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A Validated Stability-indicating RP HPLC Assay Method for the Determination of Memantine hydrochloride Drug with Refractive index detection and Peak purity by LC-MS (LC/MS/MS)

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ABSTRACT

This paper deals with the development and validation of stability indicating an isocratic high performance liquid chromatographic method for the quantitative determination of memantine hydrochloride. The method is simple, highly sensitive, selective and is capable of quantitative determination of memantine hydrochloride. The chromatographic separation is achieved by injecting 20µL standard solution of memantine hydrochloride into HPLC system with refractive index detector using a YMC ODS-AQ, 5µm (150 x4.6)mm column. The mobile phase consists of 10 mL Triethylamine in 1L water(pH 5.5 adjusted with glacial acetic acid):MeOH in the ratio of 40:60 v/v. The flow rate was set at 0.9 ml/min with column and cell temperatures at 40˚C and 50 ˚C respectively and runtime was optimized to 10 min. Forced degradation studies were performed on bulk sample of memantine hydrochloride using acid (5.0Nhydrochloric acid) ,alkali (1.0N sodium hydroxide),oxidation (30% hydrogen peroxide),thermal(105 ˚C),photolytic and humidity conditions. The peak purity was checked with LC-MS and LC-MS/MS. The developed LC method was validated with respect to specificity, precision, linearity, ruggedness, stability of analytical solution and robustness.

Keywords: Memantine hydrochloride, HPLC,LC-MS, Assay

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INTRODUCTION

Memantine hydrochloride (1-amino 3,5 – dimethyl adamantane hydrochloride) (Fig. 1) is a tricyclic amine, chemically and pharmacologically related to the antiviral prototype amantadine and its α-methyl derivative rimantadine. FDI of US has approved Amantadine and Rimantadine for the prophylaxis and treatment of influenza. Amantadine is also approved in Parkinson's disease and movement disorders.[1] It has been demonstrated to be useful in dementia syndrome.[2] Memantine is a non-competitive NMDA (N-methyl D-aspartate) antagonist in clinical use for many years in European countries. This drug produces few side effects, even among the geriatric patients, who are typical candidates for this drug.[3,4]

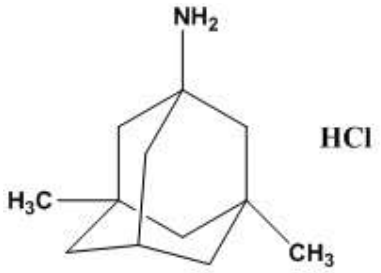


Figure 1 : Structure of Memantine hydrochloride. Molecular formula: $C_{12}H_{21}N$. HCl: molecula mass:215.76 (free base: 179.20): partition coefficient, log(P): 3.28: basicity. pKa: 10.42

The NMDA receptor, a glutamate receptor subtype, may play a significant role in the development and maintenance of dependence on opoids, nicotine and cocaine.[5,6] Low doses of NMDA antagonists inhibit the development of opioid tolerance and dependence[7] and attenuate established morphine(mμ) opioid tolerance in laboratory animals.[8] The development of tolerance dependence and/or sensitization to virtually all psychoactive drugs can be attenuated or abolished by pre-treatment with NMDA antagonist.[9] So, memantine is a promising agent for the treatment of substance use disorders. Unlike other non-competitive NMDA antagonists, such as phencyclidine and ketamine, memantine has rarely been associated with the significant adverse side effects of agitation, confusion and psychosis.[10,11]

Memantine lacks useful chromophores, so it cannot be readily assayed by HPLC-UV techniques.[12] Memantine is both highly basic and lipophilic and suggests that it may show binding to various derivatization agents like 9-fluorenylmethyl chloroformate (FMOC), dansyl chloride etc., due to ionic interaction of its basic primary amine group. Consequently, memantine has to be derivatized for HPLC-fluorescence measurement,[13-15] determined by capillary zone electrophoresis with indirect UV detection,[16] measured by GC without derivatization,[17-21] or for

enhanced sensitivity derivatized and analyzed by GC.[22-25] Dansyl chloride and 9-fluorenylmethyl chloroformate react readily with most primary and secondary amines in alkaline buffer, and is regarded as the derivatizing reagent of choice in the preparation of highly fluorescent compounds.[26,27]FMOC reacts readily with primary and secondary amines in 0.5M borate buffer solution, giving a derivative which can be detected with UV.[28]. Memantine Hydrochloride was determined by spectrophotometric technique by reacting it with anionic dyes bromomethyl blue and solochrome black T in coloured complexes[29].

The reported analytical assays based on spectrophotometry or HPLC derivatization methods and GC as well as majority of other mentioned methods are elaborate, time-consuming and involve sophisticated equipment that might not be available in most analytical laboratories. Literature survey revealed that no attempt has been made for assay of memantine hydrochloride drug in pharmaceutical preparation.

The purpose of this study is to develop, optimize and validate [30]methods with direct Refractive index detector for quantitative determination of memantine hydrochloride. This method also has advantages over some literature technique as mentioned above reference.[13-27] Memantine hydrochloride response is measured by direct refractive index detection with enhanced sensitivity and this method is simple, highly reproducible, specific, accurate, time and cost effective, compared to complex techniques like use of fluorescence detector or capillary zone electrophoresis technique, GC technique and use of UV-Detector using pre-column derivatization technique.

MATERIALS AND METHOD

Reagents and chemicals

Methanol (99.8%) from Rankem, triethylamine (99.8%) from Spectrochem, Glacial acetic acid (99.8%) from Sd. Fine Chemicals Ltd were procured from local market. Water(MIlli-Q) and Memantine hydrochloride was obtained from Macleods pharmaceuticals Ltd(India).All the above materials were used without any further purification.

