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Article

The Development and Validation of Novel, Simple High-Performance Liquid Chromatographic Method with Refractive Index Detector for Quantification of Memantine Hydrochloride in Dissolution Samples

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Abstract

The present study was aimed to develop an analytical method for quantification of memantine (MEM) hydrochloride in dissolution samples using high-performance liquid chromatography with refractive index (RI) detector. The chromatographic separation was achieved on C18 (250 × 4.5 mm, 5 μm) column using isocratic mobile phase comprises of buffer (pH 5.2):methanol (40:60 v/v) pumped at a flow rate of 1.0 mL/min. The column effluents were monitored using RI detector. The retention time of MEM was found to be ~6.5 ± 0.3 min. The developed chromatographic method was validated and found to be linear over the concentration range of 5.0–45.0 μg/mL for MEM. Mean recovery of MEM was found to be 99.2 ± 0.5% (w/w). The method was found to be simple, fast, precise and accurate, which can be utilized for the quantification of MEM in dissolution samples.

Introduction

Alzheimer's disease (AD) is the most common type of dementia which results in damaged brain cells or connections between brain cells by various diseases and conditions. The number of persons with AD is increasing as populations age and presently ~46.8 million people are affected globally in 2015. Major concerns in AD are progressive impairment in both cognitive and functional capacities. Some of the symptoms in AD can be due to depression, delirium, drug interaction, thyroid problems, excess use of alcohol or certain vitamin deficiencies (1, 2).

Cholinesterase inhibitors are commonly used as treatment strategies for AD that includes donepezil/galantamine/rivastigmine/tacrine. The mentioned drugs were also combined with memantine (MEM) or *N*-methyl-D-aspartate (NMDA) receptor antagonist.

MEM HCl (Figure 1) chemically is 1-amino-3,5-dimethyladamantane hydrochloride. It is an anti-Alzheimer's, uncompetitive NMDA receptor antagonist with strong voltage dependency and rapid blocking/unblocking kinetics. These pharmacological features appear to allow MEM to block the sustained activation of the receptor by glutamate that may occur under pathological conditions and to rapidly leave the NMDA receptor channel during usual physiological activation (3, 4).

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

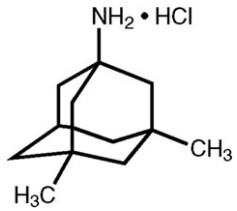

Figure 1. Structure of MEM.

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 release from dosage forms. For quantification of amount of drug release there is a need of simple, rapid and economical analytical method.

Chemical structure of MEM reveals that, it does not have chromophore hence does not show the UV absorption. This limitation of the MEM necessitates, selecting specialized analytical techniques for its quantification. A numbers of high-performance liquid chromatography (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 humor 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.

The production of counterfeit and poor-quality medicines is a major community health concern; mainly in developing countries, which lack resources for monitoring drug quality. Unavailability of advanced techniques in common laboratories create need to develop simple, accurate, specific and affordable methods to provide a practical means of speedily monitoring drug quality.

Hence our research was focused on development of a simple, accurate and specific reverse phase chromatographic method using refractive index (RI) detector for quantification of MEM in drug release profile studies of 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. The reported method can be utilized during dissolution testing in quality control of manufactured and developed dosage forms. 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

MEM hydrochloride pure drug (100.0% w/w) and tablets with label claim 30 mg of MEM 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. Glacial acetic acid and Hydrochloric acid were purchased from Merck Ltd, Mumbai, India. Sodium chloride was purchased from Merck Chemicals, Mumbai, India.

Instruments and methods

Instrumentation

Dionex—Ultimate 3000 HPLC system with Shodex DEX RI 101 RI Detector, Dissolution equipment—Electrolab 88DT.

