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TO DEVELOP HPLC METHOD FOR THE ASSAY OF MEMANTINE HYDROCHLORIDE TABLETS USING REFRACTIVE INDEX (RI) DETECTOR

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Abstract:

Objective: *To develop and validate a chromatographic method to determine the amount of drug (assay) in the tablets of memantine hydrochloride (MEM) using high performance liquid chromatography with refractive index (RI) detector.*

Methods: *The chromatographic separation was achieved on C18 (250 × 4.5 mm, 5µ) column using isocratic mobile phase comprises of buffer (pH-6.0): Methanol (45:55 v/v) pumped at a flow rate of 1.0 ml/min. The detection of effluent was monitored using RI detector.*

Conclusion: *The method was found to be simple, commercial, precise, accurate and robust which can be utilized for the determination of assay of MEM in tablets and capsules.*

Keywords: *Memantine hydrochloride, Assay, Liquid chromatography, Refractive Index detector.*

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INTRODUCTION:

Memantine Hydrochloride is often used in the treatment of Alzheimer's disease (AD).

AD can be caused due to depression, delirium, drug interaction, thyroid problems, excess use of alcohol, or certain vitamin deficiencies [1, 2].

Memantine HCl chemically is 1-amino-3,5-dimethyladamantane hydrochloride (Fig. 1).

Memantine hydrochloride occurs as a fine white to off-white powder and is soluble in water. Its molecular weight is 215.76 and molecular formula is C12H21N.HCL.

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.

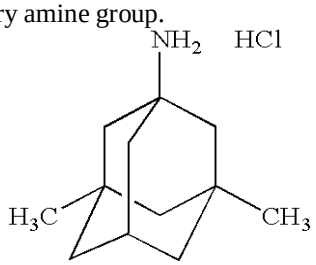

Fig. 1: Structure of Memantine Hydrochloride.

MEM is used as option in a new class of AD treatment and showed very good results in terms of efficacy and safety for patients with moderate to severe symptoms. It has been recently approved by European Union and Australia for the treatment of moderately severe to severe AD [5].

The drugs which are used in treatment of AD need to be given in appropriate dosage regimen. Hence it is very important to estimate the amount of drug present in the dosage forms. For determination of amount of drug (assay) in different formulations like tablets, capsules etc. a simple, rapid and economical analytical method is required.

MEM doesn't have chromophore. This limitation of the MEM necessitates, selecting specialized analytical techniques for its quantification. Numbers of HPLC methods were reported for quantification of MEM in pharmaceutical dosage forms with specific techniques such as pre-column derivatization with UV detector [6-8], with charged aerosol detector [9]. The MEM quantification in biological fluids such as plasma and vitreous humour were also performed using pre-column derivatization and fluorescence detection [10-14]. The reported methods are cumbersome and time consuming with tedious sample preparation process.

This research was focused on development of a simple, precise, accurate, specific and robust reverse phase chromatographic method using refractive index detector for quantification of MEM in its different pharmaceutical dosage forms. The developed method is having advantage of short run time, no derivatization required; hence more number of samples can be tested in short span of time. This method can be used in quality control and R&D. The simplicity of proposed method is economical and can be utilized by common laboratories. The developed method was validated as per International Conference on Harmonization (ICH) Q2(R1) guideline and United State of Pharmacopoeia (USP) 38 chapter <1225> and chapter <1092> [15-17]. The method found to be specific, precise, accurate and robust with compliance to acceptance criteria of ICH and USP 38.

EXPERIMENTAL:

Materials and reagents:

Memantine hydrochloride pure drug (100.0% w/w), tablets with label claim 30 mg of Memantine hydrochloride per tablet were provided by Wockhardt Ltd Aurangabad, Maharashtra India.

HPLC grade methanol was purchased from Merck Chemicals, Mumbai, India. Ultrapure water was generated from Milli-Q water purifier. Diethylamine was purchased from Spectrochem, Mumbai, India. Orthophosphoric acid and hydrochloric acid were purchased from Merck Ltd, Mumbai, India. Sodium chloride was purchased from Merck Chemicals, Mumbai, India.

Instruments and Methods:

Instrumentation:

Waters High performance liquid chromatography (HPLC) system with Refractive Index Detector.

Chromatographic parameters:

The chromatographic column used was a Hypersil-BDS, C18 (250.0 × 4.6mm; 5 µm) which was maintained at 40º C. The mobile phase was prepared by mixture of ultrapure water containing 0.2% diethylamine (pH 6.0 adjusted with dilute Orthophosphoric acid) – methanol in the ratio of 45:55 v/v. The flow rate of the mobile phase was 1.0 ml/min. The injection volume was 50.0 µL. The column effluents were monitored by refractive index detector. The detector and column oven temperature was set at 40.0º C with sensitivity of 512. The sample and reference cell were purged with mobile phase for 30min each with flow rate 1.0 ml/min.