Chromatography

The analytical separations were carried out on Agilent-1200 series HPLC system with refractive index detector. The analytical column was YMC- Pack ODS-AQ, 5µm (150 X4.6) mm. The mobile phase was a premixed and degassed solution of 10 mL triethylamine in one litre water. The pH of this solution was adjusted to 5.5 with glacial acetic acid(buffer). This buffer and methanol in the ratio of 40:60 v/v was the mobile phase after filtered through 0.45µ membrane

filter. The flow rate was 0.9 ml/min. and runtime was 10minute. Column temperature was maintained at 40˚C and cell temperature was maintained at 50˚C. Detection was measured by Refractive index detector and 20 µL sample was injected. The control of HPLC system and data collection was chemistration software and pH adjustment of aqueous phase was monitored using Elico pH meter model No: L120.

Liquid Chromatography/Mass spectrometry (LC-MS)(LC/MS/MS)) Conditions:

The LC-MS analysis was carried out on AB Sciex Instruments 4000 Q TRP Linear Ion Trap Quadrupole. LC/MS/MS Mass Spectrometer was connected with Agilent -1200 series HPLC system with refractive index detector. The column, mobile phase and column, cell temperature was as described above. The control of HPLC system and data collection was Empower software.

After chromatographic separation, the mobile phase was directly introduced into the mass spectrometer via electrospray ionization (ESI) source operating in the positive mode. Identification was performed using Q1 MS (Q1) and product ion (MS2) scan types in order to optimize all the mass parameters. The curtain gas reached 20 psi. The ion spray voltage was set at 4500V and temperature at 475 ˚C. The nebulizer gas (GS1) and turbo gas (GS2) were at 45 and 55 psi. The declustering potential (DP) and entrance potential (EP) were 25 and 10 V. Data acquisition was carried out by analyst 1.4.2 software on a DELL computer.

Preparation of Memantine Hydrochloride Standard Solution and Sample solution for Validation

Standard and sample stock solutions at 1 mg/ml were prepared by dissolving memantine hydrochloride in mobile phase. Further dilution of 5 ml standard and sample stock solution to 50 ml with mobile phase gave the final concentration of 0.1 mg/ml. These stock solutions were further diluted with mobile phase to get the required concentrations for validation studies.

RESULTS AND DISCUSSION

Method optimization parameters

An understanding of the nature of API (functionality, acidity, or basicity), the synthetic process, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting.

Different mobile phases and Stationary phases were employed to develop a suitable LC method

for quantitative determination of memantine hydrochloride in its drug. A number of columns containing various packing materials of ODS supplied by different manufacturers and different mobile phase composition were tried to obtain good peak shape and selectivity for impurities present in memantine hydrochloride. Peak tailing was observed when only water was used as buffer with various pH composition and methanol. In the next approach water as buffer at pH 5.5 and methanol in a ratio of50:50 v/v was employed using YMC Pro C-18 (150 X 4.6)mm, 5µm column. Under these conditions USP peak tailing was found to be 1.9 for memantine hydrochloride peak. In another attempt using the YMC- Pack ODS-AQ (250 X4.6) mm, 5µm column and mobile phase consisting of water as a buffer of pH 5.5 and MeOH in the ratio 40:60v/v memantine peak eluting at 8.5 min with better peak shape of memantine hydrochloride compared with other trials. In another trial using YMC –Pack ODS-AQ (150 X4.6) column with a mobile phase consisting of 10 ml of Triethylamine in 1L water at 5.5 pH adjusted with Glacial acetic acid and Methanol in ratio of (40:60 v/v) was tried. In this eluent Memantine hydrochloride gave a very good and well separated peak from all impurities. In another trial, solvent system and column are same as above and optimized the column temperature, flow and injection volume. After number of trials for flow and column temperature combination, in order to obtain best column temperature and flow, was set at column temperature 40˚C and flow rate was 0.9 ml/min. The solvent system consisting of 10 ml triethylamine in 1L water (pH 5.5 with Glacial acetic acid) and methanol in ratio of (40:60 v/v) using YMC-Pack ODS-AQ(150 x 4.6)mm, 5µm column, at a flow rate of 0.9 ml/min, runtime 10 minutes with column temperature maintained at 40˚C and cell temperature maintained at 50˚C was finally selected. Detection was measured by Refractive index detector and the sample injected was 20 µL. These chromatographic conditions were selected for validation studies. The system suitability results obtained using these chromatographic conditions are shown in Table 1 and Memantine hydrochloride peak eluting at 5.312 min. The Memantine hydrochloride and blank chromatograms are shown in Figure 2.

