of water and sonicated to dissolve and finally made up the volume with water.10 ml of the above solution is transferred to 250 ml volumetric flask and made up the volume with water.

Preparation of sample solution: Twenty commercial tablets were weighed and powdered. A quantity of the powder equivalent to 13 mg of Memantine was accurately weighed, transferred to 50 mL volumetric flask and is dissolved in 30 mL of the water. Sonicated the solution for few minutes to dissolve the drug completely.Further diluted 2.5 ml of the above solution to 25 ml with water and proceeded for Derivatization process.

Derivatization Procedure of standard and sample:
Accurately transferred 5 ml of standard stock solution and 5 ml of internal standard stock solution into 20 ml test tube separately for both sample and standard solutions. Added 2 ml of 5 N NaOH to each test tube and cyclomixed on a vortex mixer for 2 minutes and added 3 ml of diluent and cyclomixed for 2 minutes.Finally left the solutions on bench top for 5 minutes. 2 ml of upper toluene layer from the above solutions is transferred into a 20 ml test tube and added 2 ml of Derivatization reagent and cyclomixed for 2 minutes. Kept the solutions on bench top for 5 minutes. Added 3 ml of methanol to the solutions and cyclomixed for 1 minute. Injected each solution into the HPLC.

Forced Degradation Studies:
The degradation study was performed to ensure that the method was able to separate Memantine from the probable degradation products generated during the forced degradation study. Acid, base, oxidative, photolytic and thermal degradation studies were performed. A standard solution of Memantine (1 mg/ml)was prepared and used for the acid, base and oxidative Degradation studies. A solid sample of MemantineHclwas used for thermal and photolytic degradation.

