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Research Article

Multimedia Dissolution of Memantine HCl Tablets by Precolumn Derivatized HPLC Method and the Reagents Key Functional Group Assayed by Quantitative IR Spectroscopy

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ABSTRACT

The present paper deals with the development and validation of multi media dissolution of Memantine HCl tablets by precolumn derivatization by Phenoxy acetyl chloride (PAC) and Amantadine HCl, used as derivatizing agent and internal standard respectively. Memantine base was extracted from aqueous medium by basic toluene and added suitable volume of 0.15% v/v PAC solution in toluene. The solution was kept for five minutes for completion of the reaction and stopped the reaction by an organic solvent. The chromatographic was achieved by injecting 20 micro liter of derivatized mixture in HPLC column Inertsil ODS 3V [250*4.6] 5 micron. The mobile phase has 15% of 3% w/v ammonium acetate solution in methanol and the flow rate was maintained 1.5mL/minutes. Amantadine HCl was used as an internal standard, the Amantadine and Memantine peaks were eluted at the retention time of about 5.3 and 8.2 respectively. The volume, concentration and time have been optimized for derivatizing reagent and the key functional group has been assayed by Quantitative IR spectrum. After developed and validated method was used for multimedia dissolution profile was performed for 0.1N Hydrochloric acid, 0.1N Hydrochloric acid with 2% sodium chloride, pH 4.5 acetate buffer, pH 6.8 phosphate buffer and pH 7.2 phosphate buffer. Dissolution was performed 900mL of each media, Basket and 100rpm.

Keywords: Memantine HCl, Phenoxy acetyl chloride (PAC), Multimedia dissolution, Precolumn derivatization, Quantitative Infrared spectroscopy.

1. INTRODUCTION

Memantine hydrochloride (1-amino3, 5-dimethyladamantane hydrochloride) is a tricyclic amine [Structures I] chemically and pharmacologically related to the anti-viral prototype amantadine and its α-methyl derivative rimantadine. Amantadine and rimantadine have been approved in the U.S. for the prophylaxis and treatment of influenza. Amantadine is also approved for the treatment of Parkinsonism. Memantine is used in Parkinson's disease and movement disorders.[1-2]

Dissolution is pharmaceutically defined as the rate of mass transfer from a solid surface into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature and solvent composition. Dissolution testing is a critical preformulation solubility analysis research tool in the process of drug discovery that entails measuring the stability of the investigational product, achieving uniformity in production lots and determining its in vivo availability[3]. A pre column derivatized HPLC method has been developed for marketed Memantine HCL tablets in multimedia with pH range 1.2 to 7.2 and ionic strength of buffers has used same as in United States of Pharmacopeia (USP)[4] and the multimedia dissolution was performed in the dissolution condition of 900mL dissolution media, Basket and 100rpm.[5]

Quantification of Memantine HCL can not be done by direct HPLC-UV method because it is having no chromophoric group[6].

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So it has to be derivatize before analyze, and various derivatizing agent like FMOC, Dansyl chloride etc and analyzed by HPLC-fluorescence measurement[7–8]. Memantine was determined by capillary zone electrophoresis with indirect UV detection[9] and also measured by GC without derivatization or enhanced sensitivity derivatized and analyzed by GC.[10–14] Dansyl chloride and 9-fluorenylmethyl chloroformate react readily with most primary and secondary amines in alkaline buffer, and it is regarded as the derivatizing reagent of choice in the preparation of highly fluorescent compounds[7–8].