Table 1: Chromatographic Conditions of Memantine Hydrochloride

Parameter	Retention time (min)	Tailing factor	Theoretical plates	% RSD (six replicate
System suitability	5.312	1.295	6996	1.33

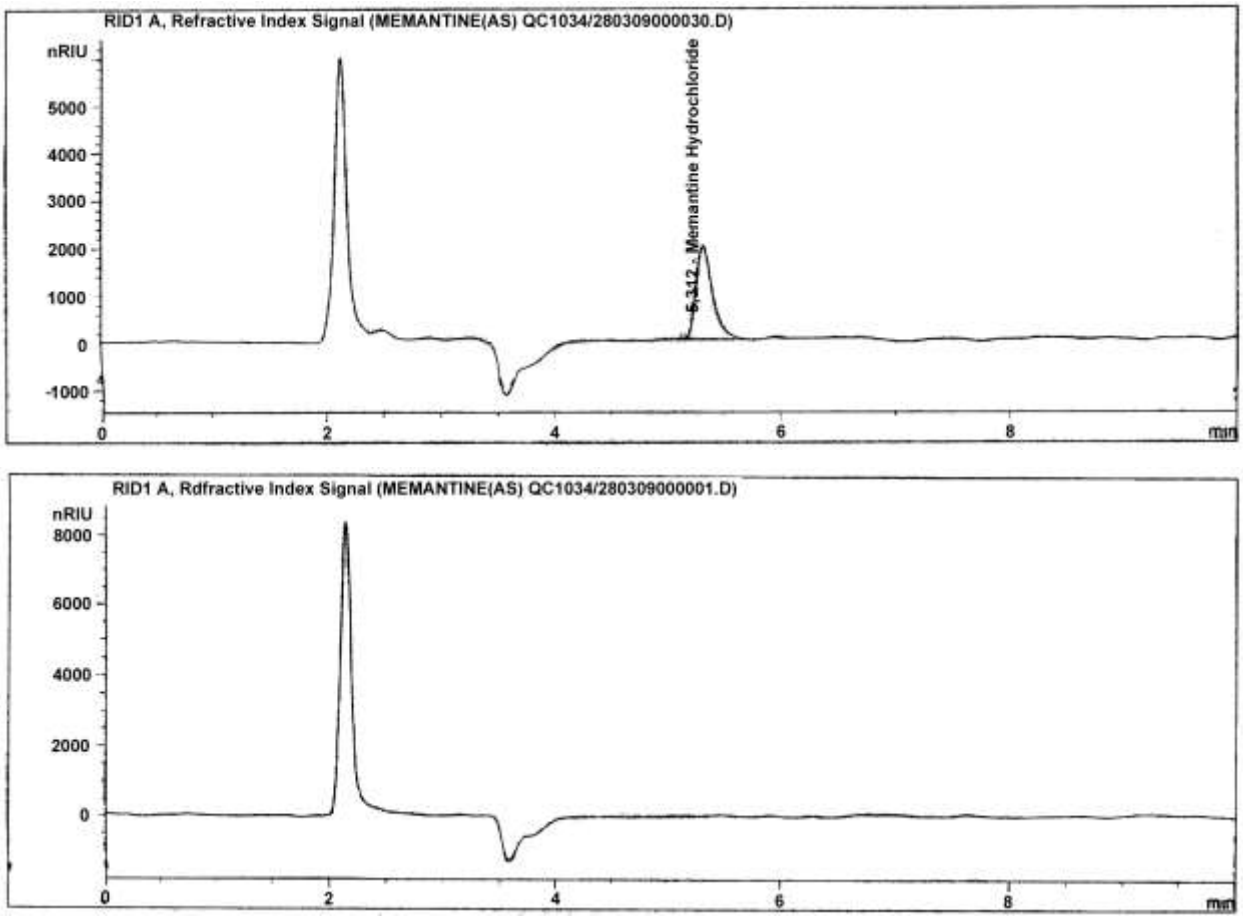


Figure 2: HPLC Chromatogram of Memantine hydrochloride and blank

System suitability Criteria:

A system suitability test was defined based on the results obtained in several representative chromatograms. The column efficiency, as determined from memantine hydrochloride peak, is not less than 3000 theoretical plates and the tailing factor is less than 2.0. %RSD for six replicate injections of standard solution are not more than 2.0. To check the system suitability, Memantine Hydrochloride standard solution was prepared at the working concentration at 100 % level (100 ppm). The standard solution was injected into HPLC system before starting every validation parameter and peak response was measured in six replicates. The mean and relative standard deviation was calculated and system suitability parameter like column efficiency and tailing factor was reported. All the system suitability criteria during validation of study was within the acceptance limits.

Method validation

Specificity

Sample solution was analyzed as per the method and peak purity of memantine peak was checked by LC-MS. Based on LC-MS mass spectrum the memantine peak was spectrally pure

and no additional mass was observed at this peak. The purity data of memantine peak indicates that the peak is homogeneous as shown in Fig 3 and mass spectra of memantine shown in Figure 4.