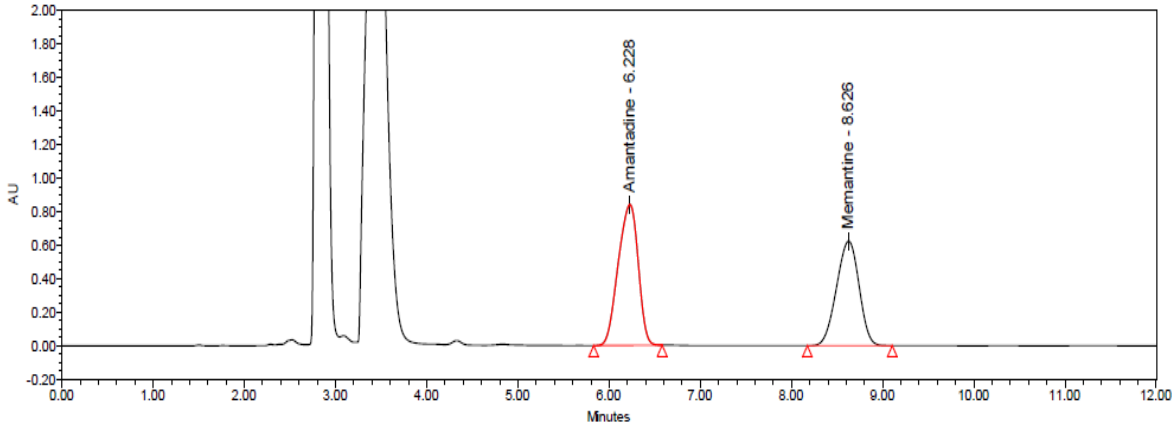


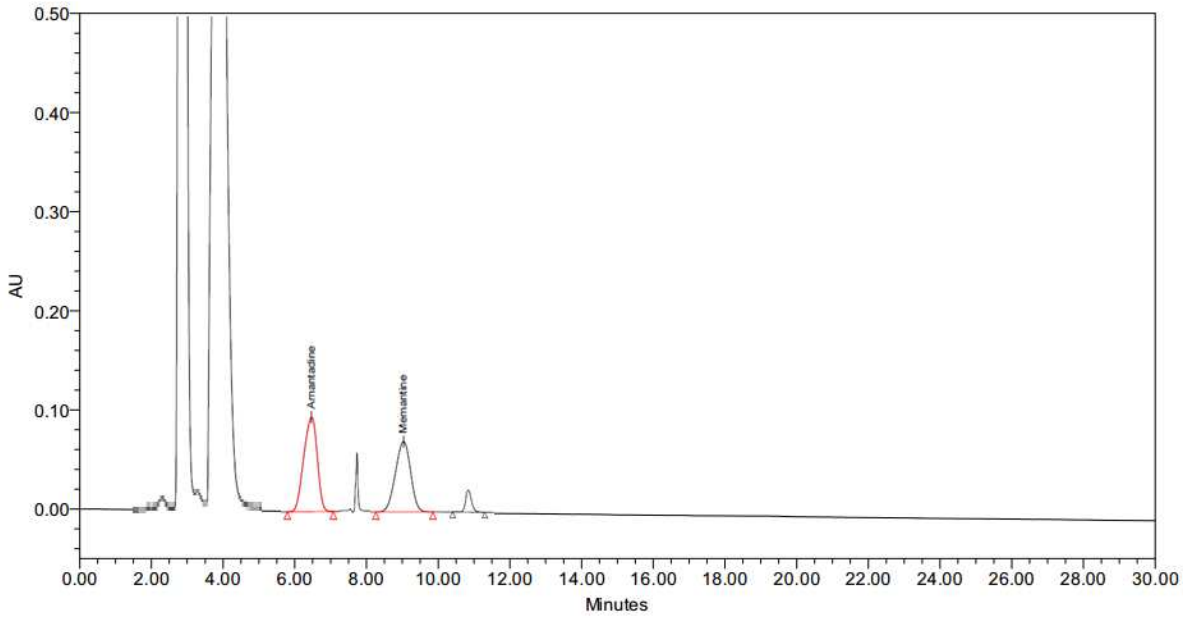
Fig: 5 Standard chromatogram of Memantine Hydrochloride

Hydrolysis

The hydrolytic degradation study of drug was carried out in 0.1N HCl with reflux at 100°C for 5 hours (**acid hydrolysis**) and in 0.1N NaOH with reflux at 100°C for 5 hours (**base hydrolysis**). Finally, the resultant solutions were neutralized by adjusting pH to 7, this was done using 0.1 N HCl for base hydrolysis and 0.1 N NaOH for acid hydrolysis. The final concentration was adjusted to 0.026 mg/ml of drug and preceded further as Directed in Derivatization procedure.

Oxidative degradation studies:

To 1 mL of stock solution of Memantine Hcl,1 mL of 10% hydrogen peroxide (H_2O_2) was added separately. The solutions were refluxed for 5 hours at 80°C. The final concentration was brought up to 0.026 mg/ml of drug and proceeded further as Directed in Derivatization procedure