Hereby plenty of methods available to determine the Memantine by GC, GC-MS, precolumn derivatization with various reagents like o-phthalaldehyde / N-acetyl-L-cysteine, Dansyl chloride, FMOC and 2-napthoxy acetyl chloride etc, at the same time each reagent having its own limitation. While compare with 2-napthoxy acetyl chloride, Phenoxy acetyl chloride is same structural similarity, but the major draw back of 2-napthoxy acetyl chloride is very moisture sensitive solid and there is no commercial manufacturer, each and every time you have synthesis its own. In this paper deals another major point investigating the assaying of the reagent's key functional it is carbonyl group hence carbonyl group is a major contribution for the derivatization. All the regulatory bodies may pose the question if the reagent used for dissolution test it has to be assayed to its equivalent of the key functional group. So carbonyl group, C-O bands has been quantified by quantitative IR spectroscopy.

Infra spectroscopy[15] is certainly one of the most important analytical techniques and its having the great advantages of IR is that virtually any sample in virtually any state may be studied. Liquids, solutions, paste, powder, films, fibrous etc shall be analyzed with a judicious choice of sample preparation. Quantitative analysis of component shall be carried out by choosing the suitable band in the spectrum of the component of the interest. The band chosen for analysis should have the higher molar absorbtivity, not overlap with other peaks from other components if it is a mixture. Most simple quantitative infrared method of analysis use the intensities of the C=O, N-H, or O-H groups. The C=O band most commonly used because its strong band in the spectral region relatively free of absorption of other functional group. In addition, the carbonyl is not as susceptible as the N-H and O-H band to chemical change or hydrogen bonding. The best peak to chose in the C=O stretching band of NAC, observed at 1000 cm^{-1}.

The principal band in the 2000-1500cm^{-1} region are due to C=O and C=C stretching, carbonyl band it is one of the strong band usually occurs in 1000-2000 cm^{-1} and find the detail table below. Hence the PAC is lies in the region of 1000-1300 cm^{-1}. So the carbonyl group has been quantified from the absorption bands of 1000 cm^{-1}.

Compounds	Functional group	Type of Vibration	Characteristic Absorptions (cm-1)
Alcohol	C-O	stretch	1050-1150
Carbonyl	C-O, C=O	stretch	1670-1820
Ether	C-O	stretch	1000-1300
Acid	C-O	stretch	1210-1320
Aldehyde	C=O	stretch	1740-1720
Amide	C=O	stretch	1640-1690
Ester	C-O	stretch	1000-1300

2. MATERIALS AND METHODS

2.1 Materials

Phenoxy acetyl chloride (98%) was purchased from Aldrich (USA), Methanol (HPLC grade), Potassium dihydrogen orthophosphate (AR grade), Sodium acetate (AR grade), Sodium chloride (AR grade), Sodium hydroxide (AR grade), Toluene (AR grade) Triethylamine (AR Grade) were purchased from Qualigen (India). Water (HPLC grade) was used for the preparation of solutions. Admenta (Memantine HCl tablets were procured form Chennai, India)

2.2 Chromatography

The analytical separations were carried out on a Waters HPLC system, equipped with a 2695 separation module and 2996 photodiode array detector. The analytical column was Inertsil C18 (150 x 4.6 mm) 5 micron. 15% of 3% w/v ammonium acetate solution in methanol was used as mobile phase. The mobile phase was filtered through a 0.45 micron membrane filter. The flow rate was 1.5 mL/min and runtime was 10 minute. Column temperature was maintained at 25°C. UV detection was measured at 265 nm and the volume of sample injected was 10microL. The control of the HPLC system and data

collection was by Empower software. Amantadine HCl was used as an internal standard, the Amantadine and Memantine peak was eluted at the retention time of about 5.3 and 8.2 respectively.

2.3 Preparation of standard

Memantine HCl and Amantadine HCl were dissolved in water and base were extracted basic toluene and extract added in derivatizing reagent kept for 5 minutes and added organic solvent for stopping the reaction and analyzed by HPLC. The final concentration of Amantadine and Memantine was about 3ppm.