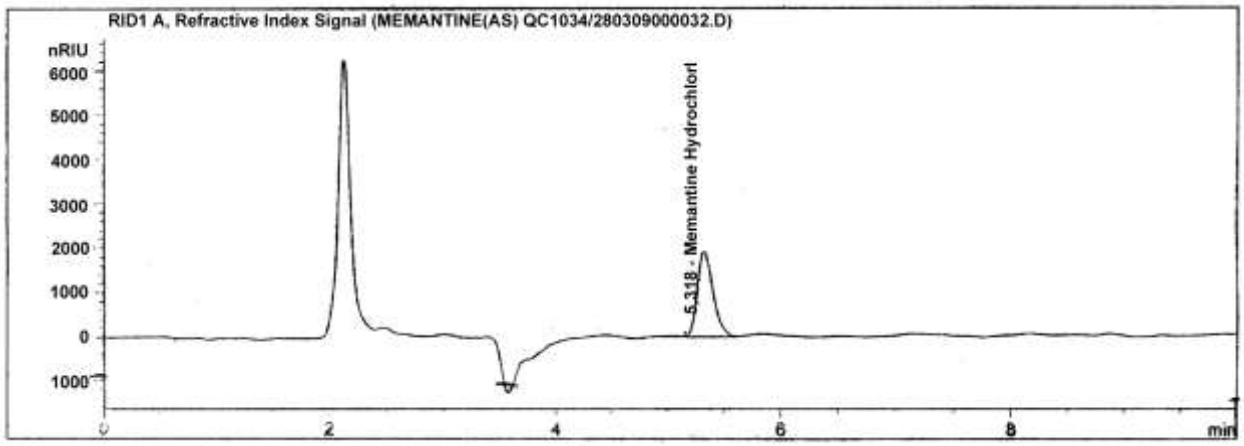
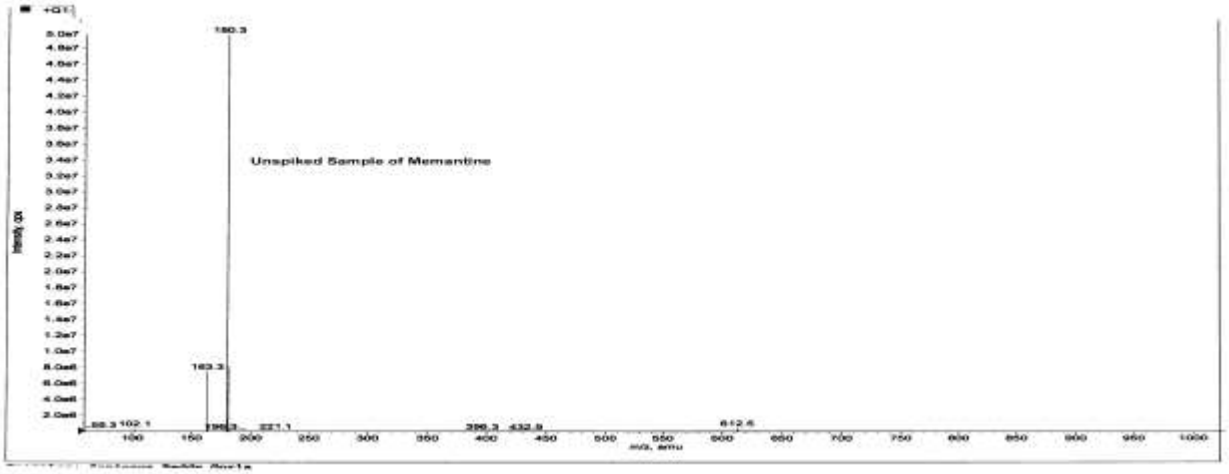
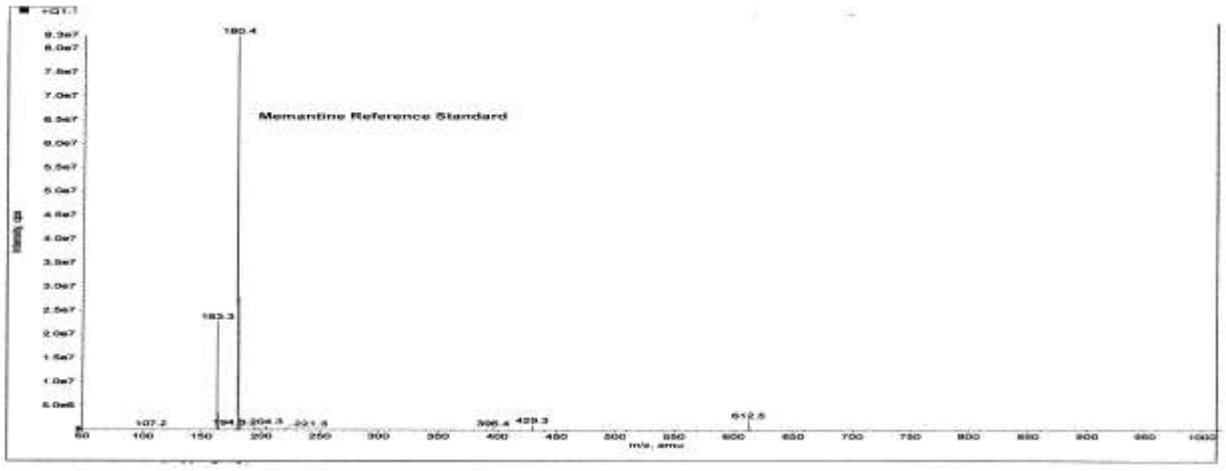