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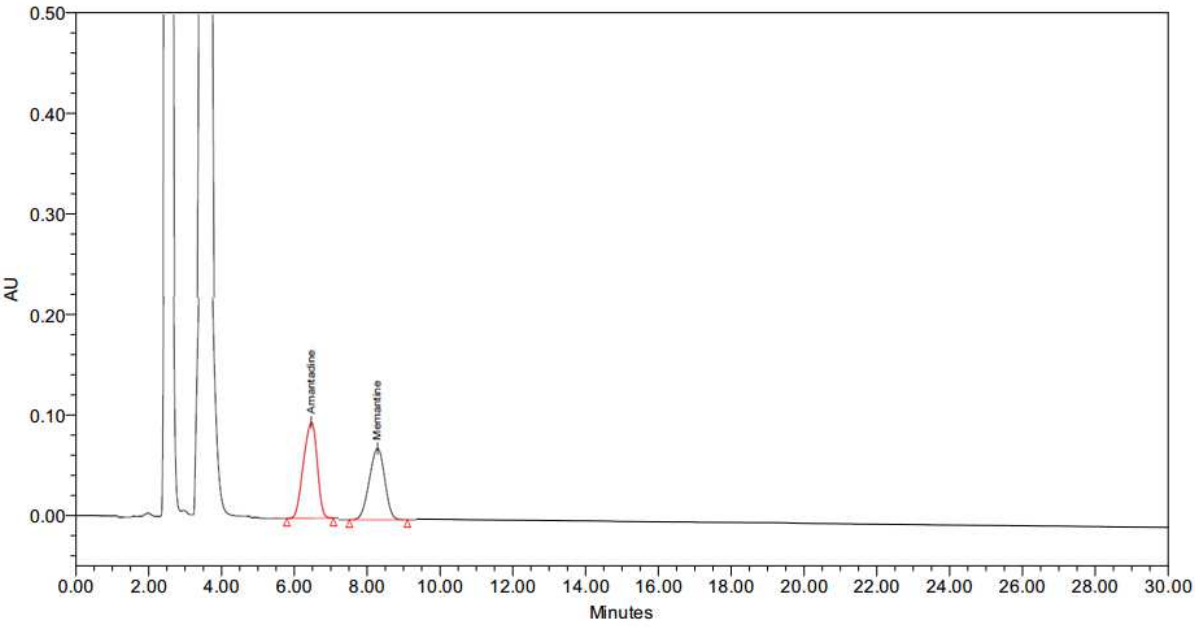
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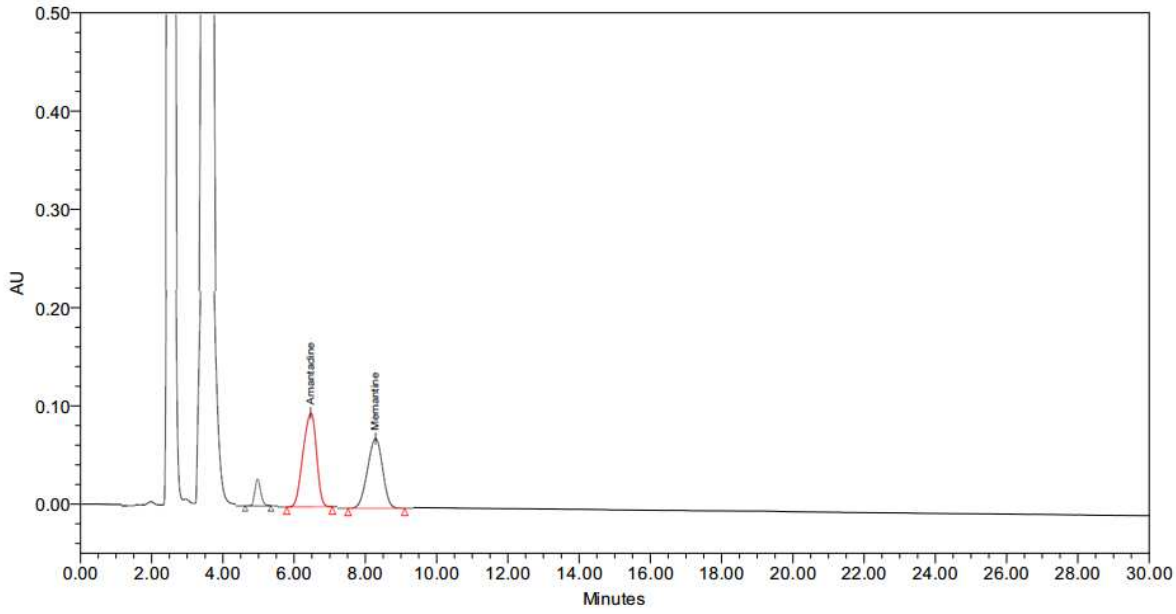
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