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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF MEMANTINE HYDROCHLORIDE IN THE PRESENCE OF ITS RELATED COMPOUNDS BY GAS CHROMATOGRAPHY

B.SRIDEVI* [1] | DR. M. AJITHA [2] | K.S.L. HARIKA [3]

[1] CENTRE FOR PHARMACEUTICAL SCIENCES, IST, JNTU-H.

[2] CENTRE FOR PHARMACEUTICAL SCIENCES, IST, JNTU-H.

[3] CENTRE FOR PHARMACEUTICAL SCIENCES, IST, JNTU-H.

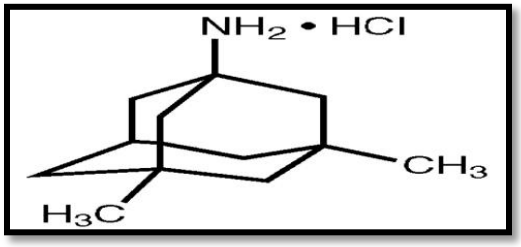
ABSTRACT:

A simple ,reliable,economical,and accurate Gas chromatographic method was developed for the estimation of Memantine Hcl Impurities in ER Tablet dosage form .The chromatographic separation was achieved on a chromatography column (stationary phase) is AT-5,length30m,internal diameter 0.25mm and film thickness1.0µ. The gas flow rate is 4.0ml/min and the load is 2µl .Diluent –n-hexane. The Rt valve of Memantine Hcl, Impurity-E was found to be 19.9 min, 28.1 min respectively. The developed method was validated for linearity, accuracy, precision, specificity, system suitability, and solution stability. Results of all validation parameters were within the limits as per ICH Guidelines.

KEYWORDS:

INTRODUCTION:

Memantine hydrochloride is a 1-amino-3, 5-dimethy ladamantane derivative developed by Merz co. for the treatment of Alzheimers disease by decreasing abnormal activity in the brain. This medication was marketed under the brand name Namenda.Memantine works by blocking the NMDA receptors in the brain and improves brain functioning in Alzheimers disease. One potential impurities Imp-E are formed during the synthesis of Memantine hydrochloride



MEMANTINE HYDROCHLORIDE

Memantine hydrochloride is an aliphatic compound and does not have any chromophores,and hence the determination of Memantine and its related substances in drug substances and drug product was a critical activity during drug testing. Several analytical methods have been reported for the determination of Memantine on high performance liquid chromatography (HPLC)in combination with mass spectrometry and ultraviolet detection or fluorescence detection usually after derivatization with a suitable chromophores or fluorophore. Most of these reported liquid chromatography-mass spectrometry(LC-MS) methods require tedious extraction procedures, which are time consuming ,complex, and expensive. There is a need to have the simple and user friendly technique to resolve all Memantine hydrochloride related impurities in drug substances. Even though other detection techniques such as capillary electrophoresis,UV labelling reagents are present, none of them can be used for the separation and quantification of Memantine hydrochloride and it process related impurities in drug substance.

EXPERIMENTAL

Materials and reagents. The investigated sample of Memantine hydrochloride and its potential process-related impurities were received from USP .In addition ,analytical reagent grade sodium hydroxide, Anhydrous sodium sulfite were purchased from Merck.n-hexane grade GC (spectrochem).Highly pure water obtained from Millipore system was used throughout the analysis.

GC (ANALYTICAL) INSTRUMENTATION AND OPERATING CONDITIONS.

The system used for method development and method validation was carried out on shimadzu GC-MS-QP 2010 Gas chromatograph and it is used by software lab solution version 5 The detector was performed by means of flame ionization detector(FID). The HP-5(30m length ×0.25mm ID,0.50µm)film thickness column has been used for method development and method validation studies. The operating conditions of the column oven was programmed as follows: initial column oven temperature,75ºc;hold for 5 min,and increased to 250ºc at the rate of 10ºc/min;hold for10min.The run time of analysis was 30minutes.the injector and detector temperature was kept at 220ºc and 300ºc,respectively.Helium was used as a carrier gas .The spilt ratio was set at 1:50 and make up gas 30ml/min,hydrogen gas flow 40ml/min and zero air flow 400ml/min.sample was injected by the instruments auto

sampler with injection volume of 2.0µl and n-hexane as the syringe cleaning solvent between injections.