2.4 Preparation of Sample

Dissolution was performed 0.1N hydrochloric acid, 0.1N hydrochloric acid with sodium chloride, pH 4.5 acetate buffer, pH 6.8 phosphate buffer and pH 7.2 phosphate buffer in 900mL of medium, 100rpm and USP apparatus I basket. Dissolution was run and collected the sample at 10, 20, 30 and 45 minutes and extracted the base and derivatized before analysis. The final concentration of Amantadine and Memantine was about 3ppm.

2.5 Method optimization parameters

Some chromatographic parameters such as derivatization process time optimization, PAC volume optimization, PAC concentration optimization were investigated to obtain a good, specific, reproduce and accurate method.

2.6 Derivatization process time optimization

Memantine and Amantadine were extracted from standard solution and added reagent and kept aside for 2, 5, 10, 30, 45 and 60 minutes and analyzed by HPLC and area ratio with respect to Memantine was obtained constant from 5 minutes.

2.7 PAC volume optimization

Memantine and Amantadine were extracted from standard solution and added reagent 1, 2, 3, 4, 5, and 6mL and kept aside for 5 minutes and analyzed by HPLC and area ratio with respect to Memantine was obtained constant from 2mL reagents.

2.8 PAC concentration optimization

Memantine and Amantadine were extracted from standard solution and added 2mL reagent at the concentration of 0.05, 0.1, 0.15, 0.2, and 0.3% v/v kept aside for 5 minutes and analyzed by HPLC and area ratio with respect to Memantine was obtained constant from 0.15% v/v.

2.9 Organic solvent volume optimization for reaction stopping

Memantine and Amantadine were extracted from standard solution and added 2mL reagent 0.15% v/v kept aside for 5 minutes and added organic solvent 0.5, 1, 1.5, 2, 3, 4 and 5mL added for stopping reaction and analyzed by HPLC and the area ratio with respect to Memantine was obtained constant from 3mL of organic solvent.

2.10 Method Validation

2.10.1 System precision

Six replicate injections of standard solution were injected into the HPLC system. Data along with the % RSD of area ratio with respect to Memantine peak shown in Table 1 indicate an acceptable level of precision for the analytical system.

Table 1: System Precision

Injection no.	Area Ratio
1	0.911
2	0.912
3	0.905
4	0.924
5	0.921
6	0.920
Mean	0.900
Standard deviation (SD)	0.0
RSD (%)	0.8

2.10.2 Method precision

Six samples of a single batch of Memantine hydrochloride drug substance were analyzed as per the proposed method. Data is shown in Table 2. The % RSD value indicates that the method has an acceptable level of precision.

Table 2: Method Precision

Sample	% Dissolved	% Dissolved **
Tablet - 1	95	97
Tablet - 2	96	96
Tablet - 3	101	97
Tablet - 4	95	95
Tablet - 5	97	95
Tablet - 6	102	100
Mean	98	97
Standard deviation (SD)	3.1	1.9
RSD (%)	3.2	1.9

** Ruggedness

2.10.3 Linearity

The linearity of the method was established response ratio with respect to Memantine was plotted against the concentration of Memantine. The linearity of response for Memantine hydrochloride was determined in the range of 1.5 ppm to 4.5ppm. Data shown in Table 3 and represented graphically in Figure 7 indicate that the response is linear over the specified range.

Table 3: Linearity

Concentration in ppm	Response ratio
1.52	0.452
2.31	0.683
3.22	0.934
3.81	1.155
4.52	1.385
Correlation coefficient (R^2)	0.999

2.10.4 Accuracy

Accuracy was established by breaking the tablets in to two pieces and dropped in to dissolution vessel and performs the dissolution in the level of 50% and 150% from the 100% level. Data is shown in Table 4. The % RSD value indicates that the method has an acceptable level of precision.