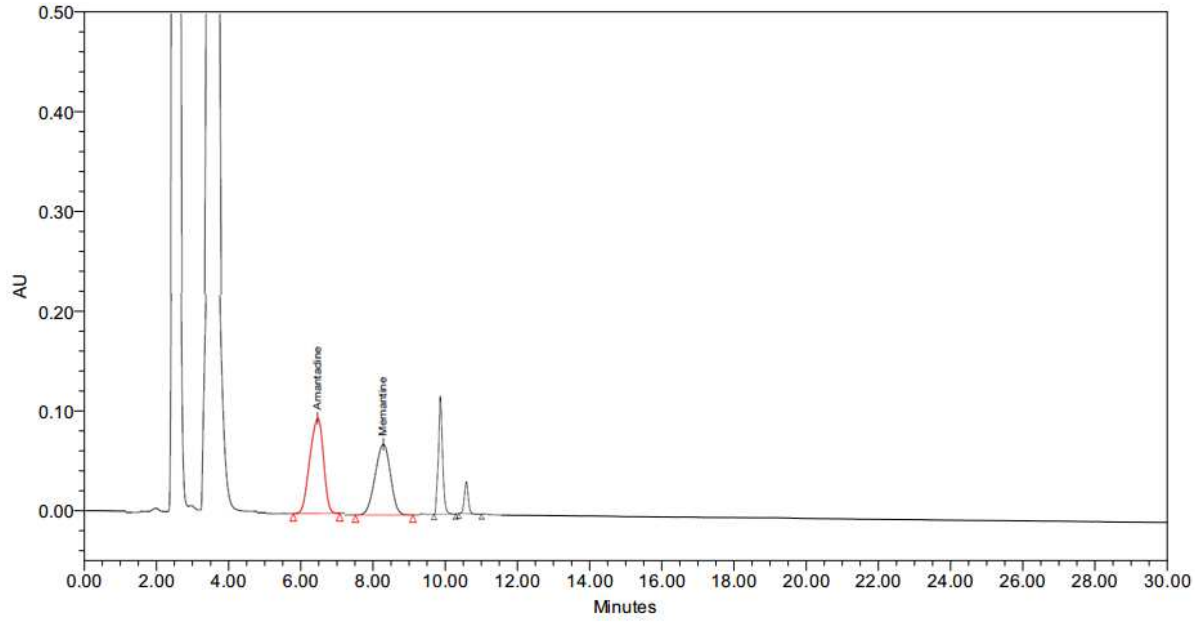
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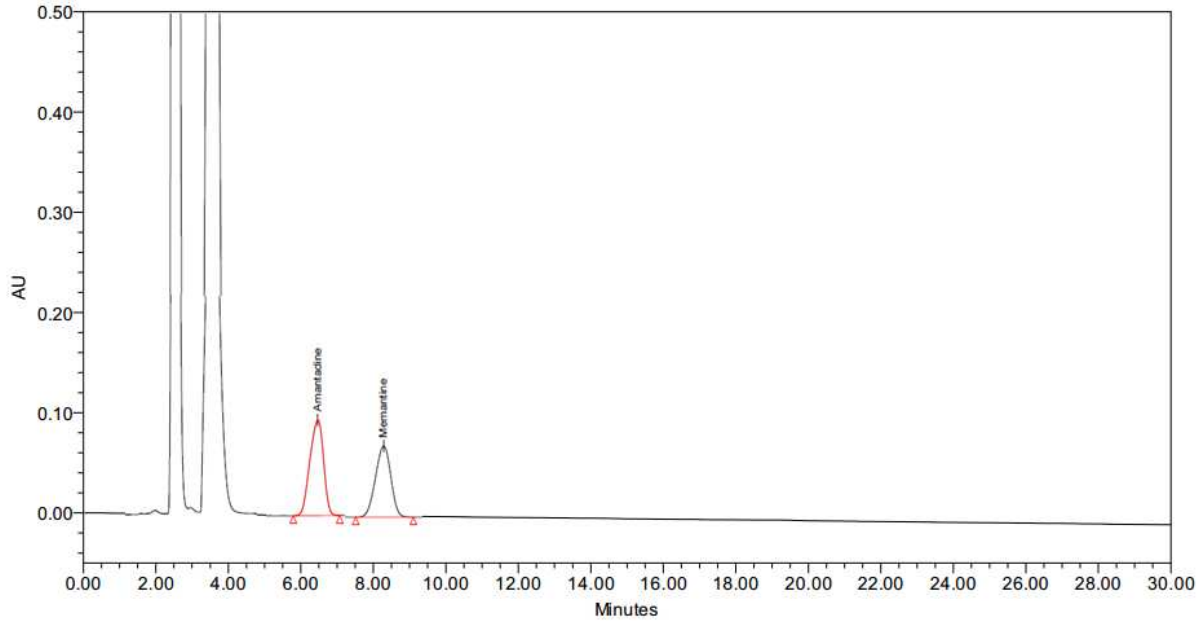
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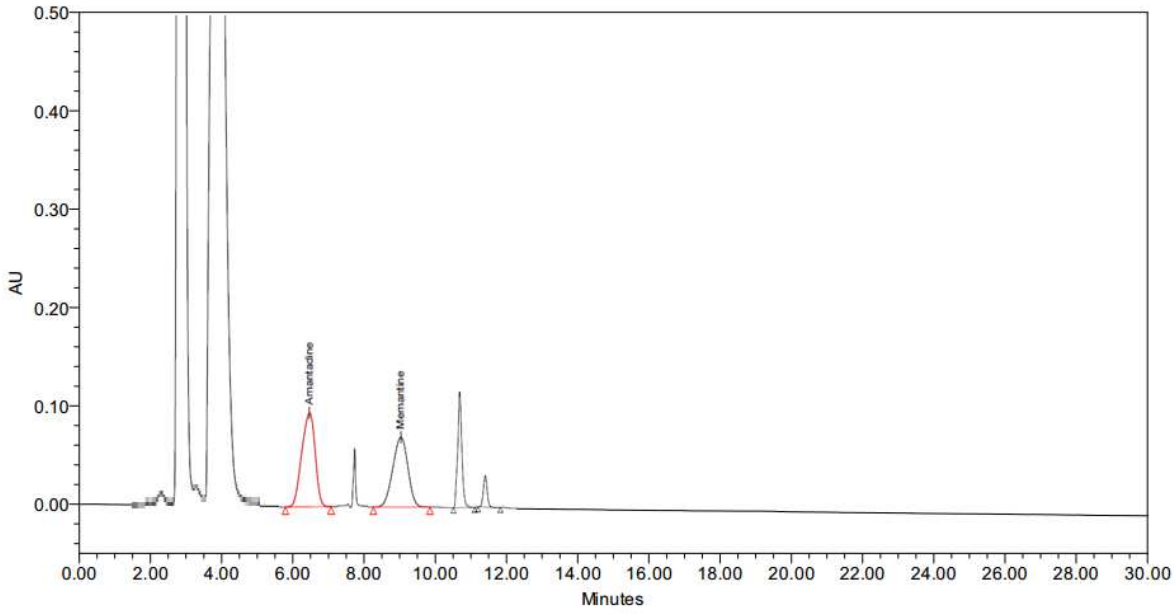