Figure 3: **Unspiked Sample of Memantine Hydrochloride chromatogram.**





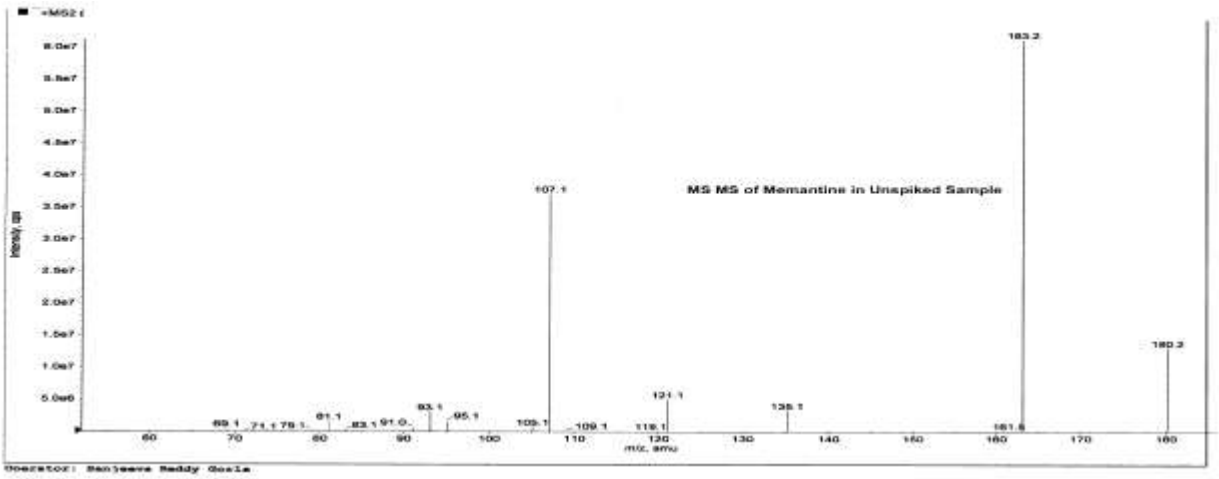


Figure 4: **Mass spectrum of Unspiked sample of Memantine hydrochloride and Memantine hydrochloride Reference standard andMS MS of Unspiked sample of Memantine hydrochloride and Memantine hydrochloride Reference standard.**

Sample solution spiked with the known related substance of memantine hydrochloride was analysed in triplicate and purity of memantine peak was checked by LC-MS, based on LC-MS mass spectrum the memantine peak was spectrally pure and no additional mass observed at memantine peak. The purity data of memantine peak indicates that the peak is homogeneous and no co eluting peaks indicating specificity of the method (Fig 5) and mass spectra of memantine is shown in Fig 6. The specificity of the method is also indicated by % difference of 0.1% between the mean of assay value of unspiked and spiked samples as shown in Table 2.(Acceptance criteria: % difference should not be more than 1)

Table 2 Specificity.