Table 4: Accuracy

S. No	% Level	% Dissolved	"mg" recovered	% mg recovered	Average	% RSD
1	50%	48	2.4	96	98.7	3.5
		48	2.40	96		
		51	2.55	102		
		52	2.60	104		
		48	2.40	96		
		49	2.45	98		
2	100%	95	4.75	95	97.7	3.2
		96	4.80	96		
		101	5.05	101		
		95	4.75	95		
		97	4.85	97		
		102	5.10	102		
3	150%	151	7.55	101	99.8	2.3
		149	7.45	99		
		147	7.35	98		
		155	7.75	103		
		145	7.25	97		
		151	7.55	101		

2.10.5 Ruggedness

Method ruggedness was verified by analyzing six samples of a single batch of Memantine hydrochloride drug substance by two different analysts using two different instruments and columns on different days. The mean standard deviation and % RSD for the two sets of data is shown in Table 2. Ruggedness of the method is shown by the overall RSD value of 2.5% between the two sets of data.

2.10.6 Stability in analytical solution

A sample solution of Memantine hydrochloride drug substance was prepared and kept at room temperature. It was analyzed initially and at different time intervals. Data is shown in Table 5. Similarity factor for standard solution and percentage difference for percentage released, it meets the acceptance criterion, it is concluded that the sample is stable in analytical solution for at least 24 hrs.

Table 5: Solution Stability

	Standard area ratio	Similarity factor	Sample (% Dissolved)	% Difference
Initial	0.920	----	95	----
Day 1	0.915	1.00	96	1.0
Day 2	0.910	1.01	96	1.0

2.10.7 Robustness

Robustness of the method was investigated by deliberately varying the instrumental conditions such as flow rate (10%), organic content in mobile phase (2%) and column oven temperature (5°C), Samples were analyzed under each condition and the mean standard deviation and % RSD are shown in Table 6. Robustness of the method is indicated by the overall RSD values of variable chromatographic condition.

Table 6: Robustness

Parameter	Flow (mL/minutes)		Organic content %		Column temperature °C	
	1.3	1.7	102	98	20	30
Area Ratio	0.931	0.932	0.924	0.907	0.906	0.941
	0.921	0.913	0.908	0.901	0.903	0.935
	0.908	0.924	0.919	0.902	0.914	0.932
	0.911	0.924	0.923	0.914	0.918	0.927
	0.924	0.911	0.912	0.915	0.902	0.924
	0.916	0.918	0.908	0.913	0.915	0.925
Mean	0.919	0.920	0.916	0.909	0.910	0.931
SD	0.009	0.008	0.007	0.006	0.007	0.007
% RSD	0.9	0.9	0.8	0.7	0.8	0.7

2.10.8 Multimedia Dissolution

Dissolution was performed for various medium like 0.1N hydrochloric acid, 1.2 simulated gastric fluid (SGF), pH 4.5 acetate buffer, pH 6.8 phosphate buffer and pH 7.2 phosphate buffer. Data is shown in Table 7 for average percentage release in different dissolution medium.

Table 7: Multimedia Dissolution

Sample	% Dissolved				
Medium	0.1N HCl	1.2 SGF	pH 4.5	pH 6.8	pH 7.2
Tablet - 1	96	95	99	96	103
Tablet - 2	97	96	95	97	102
Tablet - 3	101	101	95	98	101
Tablet - 4	100	95	97	99	100
Tablet - 5	98	97	96	101	99
Tablet - 6	96	102	95	103	97
Mean	98	98	96	99	100
SD	2.1	3.1	1.6	2.6	2.2
RSD (%)	2.1	3.2	1.7	2.6	2.2

SGF-Simulated Gastric Fluid

Table 8: Overall compilation of validation [Results of entire study]

Parameter	Acceptance criteria	Results
System Suitability	% RSD Not More Than (NMT) 2.0%	0.7% to 0.9%
Precision	% RSD (six sample preparation) NMT 5.0%	1.7 to 3.2%
Linearity	Correlation coefficient Not Less Than 0.999	0.999
Accuracy	Percent Recovery 97% to 103%	97.7 to 99.8 %
Solution Stability - Standard	Similarity factor should be 0.98 to 1.02	1.00 to 1.01
Solution Stability - Sample	Percentage difference should be NMT 3.0	1.0
Robustness & Ruggedness	% RSD Not More Than 2.0%	0.7 to 0.9%

2.11 QUANTITATIVE INFRARED SPECTROSCOPY

2.11.1 Apparatus

Instrumental analyses were performed on a Perkin Elmer Fourier transform Spectrum version infrared spectrometer with 4 cm^{-1} resolution. The sample cell used was the CIRCLE accessory with the open-boat micro cell equipped with a ZnSe crystal.