[F]

Fig. 6: Chromatograms of Memantine after Forced Degradation Studies (A) Acidic Degradation (B) Alkaline degradation (C) Oxidative degradation (D) Thermal degradation (E) Photolytic degradation

Photo Degradation

A solid portion of the drug was spread in 1 mm thickness in a Petri dish under the exposure of 1.2 lux watt hours of UV light for 24 hrs in the photo stability chamber. The sample solution was prepared to contain 0.026 mg/ml of drug and proceeded further as Directed in Derivatization procedure.

Thermal Degradation

The standard Memantine Hclwas spread in 1 mm thickness in a Petridish and kept in a hot air oven for 48 hours at 80 °C.The sample solution was prepared to contain 0.026 mg/ml of drug andpreceded further as Directed in Derivatization procedure.

Method Development & Optimization

The present investigation was carried out with a view to develop a RP-HPLC-PDA method for the quantification of Memantine in the form of derivatised complex. Mobile phase Optimization initially carried out with Inertsil ODS-3V, 250 x 4.6, 5µm using 0.02 M ammonium acetate buffer and Acetonitrile combination (12:88% v/v) at a flow rate of 1.5 mL/minand Toluene as diluent. Under these conditions the peak of Memantine-NACcomplex was elutes with fronting at 8.7 min.

In other trial, Acetonitrile wasreplaced with methanol and keeping 0.02 M ammonium acetate buffer (88:12% v/v) at a flow rate of 1.5 mL/min andunder these conditions a unsymmetry Memantine-NAC complex peak was eluted at 8.7 min.Finally, the mobile phase of 0.02 M ammonium acetate buffer and Methanol combination (12:88% v/v) at flow rate of 1.5 mL/min using Triethylamine and Toluene(0.02:99.98) as diluent was selected and under these conditions a sharp Memantine-NAC peak was eluted at 8.6 min with a total runtime of 10 min.

Few chromatographic parameters such as Derivatization process time optimization, NAC volume optimization were evaluated to obtain a specific, linear and accurate method.

NAC volume optimization:

Different volumes of NAC solution i.e 0.5,1.0,1.5,2.0,2.5,3.0 ml was added to the above mentioned solutions(sample and standard) under Derivatization procedure to confirm complete reaction of Memantine. Peak areas ratios were monitored to obtain a value of 1.0 which indicates 2.0 ml is the optimized volume of NAC Reagent.