Chromatographic parameters

The chromatographic column used was a Kromasil 100, C18 (250.0 × 4.6 mm; 5 µm), which was maintained at 35°C. The mobile phase was prepared by mixture of ultrapure water containing 1.0% diethylamine (pH 5.2 adjusted with dilute glacial acetic acid)–methanol in the ratio of 40:60 (v/v). The flow rate of the mobile phase was 1.0 mL/min. The injection volume was 100.0 µL. The column effluents were monitored by RI detector. The detector temperature was set at 35.0°C with sensitivity of 512. The sample and the reference cell were purged with mobile phase for 30 min each with flow rate 1.0 mL/min.

Dissolution parameters and procedure

The dissolution test was performed in an Electrolab multi-bath ($n = 7$) dissolution test system (Mumbai, India) using dissolution apparatus of USP Type II in accordance with USP general methods.

The dissolution method was used according to USP 38 monographs for MEM tablets. The dissolution medium was composed of 0.1 M HCl with sodium chloride (2 g/L of sodium chloride in water) of pH 1.2 (adjusted with hydrochloric acid). The volume of dissolution medium was 900 mL maintained at 37°C. The paddle was rotated with speed of 75 r.p.m. The dissolution study was performed at single point with sample aliquot drawn after 30 min.

Preparation standard solutions

Weighed accurately ~50 mg of MEM working standard and transferred to 100 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 3.0 mL of prepared standard primary stock solution was pipetted and transferred to 50 mL volumetric flask and made up to the mark with dissolution media to get nominal concentration ~30 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 HPLC method for MEM was carried out by dissolving 10 mg placebo in 10 mL of volumetric flask in dissolution medium and sonicated. The volume was made up to the mark. The resulting solution was filtered through 0.45 µm syringe filter.

The mobile phase, dissolution medium, placebo solution and standard drug solution (30 µg/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 (intraday), 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.

Chromatographic method precision was evaluated by carrying out dissolution on six tablets of MEM (30 mg/tablet) test sample and calculated % released for six tablets. The %RSD for set of six tablets for their % drug released was calculated.

The intraday precision was evaluated by analyzing six dissolution sample solutions of MEM ($n = 6$) in two different set in a day. The acceptance criteria for %RSD is not >5%.

The intermediate precision of the method was also evaluated using different analyst and a different instrument in the same laboratory by carrying out dissolution test on six tablets of MEM sample and calculated % released for six tablets. Calculated the %RSD for 12 results. The acceptance criteria for %RSD is not >5%.

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 dissolution, followed by analysis using the proposed method.

The recovery studies were done for three different levels at 80, 100 and 120% with three determination of working level concentration using standard spiking method.

The placebo was accurately weighed ~120 mg for all level. For 80% (26.7 µg/mL) level of recovery studies 24.0 mg of MEM standard was spiked along with 120 mg placebo in 900 mL of dissolution media. In the same manner for 100 and 120% recovery studies, 30 mg (33.3 µg/mL) and 36 mg (40.0 µg/mL) of MEM were spiked, respectively. The dissolution test was performed and samples were analyzed by proposed chromatographic method. The recovery at each level was calculated by using the theoretical value from exact weight taken for spiking. The prepared samples were analyzed using proposed chromatographic condition. The % recovery was calculated with respect to amount added. The acceptance criteria for % recovery is in the range of 95–105%.

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 5–45.0 µg/mL. The primary stock solution of 500 µg/mL of MEM was prepared in mobile phase. From the primary stock solution, secondary stock solution was prepared to get the concentration of 100.0 µg/mL of MEM in mobile phase. Appropriate dilution of the primary and secondary stock solution was carried in dissolution medium to get concentration of 5.0, 10.0, 15.0, 20.0, 30.0 and 45.0 µg/mL for MEM in dissolution media. The calibration curve was plotted as concentration of the respective drug solutions versus the peak area at each level. The correlation coefficient determination (r^2), slope and y-intercept values were calculated and statistically evaluated.