2.11.2 Preparation of sample

2mg/ml of NAC solution prepared in toluene. Concomitantly determine the absorbance of sample solution in 0.5mm cells at the wavelength of absorbance at about 7.9 µm in the IR spectrophotometer. This solution was scanned at the intervals of Initial, 5, 10, 15 and 24 hours and the absorbance was measured at 1000 cm^{-1}. Data is shown in Table 9 for absorbance and percentage difference.

2.11.3 Linearity

Linearity was established from the five different concentration of NAC solution in toluene at the level of 50%, 75%, 100%, 125% and 150% from the target concentration. Data of linearity shown in Table 10 and plot of linearity was shown in Figure 8 respectively.

3. RESULTS AND DISCUSSION

3.1 Multimedia dissolution

Multimedia dissolution was performed for Memantine hydrochloride tablets by precolumn derivatization and analyzed by HPLC method for the determination and the method was validated as per ICH guidelines and the parameter was explained above. System suitability was performed and the percent related standard deviation and bracketing standard was found below 2.0% for the entire activity. Precision was performed in tablets and the percent relative standard deviation of six sample preparations was found 3.2 and 1.9% for precision and intermediate precision respectively. Linearity was established from 50% to 200% of the target concentration and the correlation coefficient was found 0.999. Accuracy was performed from 50% to 150% at three levels from the target concentration in broken tablets; recovery was found 97.7% to 99.8%. Range was covered from the precision, linearity and accuracy section. Robustness was proven by the suitability of the method and the percent related standard deviation was found 0.7% to 0.9% for this activity. Derivatized sample was analyzed by LC-MS and find the mass number of adduct of Memantine peak was found about 350, and it is matching with theatrical molecular weight of Memantine adduct. Dissolution profile in various media was performed and the percentage release found above 95% in all the media and percent RSD was found 1.7% to 2.6. HPLC analysis related data was shown in Table 1 to Table 7 and overall compiled validation result tabulated in Table 8. (For typical chromatogram, mass spectrum, structure of drug and adducts and linearity plot refer Figure 1 to Figure 8).

3.2 Quantitative IR Spectroscopy

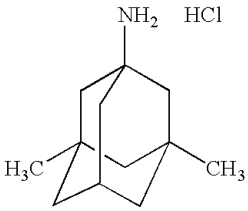
Active derivatization group carbonyl was assayed by quantitative IR spectroscopy and standard stability was proven from the absorbance of 24 hours in the solution, no significance change in the absorbance proves there are no changes in the potency of the reagent. (Acceptance Criteria: The absorbance of 2mg/ml of NAC solution should be not less than 0.25 at in 0.5mm cells at the wavelength of absorbance at about 7.9 µm in the IR spectrophotometer at 1000 cm^{-1}.

Table 9: Sample Stability – Quantitative IR

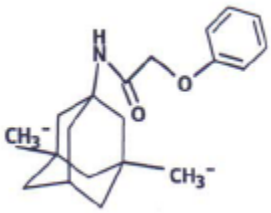
Sample	Absorbance	Difference	% Difference
Initial	0.2736	----	----
5 Hours	0.2612	0.0124	0.1
10 Hours	0.2754	0.0018	0.0
15 Hours	0.2841	0.0105	0.0
24 Hours	0.2631	0.0105	0.1