Derivatization process time optimization:
Sample and standard solutions after addition of Derivatization reagent were stored at room temperature for different time intervalsi.e. 0.5,1.0,1.5,2.0,2.5,3.0minutes to fix the reaction time.Peak areas ratios were monitored to obtain a value of 1.0 which indicates 2.0 min is optimized time to complete the reaction.

Table-1: **Optimized chromatographic conditions**

Parameters	Condition
Mobile phase	0.02 M ammonium acetate buffer and Methanol in the ratio(12:88)
Diluent	Triethylamine and Toluene
Column	Inertsil ODS-3V, 250 x 4.6, 5µm
Flow rate	1.5 mL/min
Wavelength	226 nm
Injection volume	20µL

Method Validation:
The method was validated for specificity, linearity and range, precision, accuracy, LOD and LOQ, robustness, and system suitability as per International Conference on Harmonization (ICH) guidelines.

Specificity
The specificity of the developed HPLC method for the determination of Memantine in bulk drug and pharmaceutical preparation (ADMENTA XR)was evaluated by non-interference of placebo, and forced degradation studies.

Non-Interference of Placebo
To check the non-interference of placebo, the placebo solution was prepared in the same way as that of the sample solution in the presence of all inactive ingredients of the tablet formulations, but without Memantine.

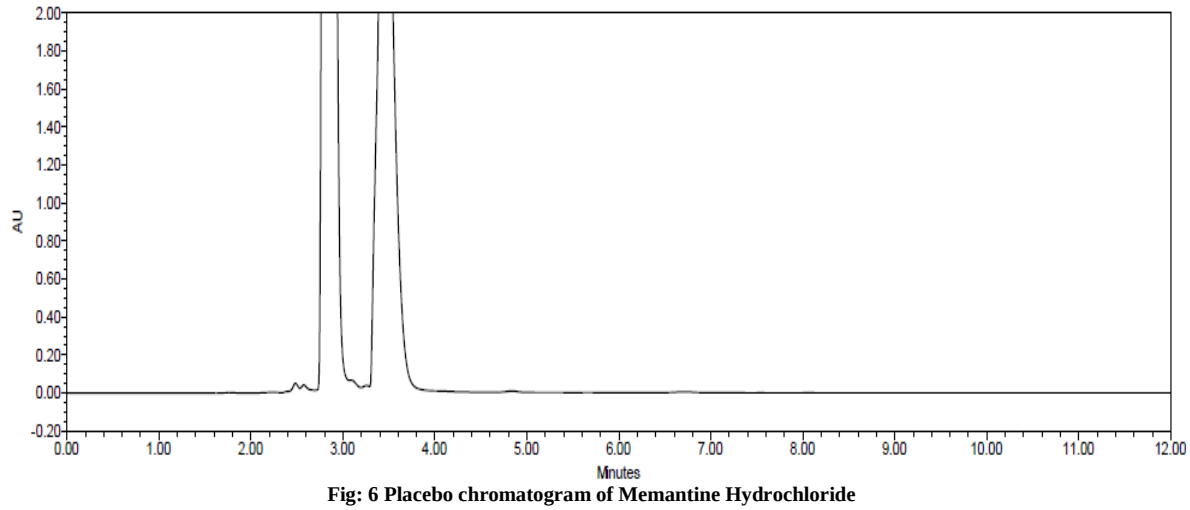
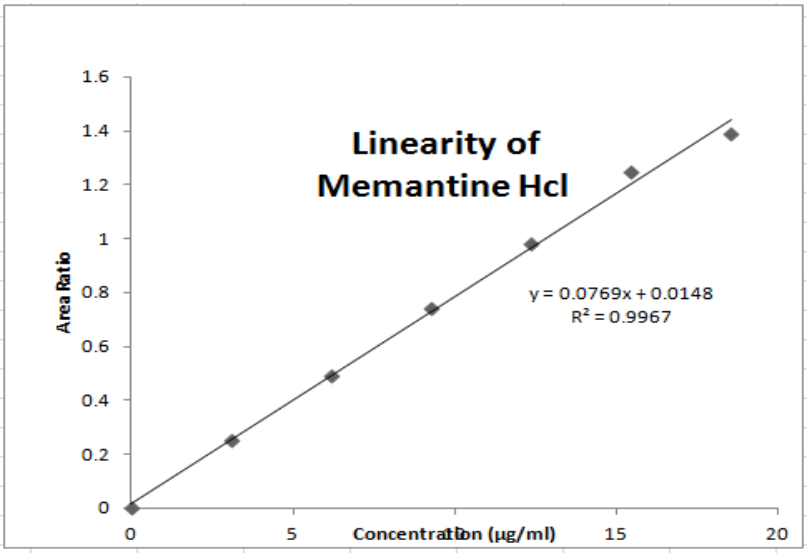