Sample	n	Mean	%difference
Unspiked sample	3	100.56	
Spiked sample	3	100.44	0.12

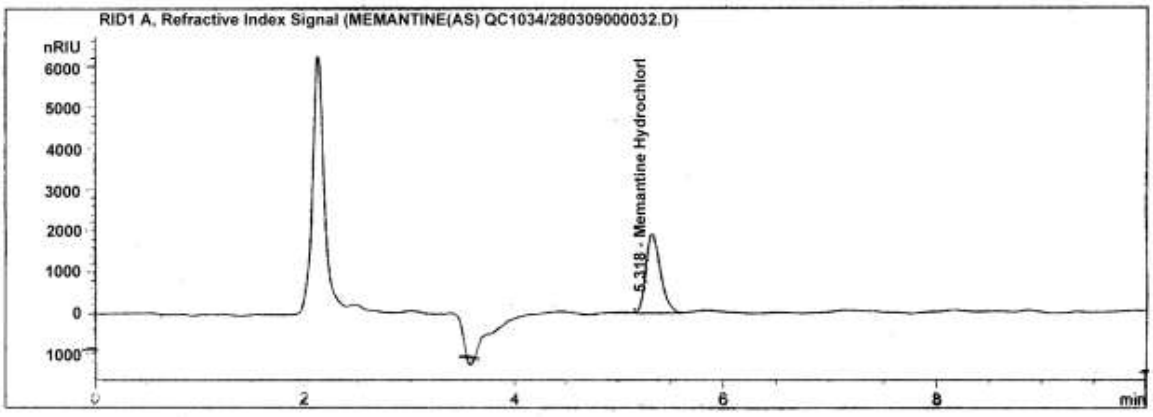


Figure 5 Spiked sample of Memantine hydrochloride

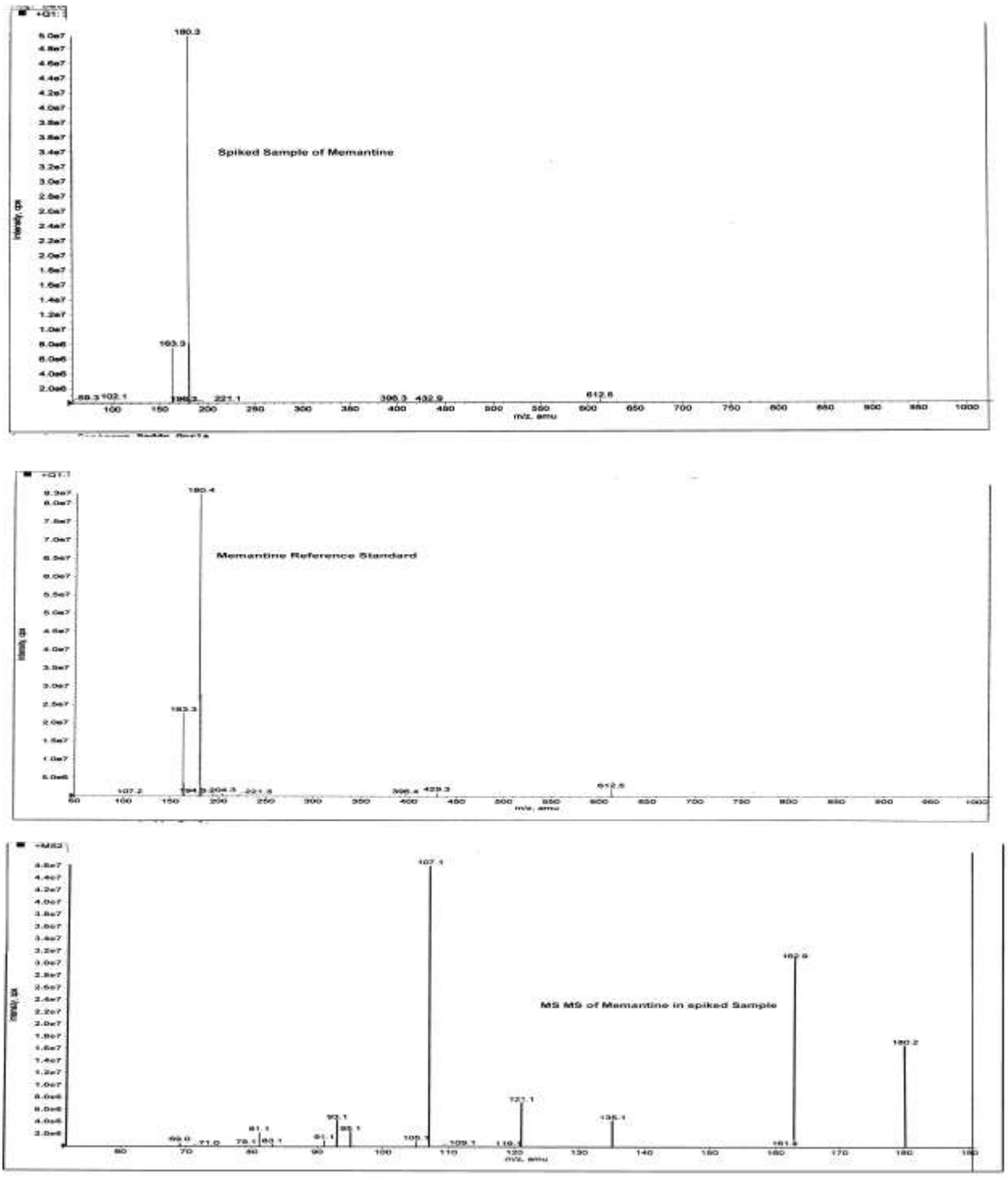


Figure 6 Mass spectrum of spiked sample of Memantine hydrochloride and Reference standard Memantine hydrochloride and MS MS of spiked sample of Memantine hydrochloride and Reference standard Memantine hydrochloride.

Forced Degradation study

A forced degradation study was carried out on Memantine hydrochloride with the following conditions. i. Control sample - No treatment and analysed by proposed method, ii. Solid state

degradation – a. Thermal degradation - Sample was subjected to thermal degradation by keeping at 105˚C for 114 hrs, followed by analysis by the proposed method. b. Photolytic degradation - Photolytic degradation study was carried out by exposing the sample to light in a photolytic chamber at 2600 LUX for 114 hrs. and analyzed by the proposed method. c. Humidity degradation - Sample was subjected to humidity degradation by keeping at 25 ˚C at 92% RH for 114 hrs., followed by analysis by the proposed method. iii. Liquid degradation – a. Hydrolytic and Oxidative degradation - Sample was separately treated with 5.0N hydrochloric acid, I.0N Sodium hydroxide and 30% w/v hydrogen peroxide solutions.

Solutions of these samples were prepared as per the conditions given in Table3 and analyzed by the proposed method and the data is summarized in the Table 3.

Table 3. Forced degradation of Memantine Hydrochloride

Mode of degradation	Condition	Assay (% w/w)	% degradation	Peak purity by LC-MS
Control	No treatment	100.25	-	Mass spectrum similar to the mass spectrum of reference standard
Acid degradation 5.0 N HCl/5mL	Initial	89.86	10.39	Mass spectrum similar to the mass spectrum of reference standard
Alkali degradation 1.0 N NaOH/2.5mL	Room temperature(RT)/5min	78.12	22.13	Mass spectrum similar to the mass spectrum of reference standard
Peroxide degradation 30% w/v H_2O_2/5mL	80˚C/15 min.	71.52	28.73	Mass spectrum similar to the mass spectrum of reference standard
Thermal degradation	105 ˚C/114 hrs.	100.53	No degradation observed	Mass spectrum similar to the mass spectrum of reference standard
Photolytic degradation	2600 Lux/114 hrs.	100.78	- No degradation observed	Mass spectrum similar to the mass spectrum of reference standard
Humidity degradation	25 ˚C/92%RH/ 114 hrs.	100.35	No degradation observed	Mass spectrum similar to the mass spectrum of reference standard

Using peak purity test (by LC-MS), the purity memantine peak was checked at every stage of the above mentioned study. The peak purity data show that the memantine peak is homogeneous and has no co eluting peaks indicating that the method is stabile and specific.

Precision

System Precision

Six replicate injections of standard solution were given into the HPLC system. Data along with the % RSD of area of memantine peak are shown in Table 4. The peak area indicate an acceptable level of precision for the analytical system (Acceptance criteria: Relative standard deviation(RSD) should not be more than 2.0%).

Table 4 System Precision of Memantine Hydrochloride

Injection No.	Peak Area
1	19088.498
2	19233.193
3	19087.051
4	19522.783
5	19596.502
6	19650.764
Mean	19363.132
% RSD	1.33

Method Precision

Six samples of a single batch of memantine hydrochloride were analysed as per the proposed method. Data is presented in Table5.The % RSD value indicates that the method has an acceptable level of precision. (Acceptance criteria: RSD should not be more than 2%)

Table 5 Method Precision of Memantine Hydrochloride

Preparation	Sample weight (mg)	Peak Area	% Assay
Sample preparation-1	25.012	19489.010	100.35
Sample preparation-2	25.125	19662.298	100.79
Sample preparation-3	24.523	19087.810	100.25
Sample preparation-4	25.120	19646.147	100.73
Sample preparation-5	24.956	19463.490	100.45
Sample preparation-6	25.325	19825.042	100.82
Mean			**100.57**
% RSD			**0.24**