Table 10: Linearity – Quantitative IR

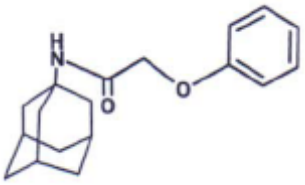
Concentration in ppm	Absorbance
1.0	0.1321
1.5	0.2081
2.0	0.2736
2.5	0.3481
3.0	0.4101
Correlation coefficient (R^2)	0.998



Memantine HCl



Memantine – NAC Adduct



Amantadine – NAC Adduct

Fig.1 (a): Structure of Memantine, Amantadine and derivatized Product

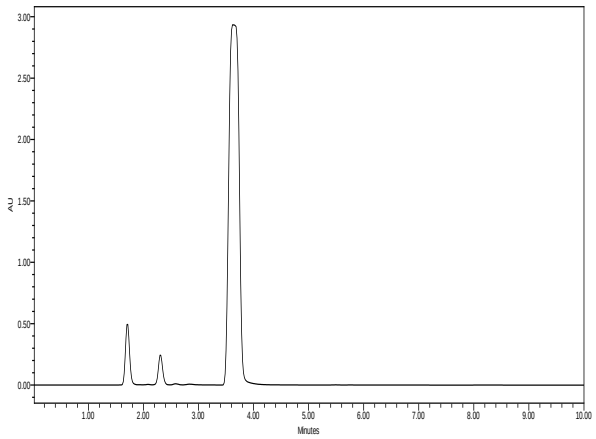


Figure 1 (b) : Typical chromatogram of Blank

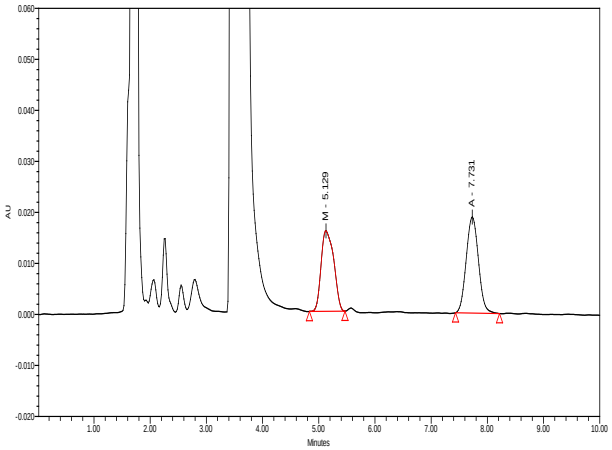