PREPARATION OF SOLUTION –A (5N NAOH):

Weighed 50g of NaoH in 250mL of DM water and mix well.

PREPARATION OF BLANK:

Weighed about 15mL of solution- A and 20mL of n-hexane into 100mL volumetric flask and shake for 10mints and transfer the contents into separator. Allow the layers to separate ,take a portion of the top hexane layer, dry the organic layer by agitate with anhydrous sodium sulfite and allow to stand for a few minutes to ensure all the remaining water gas been removed, Used the clear filtrate

PREPARATION OF STANDARD STOCK SOLUTION:

Weigh and transfer about 26mg of Memantine Hcl working standard into 50mL of volumetric flask add 15mL of solution-A shake for 5min and sonicate for 5mints, add 20mL of n-hexane and shake for 10min,transfer the contents to a separator , take a portion of the top hexane layer, dry the organic layer by agitate with anhydrous sodium sulfite and allow to stand for a few minutes to ensure all the remaining water gas been removed. Used the clear filtrate.

PREPARATION OF STANDARD SOLUTION:

2mL of above solution into a 100mL of volumetric flask make up the volume with n-hexane.

PREPARATION OF PLACEBO SOLUTION:

weigh and transfer the crushed placebo powder equivalent to about 100mg of Memantine HCl into a 100ml volumetric flask add about 15ml solution-A and shake for 5min and sonicate for 5min,add20ml of n-hexane and shake for 10min,transfer the contents into a separator. Allow the layers to separate ,take a portion of the top hexane layers ,dry the organic layer by agitate with anhydrous sodium sulfite and allow to stand for a few minutes to ensure all the remaining water gas been removed.

PREPARATION OF SAMPLE SOLUTION:

weigh the capsules and open the shells and transfer the crushed placebo powder equivalent to about 100mg of Memantine HCl into a 100ml volumetric flask add about 15ml solution-A and shake for 5min and sonicate for 5min,add20ml of n-hexane and shake for 10min,transfer the contents into a separator. Allow the layers to separate ,take a portion of the top hexane layers ,dry the organic layer by agitate with anhydrous sodium sulfite and allow to stand for a few minutes to ensure all the remaining water gas been removed.

RESULTS AND DISCUSSION:

Detection of impurities. Laboratory batches of crude Memantine hydrochloride were analyzed for their related substances identification using the developed GC-FID method. Samples were subjected to GC analysis. Imp-E was detected in the crude Memantine hydrochloride batch sample. Impurity and sample were co injected with Memantine hydrochloride to confirm the retention times.

Development of chromatographic conditions:

The main objective of present study id to develop a simple, rapid method capable of eluting Memantine and its related impurities within the short run time which complies with the requirement of system suitability. The HP-5(30m length ×0.25mm ID,0.50µm)film thickness column has been used for method development and method validation studies. The operating conditions of the column oven was programmed as follows: initial column oven temperature,75ºc;hold for 5 min,and increased to 250ºc at the rate of

10ºc/min;hold for10min.The run time of analysis was 30minutes.the injector and detector temperature was kept at 220ºc and 300ºc,respectively.Helium was used as a carrier gas .The spilt ratio was set at 1:50 and make up gas 30ml/min,hydrogen gas flow 40ml/min and zero air flow 400ml/min.sample was injected by the instruments auto sampler with injection volume of 2.0µl and n-hexane as the syringe cleaning solvent between injections. The blank and standard solution chromatograms were representedin fig.2 and fig.3 and system suitability parameters were summarized in Table-1

