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Research Article

Development and Validation of Analytical Method for Estimation of Memantine Hydrochloride

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Abstract

An accurate HPTLC analytical method for estimation of Memantine Hydrochloride was established and validated. Memantine Hydrochloride is an NMDA receptor antagonist and widely use for Alzheimer's disease. The HPTLC method was developed using aluminum plates pre-coated with *silica gel G60F254* as a stationary phase and n-Hexane: Ethyl acetate: Diethylamine (5:5:0.7 % v/v/v) as mobile phase. The separated spots were visualized as orange spots after dipping with Dragendorff's reagent solution. The method was found to be Linear, Accurate, Precise, and Robust according to ICH Guideline. Linearity was found to be 5000-30000 ng/band for Memantine HCl. The LOD and LOQ were found to be 80.07ng/band and 242.637ng/band for Memantine HCl. So, developed method is applicable for the estimation of Memantine Hydrochloride.

Keywords: HPTLC; Memantine HCl; Alzheimer; Validation

Abbreviations

API: Active Pharmaceutical Ingredient; AR: Analytical Reagent Grade; B.P.: British Pharmacopoeia; CDSCO: Central Drug Standard Control Organization; HPLC: High Performance Liquid Chromatography; HPTLC: High Performance Thin Layer Chromatography; MEM: Memantine; ICH: International Conference on Harmonization; LOD: Limit Of Detection; LOQ: Limit Of Quantification; MS: Mass Spectroscopy; *Rf:* Retention Factor; RSD: Relative Standard Deviation; SD: Standard Deviation; UV: Ultra Violate; U.S.P.: United States Pharmacopoeia; USFDA: United States Food and Drug Administration.

Introduction

Memantine Hydrochloride is chemically 3,5-dimethyladamantan-1-amine hydrochloride and it is an NMDA receptor antagonist and widely used for Alzheimer's disease. It has empirical formula C12H21N•HCl with molecular weight 215.76 (g/mol) (Figure 1) [1]. Memantine HCl was first approved by FDA for treatment of Alzheimer's disease on October 2003. Memantine HCl was approved by CDSCO on 6th September, 2010. Memantine HCl exerts its action through uncompetitive NMDA (N Methyl D aspartet) receptor antagonism, binding preferentially to the NMDA receptor-operated action channels. Prolonged increased levels of glutamate in the brain of demented patients are sufficient to counter the voltage-dependent block of NMDA receptors by Mg2+ ions and allow continuous influx of Ca2+ ions into cells, ultimately resulting in neuronal degeneration. Memantine binds more effectively than Mg2+ ions at the NMDA receptor, and thereby effectively blocks this prolonged influx of Ca2+ ions through the NMDA channel whilst preserving the transient physiological activation of the channels by higher concentrations of synoptically released glutamate. Thus memantine HCl protects against chronically elevated concentrations of glutamate. This research article reports a precise, accurate and sensitive HPTLC method, useful for routine quality control of Memantine HCl. The method was validated by parameters such as linearity, accuracy, precision and robustness. Memantine is official in USP-37 NF-32 [2]. Literature review reveals that the methods like HPLC, UV-Spectroscopic, LC-MS/MS, RP-HPLC, and UPLC have been reported for estimation of Memantine HCl in bulk and tablet dosage form [3-15]. But, No HPTLC method has been reported for the same. HPTLC method is widely used for routine drug analysis. Less amount of mobile phase is required which is one of the advantages of this method. Solvents need no prior treatment like filtration and degassing. High sample through put of similar or different nature of samples. Low cost pre coated HPTLC plates are available. Hence, this method is more economic and lower analysis time as compared to reported HPLC methods. This work deals with the validation of the developed method for the assay of Memantine HCl from tablet dosage form. Hence, the method is useful for routine quality control analysis. So, aim of dissertation work is to develop and validate precise and accurate HPTLC method.

Experimental

HPTLC method development

Instruments: A Camag HPTLC System (Switzerland) HPTLC instrument consists of CAMAG (Muttenz, Switzerland) Linomat V sample applicator with 100 µl applicator syringe (Hamilton, Bonadauz, Switzerland). Chromatography was performed on 10 cm × 10 cm aluminum TLC plates precoated with silica gel G*60F254* (E.

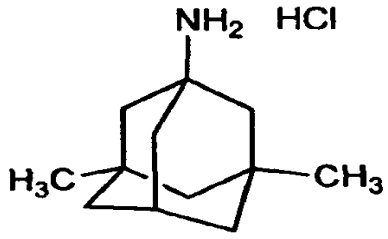

Figure 1: Memantine HCl.

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Merck, Darmstadt, Germany). CAMAG TLC scanner 3 was used for the densitometric scanning of the developed chromatogram. All drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., and Japan). Detection by UV cabinet with dual wavelength UV lamp (254 nm & 366 nm) and data analysis by Camag win-CATS software.