Figure 2: Typical chromatogram of Standard

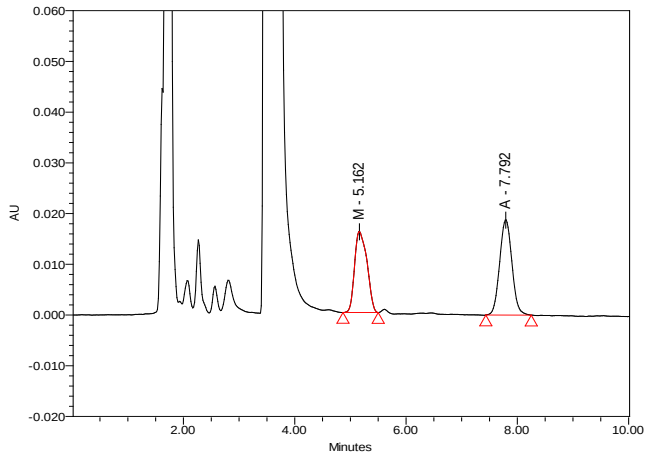


Figure 3: Chromatogram/Sample/pH 1.2 SGF

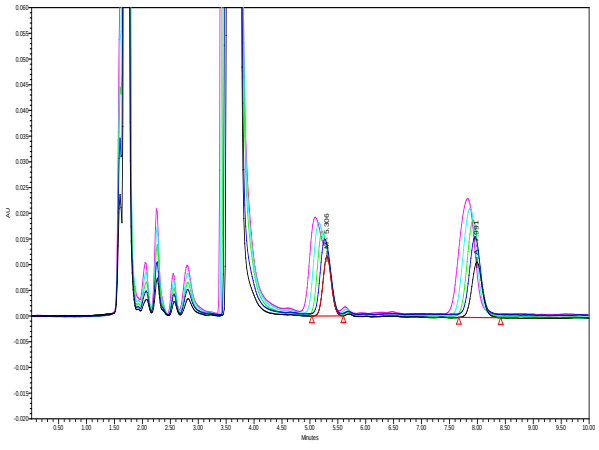


Figure 4: Linearity - Overlay

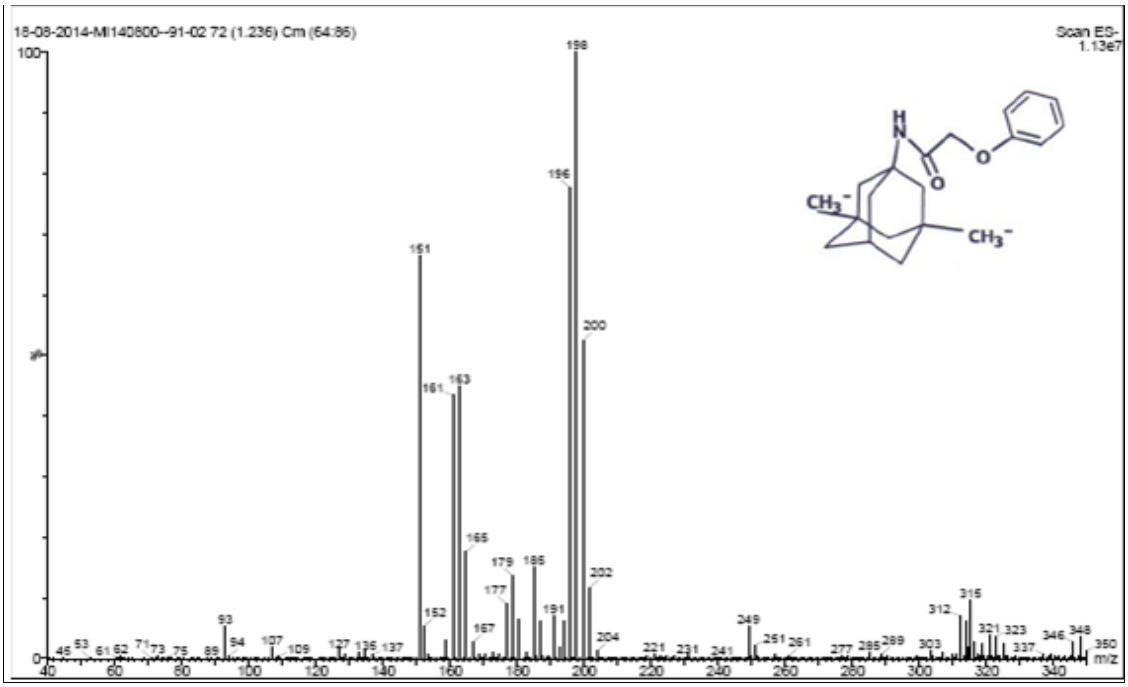


Figure 5: Typical Mass spectrum Memantine Adducts

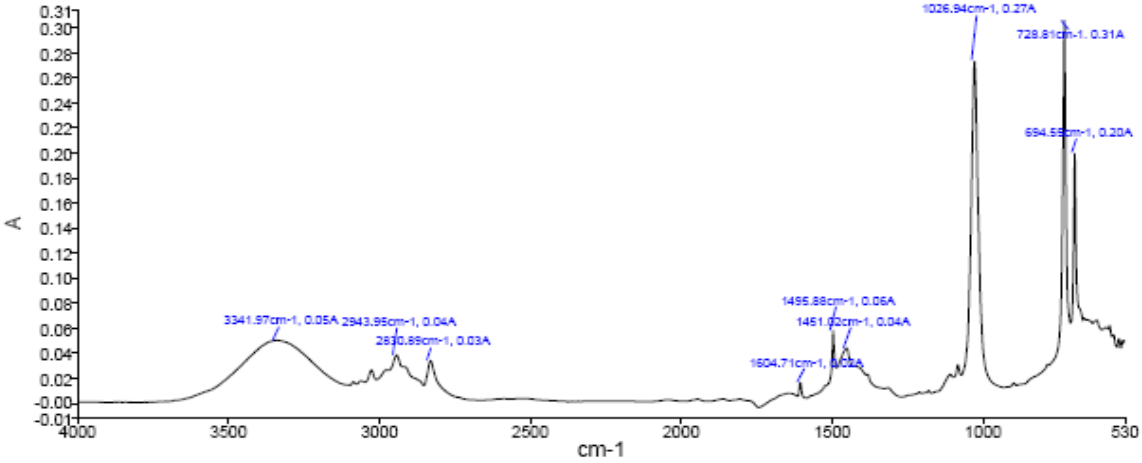


Figure 6: Typical IR Spectrum of Carbonyl function

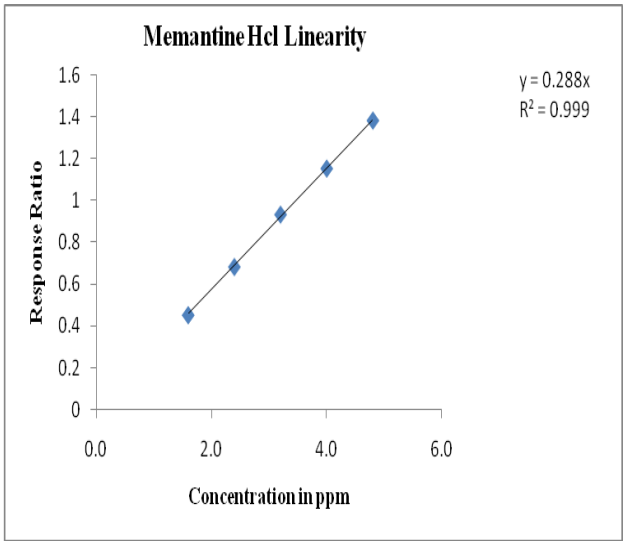


Figure 7: Linearity - Memantine