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 30.0 µg/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 mobile phase composition (±2%)
- Effect of flow rate (±10%)
- Column oven temperature (±2°C)

Solution stability in dissolution media

The solution stability of MEM standard and samples was performed in dissolution medium to understand stability, which will be helpful to understand sample handling using proposed chromatographic method. A solution stability of MEM was carried out for standard and sample solution (30.0 µg/mL) in a tightly capped volumetric flask at ambient temperature for 72 h. The sample and standard solution after preparation were injected immediately to the system considering as an initial at 0 h as baseline.

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 SST (Table I) was performed in accordance with USP (18).

Table I. System Suitability Parameters

Parameter	Limit	Obtained results for MEM
Capacity factor	$k' > 2$	3.6
Injection precision	RSD < 1% for $n > 6$	0.52%
Tailing factor	$T < 2$	1.10
Theoretical plates/meter	$N > 2{,}000$	7178

Application of chromatographic method for quantification of MEM in dissolution samples

Dissolution test was performed using methodology as per section "Dissolution parameters and procedure" on six tablets. Sample aliquot was withdrawn after 30 min and filtered through 0.45 µm syringe filter and injected (100.0 µL) directly to HPLC system with RI detector. Testing was performed at ambient temperature.

Results

Validation of chromatographic method

Specificity

The overlay chromatogram (Figure 2) of diluent, placebo and standard solution was reveled that there is no interference at the retention of MEM. The developed chromatographic method was found to be highly specific for quantification of MEM in dissolution samples.

Precision

The intraday precision was evaluated as %RSD by analyzing six dissolution sample solutions of MEM ($n = 6$) in two different set in a day was found to be 0.945%.

The %RSD for inter-day precision of MEM for two sets of six tablets ($n = 12$) for their % drug released was found to be 1.8%.

The absolute difference between results for intermediate precision found 3.4% (Table II).

Accuracy (recovery)

The % recovery at 80, 100 and 120% was found to be **98.8 ± 0.6**, **99.2** ± 0.5 and **99.7 ± 1.5**%, respectively (Table III). The overall

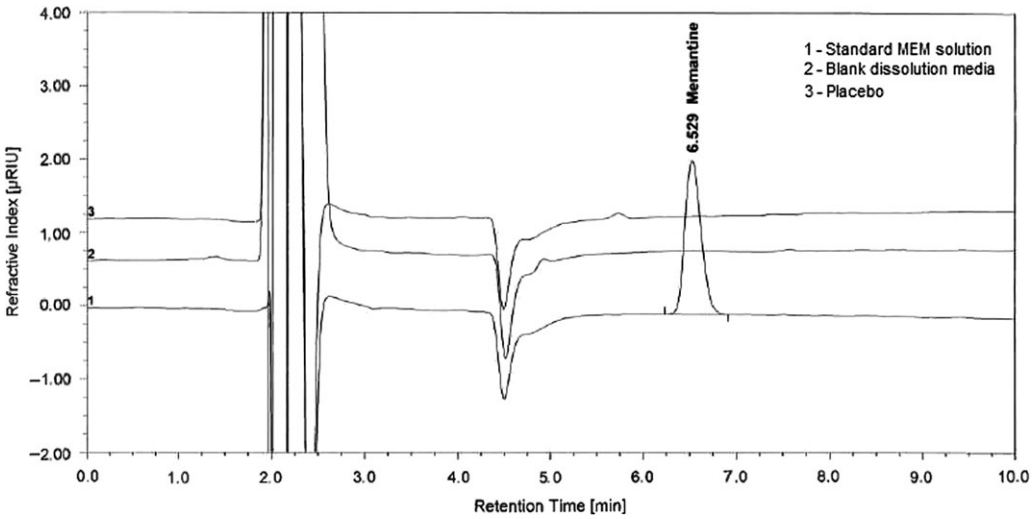

Figure 2. Overlay chromatogram of standard MEM, blank dissolution media, placebo.