Preparation Standard solutions:
Weighed accurately about 50 mg of MEM working standard and transferred to 50 ml volumetric flask. The content of the flask was dissolved with mobile phase with sonication and volume was made up to mark with mobile phase as primary stock solution. Further 5.0 ml of prepared standard primary stock solution was pipetted and transferred to 50 ml volumetric flask and made up to the mark with mobile phase to get nominal concentration about100 µg/ml.

Method Validation

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities or expected to present. The specificity of the developed LC method for MEM was carried out by injecting placebo, known impurities of MEM (Admantamine) The placebo was prepared by dissolving 10mg placebo in 10 ml of volumetric flask in mobile phase and sonicated. The volume was made up to the mark with mobile phase. The resulting solution was filtered through 0.45µ syringe filter.
Each impurity was prepared by weighing 1mg of individual impurity in 10 ml volumetric flask and dissolved in mobile phase by sonication. The volume was made up to the mark with mobile phase. Further diluted 1ml of this solution to 10 ml with mobile phase.

The mobile phase (blank), placebo solution, individual impurities (10µg/ml) and standard drug solution (100µg/ml) were injected in sequence for evaluation of specificity of proposed method. The chromatograms were monitored for any peak eluted at the retention time of drug.

Precision

Precision express the measure of how close the analytical results are to each other from a set of measurements under controlled analytical conditions. Precision proves random errors of the measurement.
Precision is a measure of the degree of repeatability (Intra-day), intermediate precision and reproducibility (inter-day) of the analytical method under normal operating circumstances.
Precision is usually measured as the relative standard deviation (RSD) of analytical results acquired from independently prepared quality control standards.
Method precision was evaluated by six sample preparations of same homogeneous test sample of MEM (30mg/tablet) and calculated % assay for each sample preparation. The % RSD for set of six preparations was calculated.

The intra-day precision was evaluated by analyzing six preparations of MEM (n = 6) in two different set in a day. The acceptance criteria for % RSD is not more than 2%.

The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory by carrying out six sample preparation of same test sample of MEM tablets and calculated % assay for all preparations. Calculated the %RSD for 12 results. The acceptance criteria for % RSD is not more than 2%.

Recovery (Accuracy)

Accuracy is extremely important in analytical method validation as it assures the closeness of agreement between a test result and the accepted reference value. Accuracy is expressed as trueness and involves a combination of random components and a common systematic error or bias component. The accuracy of the method was performed by recovery studies.
In order to evaluate the accuracy of the proposed methods, a recovery test was performed by adding known amounts of standard solutions to the placebo formulation sample, followed by analysis using the proposed chromatographic method.
The recovery studies were done for three different levels at 80%, 100% and 120% with three determinations of working level concentration using standard spiking method.

The placebo was accurately weighed about 120mg for all level. For 80% (80µg/ml) level of recovery studies, 40.0 mg of MEM standard was spiked along with 120mg placebo and dissolved in 50ml of mobile phase. Further diluted 5.0 ml of this solution to 50 ml with mobile phase. The resulting solution was filtered through 0.45µ syringe filter. In same manner for 100% and 120% recovery studies 50mg (100µg/ml) and 60mg (120.0µg/ml) of MEM was spiked with 120mg of placebo respectively and prepared the solutions. All the above solutions were prepared in triplicate and were analyzed using proposed chromatographic condition. The recovery at each level was calculated by using the theoretical value from exact weight taken for spiking. The % recovery was calculated with respect to amount added. The acceptance criteria for % recovery are in the range of 98 - 102%.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analytes in the samples within a given range.

The linearity plot was constructed for MEM in the range of 50 to 150µg/ml. The primary stock solution of 1000µg/ml of MEM was prepared in mobile phase. From the primary stock solution, appropriate dilutions were made to get concentration of 50.0, 70.0, 100.0, 120.0 and 150.0. The calibration curve was plotted as concentration of the respective drug solutions versus the peak area at each level. The results were statistically evaluated and correlation coefficient determination (r^2), slope and y-intercept values were calculated.

Robustness

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. One consequence of evaluation of robustness is that a series of system suitability parameters is established to ensure that the analytical procedure is maintained whenever used. In the present study the working concentration 100.0µg/ml of MEM was used for the determination of the robustness of the method. The following parameters were considered for the robustness of the proposed chromatographic method.