Fig: 6 Placebo chromatogram of Memantine Hydrochloride

Linearity:
Linearity of detector response for Memantine was established by analyzing serial dilutions of a stock solution of the standard. Six concentrations ranging from 25% (3.10µg/mL) to 150% (18.57µg/mL) of the test concentration of 12.38µg/mLwere prepared. Internal standard solution was added to each dilution as described in the Derivatization procedure, such that each solution contains 12.38 µg/mL of AmantadineHCl and proceeded for Derivatization. The final concentration of each solution in mg per mL was calculated and plotted against peak area ratio of Memantine to Amantadine. The slope, y-intercept and correlation coefficient (R) were calculated.

Fig:7 Calibration curve of Memantine Hydrochloride



Precision:
The intra-day precision of the assay method was evaluated by carrying out 6 independent assays of a test sample of Memantine at100% concentration level against a standardMemantine. The %RSD of the obtained assay values was calculated. The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at 100% concentration level .The % RSD of the obtained assay values on 100% concentration level was calculated.

Accuracy:
The accuracy of the method was performed by recovery study of Memantine in the dosage form at three concentration levels. A fixed amount of preanalyzed sample was taken and standard drug was added at 50%, 100% and 150% levels.Internal standard solution was added to each set as described in the Derivatization procedure.Each level was repeated three times. The content of Memantine per tablet was calculated. The percentage recovery ranges from 98.50-99.90% and the mean recovery of Memantine was 99.40% that shows there is no interference from excipients and the lower values of RSD of assay indicate the method is accurate.

Robustness:
The robustness test was performed by deliberately making the changes in the flow rate and buffer concentrations. Retention time, tailing factor, resolution, and theoretical plates were measured to demonstrate the robustness of the method. Robustness was conducted on the sample solutions prepared from the tablet formulation.

System suitability:
System suitability was carried out by injecting a standard concentration of 12.38µg/mL at six replicates and system suitability parameters were determined. The system suitability test parameters were noted and % RSD was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ) :
It was performed based on the signal-to-noise ratio. A standard solution of 3.1 µg/ml of Memantine solution was prepared to check the signal-to-noise ratios of the analytes. Then further dilutions were made for LOD and LOQ determination.

Application to Analysis of Pharmaceutical Formulations
The proposed method was applied for the estimation of Memantine(Commercial Tablets- ADMENTA XR) in their tablet formulations. About twenty tablets were taken and pulverized to a fine powder, and then tablet powder equivalent to the average weight of one tablet was taken. The drugs were extracted with mobile phase for carrying out the analysis.

RESULTS AND DISCUSSION

Stability-indicating assay methods are useful for determining the integrity of the drug substance and drug product during accelerated shelf life studies. It provides information about the drug quality. Therefore, there is a need for developing a stability-indicating HPLC method for the estimation of Memantine in pharmaceutical formulations. A reverse phase isocratic liquid chromatographic technique was developed, optimized and validated for the determination of Memantine in bulk and tablet dosage forms with UV detection at 226 nm by using Inertsil ODS-3V, 250 x 4.6, 5µmwith mobile phase composition of 0.02 M ammonium acetate buffer and methanol in the ratio(12:88) in optimized isocratic program. The results of optimized HPLC condition were shown in Table No 1.Linearity for the proposed method was established by least squares regression analysis of the calibration curve. Calibration curves were linear over the concentration range of 3.1–18.57 µg/mL for Memantine with a correlation coefficient (r2) of 0.9967± 0.002.The calibration curve was shown in Fig No 7 and the results of Linearity were given in Table No.2. The precision of proposed method was good with a % RSD of below 1.0%which indicates the method is precise. The results are presented in Table No 3.The % recoveries of Memantine were found in the range of 98.5 -99.9 and the % mean recoveries found to be 99.4 for Memantine, which indicates the method is accurate. The results of recovery studies were shown in Table No 4**.** The Number of theoretical plates was 6280, Resolution between Amantadine and Memantine is 6.2and the tailing factor was 1.0 for Memantine which assures efficient performance of the column.Results of system suitability parameters were given in Table No. 5. Typical chromatogram of StandardMemantine was shown in FigNo: 5.Specificity was demonstrated by the placebo studies and through forced degradation studies. The LOD and LOQ of Memantinewere found to be0.12 and 0.32 µg/mL. In all the deliberate varied chromatographic conditions (flow rate, pH variation, buffer concentration), the system suitability parameters like tailing factor, resolution, and theoretical plates were not much affected, which shows that the method is robust.