Table II. The Precision Data of MEM by the Proposed HPLC Method

Concentration (µg/mL)	%RSD		Absolute % difference in mean value for intermediate precision study
	Intraday ($n = 6$)	Inter-day ($n = 12$)	
33	0.945%	1.8%	3.4%

Table III. The Accuracy Data of MEM by the Proposed HPLC Method

Spiked level	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % recovery
80%	26.35	25.95	98.5	98.8 ± 0.6
	26.35	26.21	99.5	
	26.35	25.91	98.3	
100%	33.38	33.18	99.4	99.2 ± 0.5
	33.38	33.23	99.6	
	33.38	32.95	98.7	
120%	39.6	40.1	101.3	99.7 ± 1.5
	39.6	39	98.5	
	39.6	39.3	99.2	
% Mean recovery				99.2 ± 0.5

mean recovery was found to be **99.2 ± 0.5**%. The recovery results were found within acceptance criteria. The developed method found to be accurate for quantification of MEM in dissolution study.

Linearity

The linearity of the MEM in dissolution medium was performed in the range of 5–45 µg/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 0.997. 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 IV. Robustness Parameter of the HPLC Method

Parameter		%RSD
Flow	0.9 mL/min	0.72
	1.1 mL/min	0.66
pH	5.0	1.10
	5.4	0.76
Mobile phase composition (±2%)	38:62	0.52
	42:58	0.34
Column oven temperature (±2°C)	33°C	0.43
	37°C	0.32

Table V. %MEM in Dissolution Samples

Serial no.	% MEM	Limit
1	94	≥85% drug release at 30 min
2	96	
3	94	
4	103	
5	94	
6	96	

Solution stability in dissolution media

The MEM found to be stable up to 72 h in dissolution medium at ambient temperature. The results after 72 h were found to be **99.6 ±** 0.7 and **98.8 ± 0.1**% for standard and sample, respectively.

Application of developed method

The proposed chromatographic method was used for quantification of MEM in dissolution samples of MEM 30 mg tablets and the results were found within the specification (Table V).

Discussion

The RI detector is used as universal detector in HPLC. The detection technique is based on measurement of the change in RI of mobile phase. RI detectors consist of two-path cell–sample and reference. The RI of sample path cell is continuously compared with the reference cell. The greater the RI differences between sample and mobile phase, higher the sensitivity. The RI detector is having advantages as a universal detector with low sensitivity to air bubbles in the flow cells; and also covers the RI range from 1.000 to 1.750 RI. The only limitation is the comparatively low sensitivity (19).

In this study, the chromatographic method optimization was carried out by utilizing different stationary phase, with organic modifier and pH. The optimized chromatographic conditions obtained was Kromasil 100, C18, (250 × 4.6 mm, 5 µm) with mobile phase, mixture of ultrapure water containing 1% diethylamine (pH 5.2 adjusted with dilute glacial acetic acid)–methanol in the ratio of 40:60 (v/v). The retention time of MEM in the optimized condition was found was 6.5 min (Figure 3). For the present the study, the 30 µg/mL of MEM as the standard concentration was injected six times to record the system suitability parameter. The system suitability was found to be with acceptance criteria (Table I). The representative chromatogram for Limit of Quantification (LOQ) (Figure 4).

Conclusion

The proposed liquid chromatographic method provides simple, accurate and reproducible methodology for quantitative measurement of