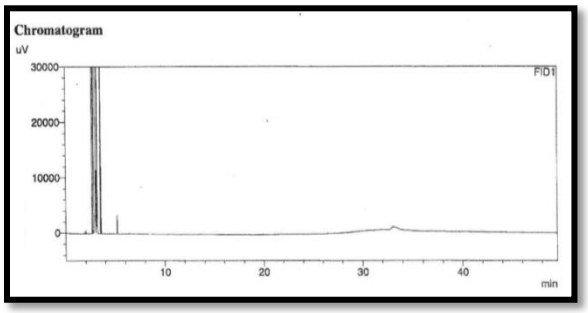


FIG.1 BLANK SOLUTION CHROMATOGRAM

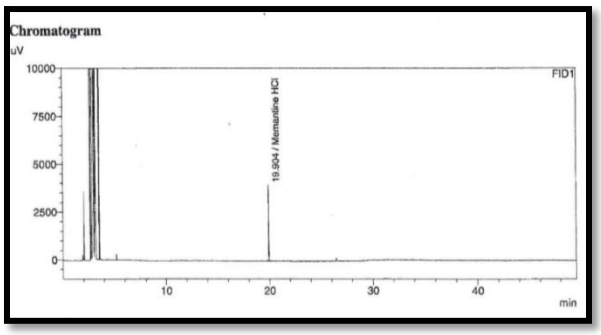


Fig.2 Standard solution chromatogram

Table.1 System suitability parameters

Parameter	Result	Acceptance criteria
Tailing factor	1.1	NMT 2.0
%RSD for six standard injections	1.6	NMT 10.0%
System performance	7.7	NMT 10.0%

METHOD VALIDATION:

The developed and optimized GC method validated according to ICH guidelines for the following parameters:

1. Specificity
2. Precision
3. Accuracy
4. Linearity
5. Solution stability
6. LOQ& LOD

SPECIFICITY:

Blank, placebo, sample and impurity were injected and compared, there is no peak observed at the retention time of Memantine peak from the analysis of the Diluent (blank) and placebo.so,the developed method was specific and do not have any interference. Chromatograms were represented in Fig.3, 4 & 5 .

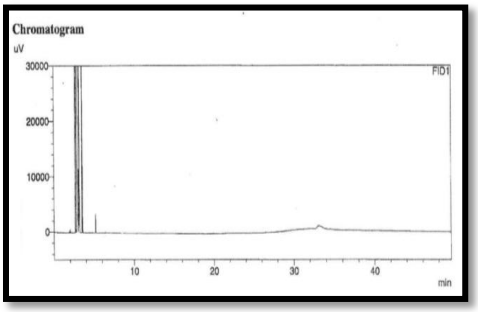


FIG.3 BLANK SOLUTION CHROMATOGRAM

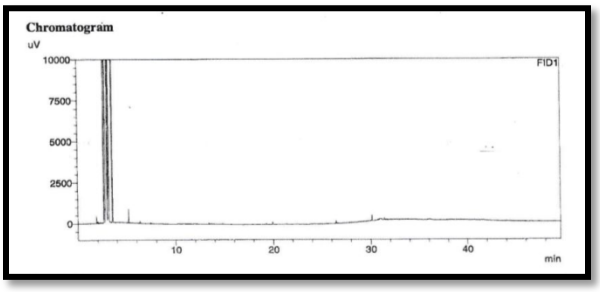


FIG.4 PLACEBO CHROMATOGRAM

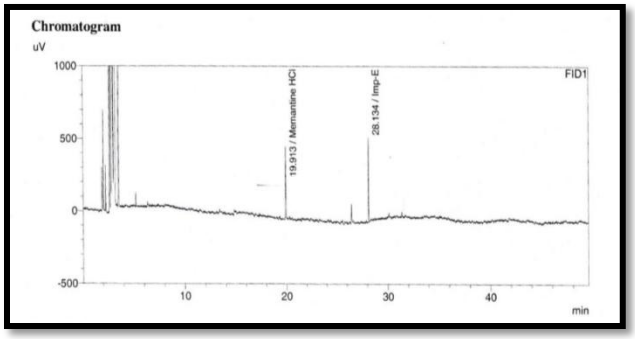


FIG.5 IMPURITY INTERFERENCE CHROMATOGRAM

PRECISION:

METHOD PRECISION:

To evaluate the method precision for related compounds method, six replicate test preparations for Memantine hydrochloride. By spiking the known impurity at specification level were prepared and analyzed as per test method. The %RSD for known impurity and % of total impurities were calculated and found to be within the acceptance criteria.