- Effect of pH in the mobile phase (±0.2)
- Effect of organic modifier in mobile phase composition (± 2%)
- Effect of flow rate (±0.1)
- Column oven and detector temperature (±2°C)

Solution stability of sample and standard in mobile phase

The solution stability of MEM sample was performed to understand stability which will be helpful to understand sample handling in proposed chromatographic method. The sample and standard solution after preparation were injected immediately to the system considering as an initial at 0 hr as baseline. A solution stability of MEM was carried out for sample solution (100.0µg/ml) in a tightly capped volumetric flask at ambient temperature for 72 hr. The results were calculated against freshly injected standard.

System Suitability

The rationale of the system suitability assessment is to make sure that the complete testing system (including instrument, reagents, columns, analysts) is appropriate for the intended application.

System suitability tests (SST) are vital part of liquid chromatographic methods. They are used to verify the reproducibility of the chromatographic parameters and system is satisfactory for the analysis to be done. SST is support on the concept that the equipment, electronics, analytical operations and samples to be analyzed comprise an integral system that can be evaluated as such.

The system suitability test was performed in accordance with USP [18].

Application of chromatographic method for determination of assay of MEM tablets:

Assay test of MEM tablets was performed using developed method.

RESULTS:

Validation of chromatographic method

Specificity:

The overlay chromatogram (Fig. 2) of diluent, placebo, known impurities and standard solution were revealed that there is no interference at the retention of MEM. The developed chromatographic method was found to be highly specific for determination of assay for MEM tablets and capsules.

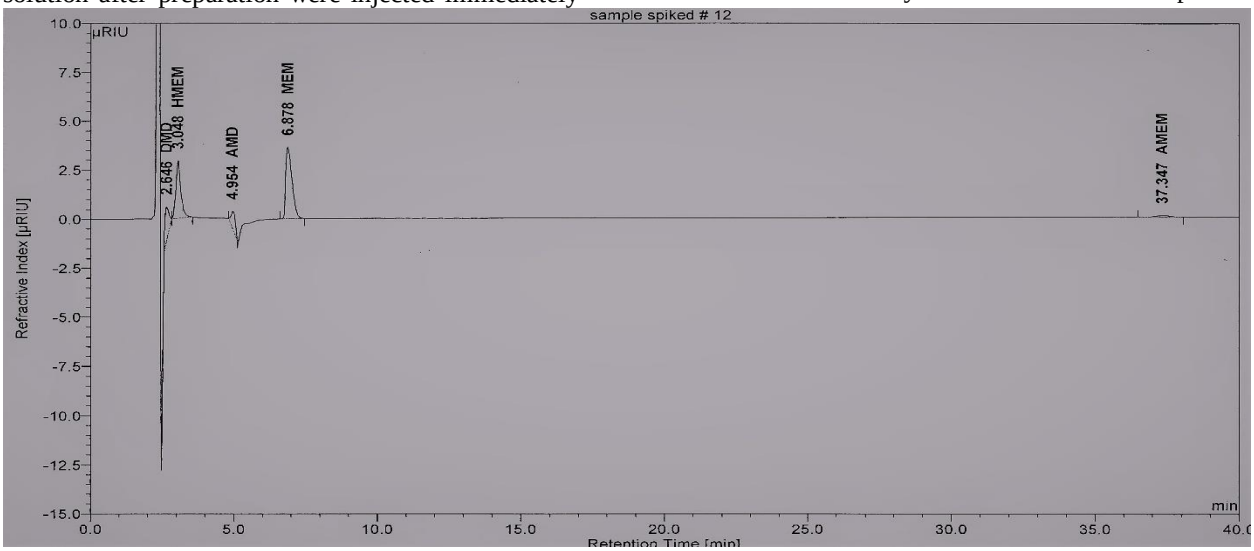

Fig. 2: Chromatogram of Memantine HCl along with diluents, placebo and known impurities.

Table 2: The precision results

Concentration (µg/mL)	% RSD		Absolute % difference in mean assay value
	Intra-day(n=6)	Inter-day (n=12)	
100	0.47%	0.53%	0.4%

Precision:

The intra-day precision was evaluated by performing six (n = 6) assay determinations on same homogeneous sample of MEM tablets and the % RSD was found to be 0.47%.

The % RSD for inter day precision of assay test for two sets (n=12) for their % assay was found to be 0.53%.

The absolute difference between results for intermediate precision found 0.4% (Table no. 2).

Accuracy (Recovery)

The % recovery at 80, 100 and 120% was found to be 99.8±0.5, 100.1±0.5, and 100.1±0.1% respectively (Table no. 3). The overall mean recovery was found to be 100.0±0.2%. The recovery results were found within acceptance criteria. The developed method found to be accurate for determination of assay in MEM tablets (Table no. 3).