Degradation studies of Memantine under different stress conditions indicates the following degradation behavior. In acidic and photolytic degradation conditions Memantine is slight sensitive compare to other stress conditions like alkaline, oxidation and thermal degradation. The summary of degradation studies is given in Table No 6and the degradation behavior of Memantine in stress conditions is given in Fig No 6. The proposed method was successfully applied to the assay of Memantine in commercial tablets (ADMENTA -XR). The percentage recoveries of the drug was based on the average of five replicate determinations. The results are shown in Table No 8.The analysis of commercial tablets results were well within the limits which indicates that the method can be applied for Routine commercial analysis.

Table-2: **Results of Linearity**

S.No	Concentration (µg/ml)	Memantine Area	Amantadine Area	Area Ratio
1	18.57	3459725	2489011	1.39
2	15.48	3110001	2488001	1.25
3	12.38	2538900	2488122	0.98
4	9.29	1859610	2488652	0.74
5	6.19	1239120	2489122	0.49
6	3.10	620503	2488065	0.25

Table-3: **Results of Precision**

S.No	Intraday precision		Interday precision	
	Area Ratio	% of Assay	Area Ratio	% of Assay
1	0.963	96.3	0.954	95.4
2	0.952	95.2	0.931	93.1
3	0.941	94.1	0.961	95.1
4	0.940	94.0	0.950	95.0
5	0.960	96.0	0.971	97.1
6	0.952	95.2	0.960	96.0
Average		95.1	Average	95.3
Standard Deviation		1.0	Standard Deviation	1.3
% RSD		0.9	% RSD	1.3

Table-4: **Results of Accuracy**

Level	Concentration added (µg/mL)	Concentration found (µg/mL)	% Recovery	Mean recovery
50 %	6.19	6.10	98.5	99.4
100%	12.38	12.35	99.8	
150%	18.57	18.55	99.9	

Table-5: **Results of System suitability**

Parameters		USP limits	Results
Theoretical plates		NLT 2000	6280
Tailing factor	Memantine	NMT 2.0	1.0
	Amantadine	NMT 2.0	1.0
Resolution		NLT 2.0	6.2
Retention time	Memantine		8.6
	Amantadine		6.2
% RSD of Peak Response Ratio		NMT 2.0%	0.8

Table-6: **Results of degradation studies**

Stress conditions	Memantine peak area	Amantadine peak area	Area Ratio	% Degradation	% of active drug present after degradation
Standard Drug	2478611	2487022	0.996	--	--
Acidic degradation	2305691	2487262	0.927	7.3	92.70
Alkaline degradation	2347654	2486922	0.944	5.6	94.4
Oxidative degradation	2372808	2487221	0.954	4.6	95.4
Thermal degradation	2340484	2487231	0.941	5.9	94.1
Photolytic Degradation	2407562	2487151	0.968	3.2	96.8

Table-7: **Assay Results of Market formulation**

Market formulation	Label claim (mg)	Quantity of API found (mg)	Assay %
ADMENTA XR-14 mg, Sun pharma	Memantine Hcl	Memantine Hcl	Memantine Hcl
NA	14	13.8	98.5

CONCLUSION

A simple ,precise and Accurate Stability indicating RP-HPLC method was developed and validated as per ICH Guidelines by using (2-Napthoxy) Acetyl chloride as Derivatization agent due to lack of chromophores groups in Memantine molecule. Amantadine is used as an Internal standard which increases the sensitivity of the method. Developed method can be used for the routine commercial analysis of Memantine formulations and also for long-term and accelerated stability studies.

Acknowledgments
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