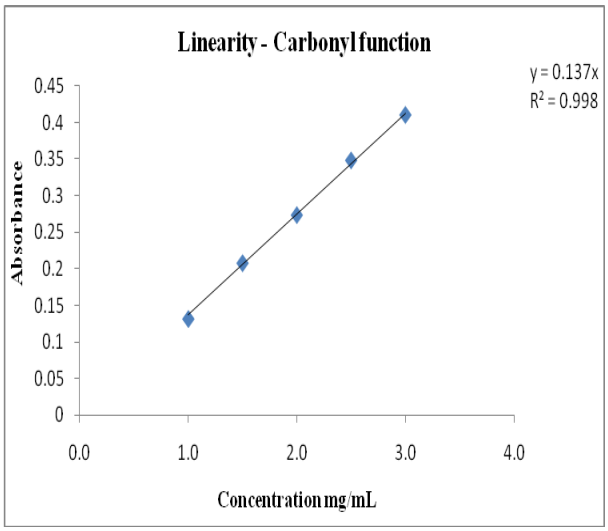


Figure 8: Linearity - Carbonyl function

CONCLUSION

This study presents a simple and validated HPLC method for performing the dissolution of Memantine HCl tablets in various dissolution media. The method developed is specific, accurate, precise and rugged. Key functional group carbonyl was quantified by quantitative IR spectroscopy and the stability and linearity was proven.

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