Linearity of response

The linearity of the method should be tested in order to demonstrate a proportional relationship of response versus analyte concentration over the working range. It is usual practice to perform linearity experiments over a wide range of analyte. This gives confidence that the response and concentration are proportional and consequently ensures that calculations can be performed using a single reference standard/working standard, rather than the equation of a calibration line. The linearity of response for Memantine hydrochloride was determined in the range of 50µg/ mL to 150 µg/mL. Each solution was injected in duplicate into the HPLC system and the mean area, corrected area of the peak due to memantine hydrochloride was calculated. Data shown in Table

6 and represented graphically in Figure 7 indicate that the response is linear over the specified range. (Acceptance criteria: Correlation coefficient should not be less than 0.999).

Table 6 Linearity of response of Memantine Hydrochloride

Level	Concentration	Mean area	Corrected area
50 % of target concentration	50	9681.304	9705.432
80 % of target concentration	80	15883.541	15923.127
90 % of target concentration	90	17664.442	17708.467
100 % of target concentration	100	19334.521	19382.708
110 % of target concentration	110	21056.041	21108.518
120 % of target concentration	120	23064.524	23122.007
150 % of target concentration	150	28613.236	28684.548
SLOPE			**187.8223**
INTERCEPT			**594.1692**
CORRELATION COEFFICIENT			**0.9994**
RANGE: 50 % to 150 % of target concentration (i.e 50 ppm to 150 ppm)			

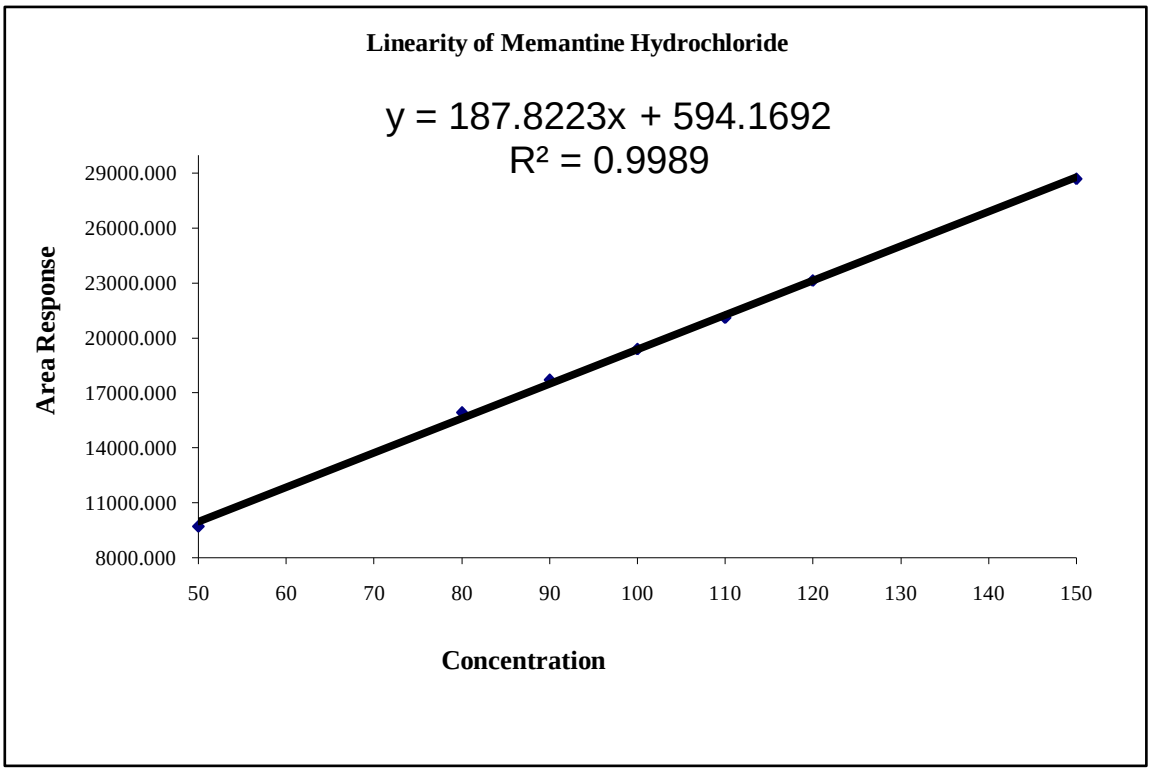


Figure7: Linearity Graph of Memantine Hydrochloride

Ruggedness

Method ruggedness was verified by analyzing six sample preparations of single batch of memantine hydrochloride by two different analysts using two different instruments and columns on different days. The mean standard deviation and % RSD for two sets of data is shown in Table 7. Ruggedness of the method is shown by the overall RSD value between the two sets of data (Acceptance criteria: Overall RSD should not be more than 2%).