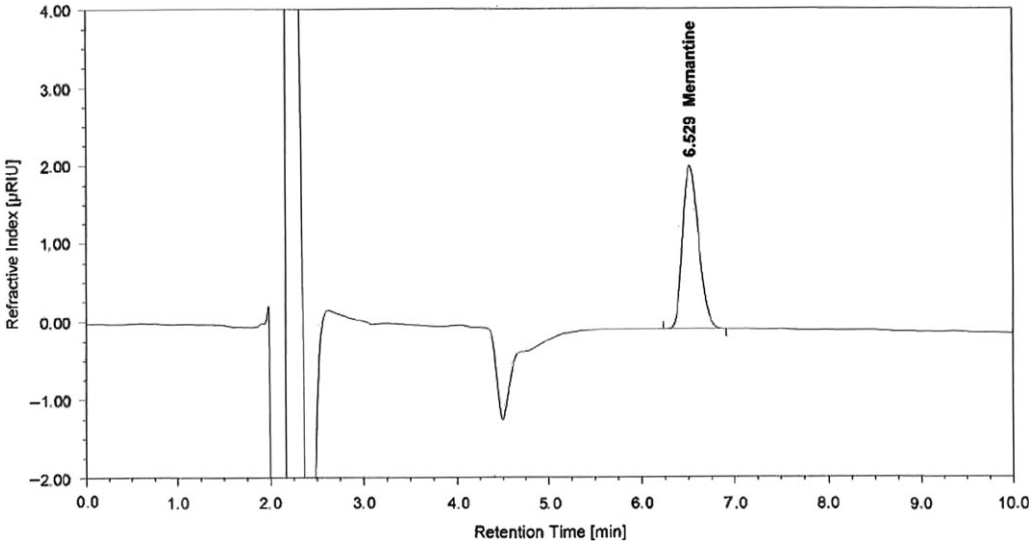

Figure 3. Representative chromatogram of MEM standard.

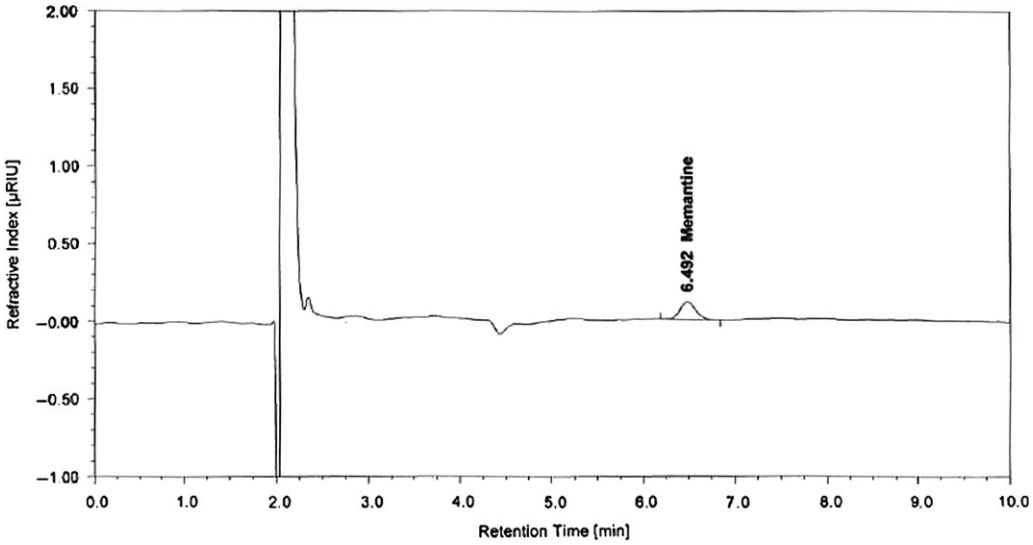

Figure 4. Chromatogram of MEM at LOQ level.

MEM in dissolution sample using RI detector without any interference from the excipients. The proposed method is very simple and can be used without derivatization of compound under analysis. The response of the method was found to be linear in the range of 5.0–45 µg/mL, and it proved to be precise and accurate. The developed chromatographic method was fully validated accordingly to ICH Q2(R1) and USP 38 showing acceptable data for all the method validation parameters tested. The developed method is economical with utilization of commonly used reagents and instruments. The intended method can be used conveniently by quality control to establish the drug release in dissolution samples in regular MEM hydrochloric production samples and stability samples. The said method also can be used during developmental studies of newer formulations for quantification of MEM during dissolution profile studies.

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