Name	Area at RRT@0.71 UNKNOWN	AREA at RRT@ 1.4 IMP-E	% UNKNOWN IMP	% IMP-E
1	7374	88749	0.07	0.52
2	7569	88735	0.07	0.52
3	7952	88527	0.08	0.51
4	7455	88768	0.07	0.52
5	7841	88146	0.08	0.51
6	8199	88147	0.08	0.51

Average	**0.7**	**0.51**
STDEV	0	**0**
% RSD	0.41	**0.03**

Intermediate precision (Intra day precision): The intermediate precision for Memantine hydrochloride compound was carried out and results were tabulated

Name	Area at RRT@0.71 UNKNOWN	AREA at RRT@ 1.4 IMP-E	% UNKNOWN IMP	% IMP-E
1	7425	88536	0.07	0.52
2	7966	88961	0.07	0.52
3	7855	88364	0.08	0.51
4	7485	88953	0.07	0.52
5	7422	88542	0.08	0.51
6	7895	88182	0.08	0.51
average		**0.7**		**0.51**
STDEV		0		**0**
% RSD		0.41		**0.03**

No variations in the results were observed.

Accuracy: The mean recovery of three replicate preparations at 50 to 150% level should be between 85.0% and 115.0%.the %RSD at each level should not be more than 10.0

Parameter	Result	Acceptance criteria
Tailing factor	1.1	NMT 2.0

%RSD for six standard injections	1.6	NMT 10.0%

MEMANTINE RELATED COMPOUND-E

Level	Spiked conc µg	Area	Related conc. .(µg)	Recovered (%)
50	7.7472	3534	7.8510	101.3
	7.7472	3612	8.0262	103.6
	7.7472	3548	7.8824	101.7
100	15.4956	7246	16.1915	104.5
	15.4956	7205	16.0995	103.9
	15.4956	7434	16.6092	107.2
150	23.2426	10360	23.1824	99.7
	23.2426	10491	23.4766	101.0
	23.2426	10357	23.1510	99.6

Avg	SD	%RSD
102.2	1.2	1.2
105.2	1.8	1.7
100.1	0.8	0.8

SOLUTION STABILITY:

The sample solution is stable if the percent difference found between the impurity values of the stored sample solution quantitated against a freshly prepared standard solution and the initial impurity value for peaks > 0.05 should not be more than 20.0% and any additional (new) impurity peak should not be more than 20% of the proposed specification Limit. The standard solution is stable if the % Deviation value should me NMT 10.0% of Initial and Final Standard solutions.

SYSTEM SUITABILITY AND SYSTEM PERFORMANCE RESULTS FOR PRECISION STUDY:

Results	Tailing factor	%RSD
Acceptance criteria	1.1	1.6
	NMT 2.0	NMT 10.0

SOLUTION STABILITY DATA FOR THE STANDARD

Time station	% Assay	% Difference
Initial	98.8	NA
12^{th} hr	107.5	8.5

SOLUTION STABILITY DATA FOR THE SPIKED SAMPLE

Spiked sample for Memantine Related Compound-E		
Time	%Result	%Difference
INITIAL	0.32	NA
12^{th} hr	0.33	0.02
24^{TH} hr	0.34	0.02

Solution stability of the standard solution is stable up to 12hours, Control and spiked solutions are stable up to 24hours when stored at room temperature.

LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ):

SYSTEM SUITABILITY AND SYSTEM PERFORMANCE RESULTS FOR LOD AND LOQ STUDY:

Parameter	Result	Acceptance Criteria
Tailing factor	1.1	NMT 2.0
% RSD	1.5	NMT 10.0

The concentration and S/N ratio of Memantine HCl results mentioned in the following .

TABLE - REPRESENTS THE PRECISION DATA FOR LOQ SOLUTION.