Table 7. Method Ruggedness of Memantine Hydrochloride

Preparation	Assay(% w/w)	Assay(% w/w)
Sample preparation-1	25.046	19818.137
Sample preparation-2	25.151	19941.613
Sample preparation-3	25.125	19854.542
Sample preparation-4	24.921	19567.763
Sample preparation-5	25.010	19684.305
Sample preparation-6	24.928	19637.962
Mean	100.57	100.I8
%RSD	0.24	0.36
Overall Mean	100.34	
Overall % RSD	0.33	

Stability in analytical Solution

A standard and sample solution of memantine hydrochloride was prepared and kept at room temperature. It was analysed initially at different time intervals. Data is shown in Table 8 & 9 (Acceptance criteria: % of difference in area with reference to initial area should not be more than 1%)

Table 8 Stability of Standard solution of Memantine Hydrochloride

Time (hrs)	Standard solution			
	Room temperature		At 2-10°C	
	Mean Area	% Difference w.r.t. Initial	Mean Area	% Difference w.r.t. Initial
Initial	19602.622	---	19776.416	---
3 hours	19780.847	0.91	19812.412	0.18
6 hours	19492.294	0.56	19611.631	0.83
9 hours	19444.054	0.81	19881.904	0.53
12 hours	19657.084	0.28	19835.014	0.30
24 hours	19471.311	0.67	19645.262	0.66

Table 9 Stability of Sample solution of Memantine Hydrochloride

Time (hrs)	Sample solution			
	Room temperature		At 2-10°C	
	Mean Area	% Difference w.r.t. Initial	Mean Area	% Difference w.r.t Initial
Initial	23865.194	---	19866.462	---
3 hours	23675.188	0.80	19742.985	0.62
6 hours	23857.510	0.03	20024.218	0.79
9 hours	23748.042	0.49	20024.843	0.80
12 hours	23840.438	0.10	19697.764	0.85
24 hours	23910.518	0.19	19892.229	0.13

The % difference in area with reference to initial for memantine hydrochloride in standard solution at different time intervals are in the range of 0.18% to 0.91% when stored at room temperature and 2-10°C for 24hours, which is well within the acceptance criteria of not more

than 1.0 %.The % difference in area with reference to initial for memantine hydrochloride in sample solution at different time interval are in the range of 0.03% to 0.85% when stored at room temperature and at 2-10°C for 24 hours, which is well within the acceptance criteria of not more than 1.0 %. Based on the above data it is concluded that the standard solution and sample solution can be used up to 24 hours after preparation when stored at room temperature and 2-10°C.

Accuracy

The accuracy of the method has been established based on linearity, precision and specificity study of the method.

Robustness

Robustness of the method was investigated by deliberately varying the instrumental conditions such as changing the flow rate (0.7 mL/min. &1.1 mL/min), changing the Column oven temperature(35°C&45°C), changing the pH of buffer (5.3& 5.7) and changing the mobile phase composition - Buffer: MeOH (380:620&420:580).The absolute difference in assay results of memantine hydrochloride obtained in the normal condition (Repeatability of sample preparation-1) and altered conditions were calculated. The results are presented in the Table 10 for memantine hydrochloride.

Table 10 System suitability for Memantine Hydrochloride

Altered condition	Retention time (min)	Tailing factor	Theoretical plates	% RSD (six replicates)	Average Area	% Assay	Absolute Difference (W RT
Unaltered (Repeatability)	5.317	1.295	6996	1.33	19489.010	100.35	---
Flow rate 0.7 mL/min	7.019	1.450	8312	0.30	24604.007	100.01	0.34
Flow rate 1.1mL/min	4.452	1.361	6369	1.59	15491.870	100.15	0.20
Column Oven Temperature(35°C)	5.785	1.437	6899	0.61	18732.851	100.03	0.32
Column Oven Temperature(45°C)	5.241	1.377	6660	1.34	19349.607	99.91	0.44
Buffer pH (5.3)	5.581	1.289	7183	1.34	19155.495	100.01	0.34
Buffer pH (5.7)	5.562	1.685	7197	0.27	19952.237	100.01	0.34
Mobile phase composition 380:620)	4.947	1.409	6669	1.34	19403.640	100.44	0.09
Mobile phase composition (420:580)	5.987	1.642	7160	1.21	19287.580	99.99	0.36

The absolute difference in the results obtained by individual altered condition and repeatability sample preparation-1 are observed well within the acceptance criteria of NMT 1.0%. The system suitability parameters like % RSD for six replicate injections of standard solution, column efficiency and tailing factor are not significantly changed with altered conditions. Hence it is concluded that the method is robust to the specified changes in flow rate, mobile phase composition, column oven temperature and pH of the buffer.

Results summary

Memantine hydrochloride standard solution was prepared at the working concentration of 100 % level (100 ppm) to check the system suitability. The standard solution was injected into HPLC system before starting every validation parameter and peak response was measured in six replicates. The mean and relative standard deviation was calculated and system suitability parameter like column efficiency and tailing factor was reported. The comparative results are presented in the Table 11.

Table 11Comparative data of system suitability of Memantane Hydrochloride

Parameter	Retention time (min)	Tailing factor	Theoretical plates	% RSD (six replicate injections)
System suitability	5.317	1.295	6996	1.33
Specificity	5.317	1.295	6996	1.33
Linearity and Range	5.317	1.295	6996	1.33
Solution stability(RT)	5.309	1.367	7527	1.51
Solution stability (2-10°C)	5.479	1.643	7175	0.80
System Precision	5.317	1.295	6996	1.33
Repeatability	5.317	1.295	6996	1.33
Intermediate Precision	5.453	1.330	7379	1.06

CONCLUSION

A simple isocratic reverse phase assay method was optimized and validated; the method is selective, precise and accurate and was successfully applied to analysis of commercially available Memantine hydrochloride drug substance. Memantine hydrochloride is not easily detected by normal HPLC using UV detection because of absence of a chromophoric group. A simple rephrase isocratic HPLC method using Refractive index detection of Memantine hydrochloride. This is a simple, cost effective, time saving and very effective means of enhancing the chromatographic detection of the compound.

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