Linearity

The linearity of the MEM in dissolution medium was performed in the range of 50-150µg/ml and found to be linear. The representative regression equation was found to be y=1.6072x +1.5924 with lowest correlation coefficient (r^2) was found to be 0.999. The linearity was found with in acceptance criteria.

Robustness

The robustness parameters for chromatographic method are presented in table IV and found within acceptance criteria (Table no. 4).

Table 3: Accuracy (Recovery) results

Concentration level	% Recovery	Mean % Recovery
80%	100.2	99.8±0.5
	99.8	
	99.3	
100%	99.9	100.1±0.5
	100.6	
	99.7	
120%	100.3	100.1±0.1
	99.9	
	100.2	
% Mean Recovery		100.0±0.2

Table 4: Robustness results

Parameter		%RSD
Flow (mL/min)	0.9	0.45
	1.1	0.35
pH	5.8	0.91
	6.2	0.68
Mobile phase composition (change in organic modifier ± 2%)	53	0.85
	57	0.64
Column oven and detector temperature (±2°C)	38	0.43
	42	0.32

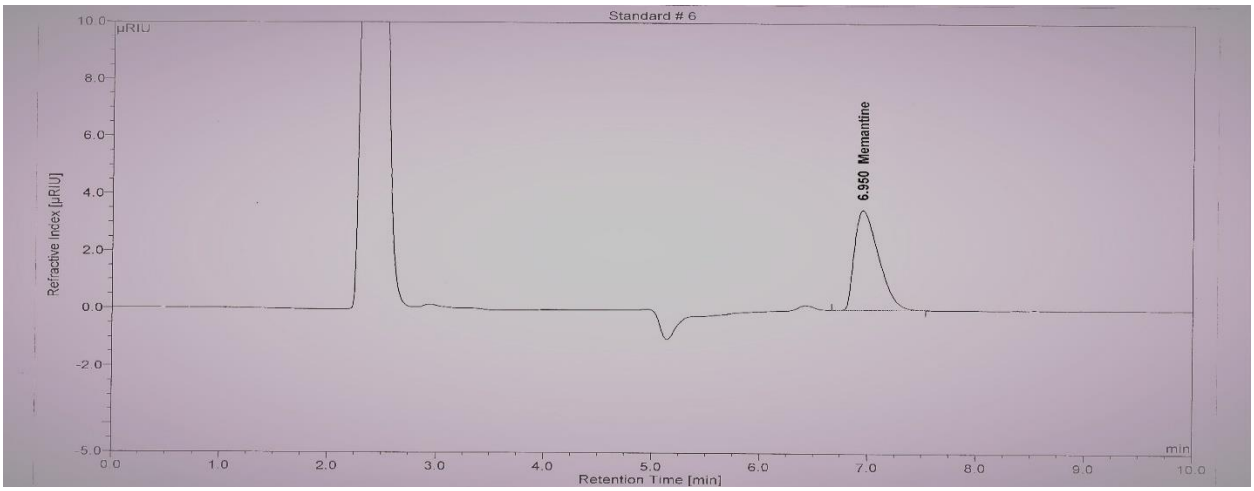

Fig. 3: Representative chromatogram of Memantine HCl standard

Solution stability of sample and standard

The MEM found to be stable up to 72h in dissolution medium at ambient temperature. The difference between the initial results and after 72h was found to be 0.5%.

Application of developed method:

The proposed validated method was used for determination of assay in MEM tablets and the results were found within the specification (98.5%).

DISCUSSION:

MEM lacs chromophore, due to this limitation, MEM cannot be readily assayed by HPLC-UV techniques and hence refractive index detector was selected for detection. During development of this method, method optimization was carried out by using different HPLC columns, by changing concentration of organic modifier (methanol) and also different pH to achieve the separation between impurity and MEM. The main peak of MEM in the proposed method was found at 7.0 min. (Fig. 3). The sensitivity of standard and sample at concentration 100 µg/mL of MEM was found good.

The method was found economical, simple and robust. The system suitability was found to be with acceptance criteria. The proposed method is more accurate and time saving than the current published methods. It can be used by laboratories to determine the assay of memantine hydrochloride tablets of different strengths. The refractive index detector is a universal detector and easily available. Analyst need not to do derivatization of drug which is complex and time consuming.

CONCLUSION:

The validation of the method proven that the method is linear in the range of 50–150 µg/ml, to be precise and accurate over the range. The validation of the method is done accordingly to ICH Q2 (R1) and USP 38. All the parameters are meeting the acceptance criteria. Since method is simple and time saving hence it can be used conveniently by laboratories to determine the assay of different formulations of MEM.

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