Component	LOQ		LOD	
Memantine HCl	Concentration(µg /ml)	S/N Ratio	Concentration(µg /ml)	S/N Ratio
	2.4	27	0.8	12

PRECISION AT LOQ DATA FOR MEMANTINE HCL

Injection	Memantine Area
1	1412
2	1488
3	1392
4	1354
5	1328
6	1646
AVG	1437
%RSD	8.1

The analyte concentration level that exhibits an average S/N ratio NLT 3 is the concentration representing the LOD. The analyte concentration level that exhibits an average S/N ratio of NLT 10 is the concentration representing LOQ.

%RSD for Precision at LOQ should be not more than 10.0%

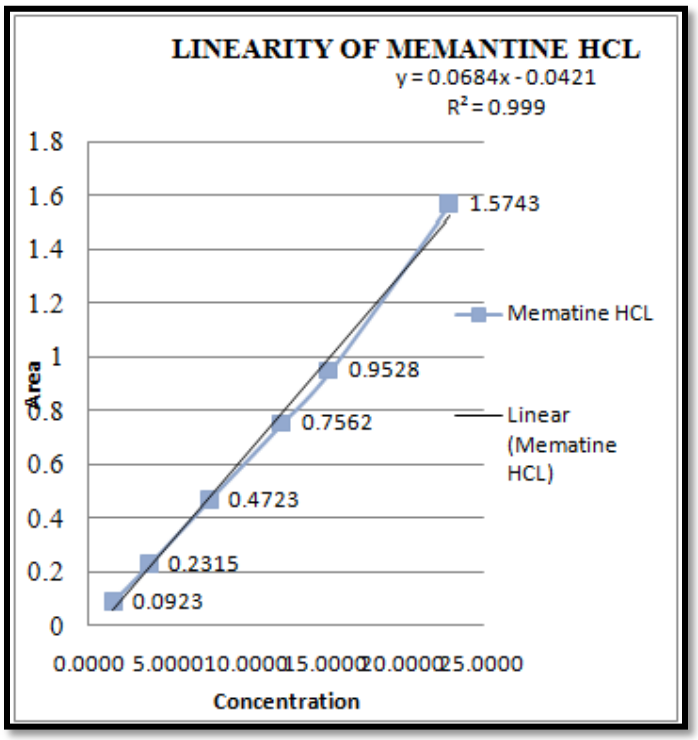
From the above data it was concluded that the analytical method for the Related compounds of Memantine HCl ER

Capsules 14mg met the acceptance criteria for LOD and LOQ.

LINEARITY AND RANGE

A series of solutions of Memantine hydrochloride were prepared from LOQ to 150% specification level and injected into GC system as per test method

Level %	CONC.(µg/ml)	AREA	% RSD
10	1.5260	0.0926	0.2
		0.0921	
		0.0922	
25	3.8151	0.2299	1.1
		0.2344	
		0.2303	
50	7.6301	0.4731	0.1
		0.4717	
		0.4719	
80	12.2082	0.7579	0.1
		0.7567	
		0.7570	
100	15.2603	0.9524	0.1
		0.9527	
		0.9534	
150	22.8905	1.5855	0.6
		1.5633	
		1.5742	



slope	**0.0684**
Y-intercept	**-0.0421**
R square value	**0.999**
Response	**0**
Y-intercept at 100% level	**-1.1**

CONCLUSION:

A simple gas chromatographic method is developed for Memantine hydrochloride impurities. This method is validated and it is found to be specific, precise, accurate, linear to detect and quantify the Memantine hydrochloride and its impurities

REFERENCES

1. V.Jagathi, B.Anupama, B.P.S.Praveen, G.D.Rao; Spectrophotometric determination of memantine in bulk and in pharmaceutical formulations, International Journal of Current Pharmaceutical Research, 2010, vol 2 (4), Pg No: 17-18

2. H.Jalalizadeh, M.Raei, R.T.Tafti, H.Farsam, A.Kebriaeezadeh, E. Souri; A Stability-Indicating HPLC Method for the Determination of Memantine Hydrochloride in Dosage Forms through Derivatization with 1-Fluoro-2,4-dinitrobenzene, Scientif Pharmaceutical, 2013, vol 82 (2), Pg No: 265-279

3. Drug Profile of Memantine Hydrochloride, November 2014