Chemicals and reagents: Standard Memantine HCl was obtained as gift sample by Alembic Pharmaceuticals Limited, Vadodara, India. The marketed formulation Admenta-10 tablets (Each film coated tablet contains Memantine HCl-10 mg) manufactured by Sun pharmaceuticals Limited was procured from local market. Methanol was obtained from Allied Chemicals Corporation, Vadodara. Ethyl acetate and n-Hexane were obtained from Chemdyes Corporation, Vadodara.

Chromatographic system

Sample application: Standard and formulation sample of Memantine HCl was applied on the HPTLC plates in the form of narrow bands of 6 mm length. The bands were applied 10 mm above from the bottom and 15 mm away from left edge of the plate. Samples were applied under a continuous drying stream of nitrogen gas.

Mobile phase and development: Plate was developed using a mobile phase consisting of *n*-Hexane: Ethyl Acetate: Diethylamine (5:5:0.7v/*v*/*v*). Linear ascending development was carried out in a twin-trough glass chamber equilibrated with the mobile phase vapors for 25 min. Mobile phase (10.7 ml) was used for development and allowed to migrate at up to 70 mm. After development, the HPTLC plate was air dried completely.

Densitometric analysis: Densitometric scanning was performed in the absorbance mode under control by win CATS planar chromatography software (CAMAG, Muttenz, Switzerland). The source of radiation was the deuterium lamp, and bands were scanned at 501 nm. The slit dimensions were 6 mm length and 0.45 mm width, with a scanning rate of 10 mm/s. Concentration of the compound was determined from the intensity of diffusely reflected light and evaluated as peak areas against concentrations using a linear regression equation.

Visualization of spots: Dipping in the detection reagent for visualization of spots. The plate was dipped in Dragendorff's reagent (approximately 10 ml was consumed) in petri dish and the plate was air-dried for 10 min. The entire plate was turned yellow, and orange spots appeared on the plate.

Preparation of standard stock solution: 50 mg of Standard Memantine HCl was weighed and transferred to a 10 ml volumetric flask and dissolved in methanol.The volume was made up to the mark with methanol to yield a solution containing 5000 µg/ml of Memantine HCl.

Test preparation

Total 20 tablets of Admenta-10 brand containing API as Memantine HCl were taken. They were individually weighed. Then average of weight of 20 tablets was taken. The tablets were crushed and powdered. The quantity of tablet powder equivalent to 50 mg of Memantine HCl was taken and transferred into 10 ml of volumetric flask and dissolved in methanol and volume was made up to the mark. The solution was filtered with whatman filter paper (0.45µm).

Method validation

Validation of the developed HPTLC method was carried out according to the International Conference on Harmonization (ICH) guidelines Q2 (R1) [16].

Specificity: Specificity of the method was ascertained by analysing standard drug and sample. The mobile phase resolved the drug very efficiently. The spot for Memantine HCl was confirmed by comparing the Rf and spectra of the spot with that of standard. The wavelength 501nm for detecting peak purity of Memantine HCl was assessed by comparing the spectra at three different levels, i.e, Peak start (s), Peak apex (M), and Peak end (E) Position of the band.

Linearity of calibration curves: Six series of standard Memantine HCl solution in the range of 1-6 µl were determined. So, linearity responses for Memantine HCl were assessed in the concentration ranges 5000-30000 ng/band of working standard solution. The area at each level was calculated and a graph of mean area v/s concentration (ng) was plotted. The correlation co efficient (r^2), intercept (c), and slope of regression line (m) were calculated and recorded.

Accuracy: Accuracy was determined by calculating recovery of both the drug by standard addition method at three different concentration levels (80%, 100% and 120%) of drug. Standard was prepared as per method. Along with standard calibration curve, assay formulation 2 µl (10000 ng/band) of solution containing Memantine HCl was spotted on TLC plate under nitrogen atmosphere. 1.6, 2 and 2.4µl of standard solution (Containing 8000, 10000, 12000ng/band) was added on succeeding spots to obtain final concentration range of 18000, 20000, 22000 ng/band for Memantine HCl. The plate was developed, dried and photometrically analyzed. The % Recovery was calculated.

Assay of formulation

Tablet powder equivalent to 50 mg Memantine HCl was taken in 10 ml volumetric flask. Methanol was added to the above flask, and the flask was sonicated for 10 min. The solution was filtered using Whatman filter paper No. 41, and the volume was made up to the mark with methanol in order to obtain a solution 5000 µg/ml of Memantine HCl. From which 3µl was applied in the form of band to obtain a conc. of Memantine HCl (15000ng/ band).

Precision

Method Precision (Repeatability): Method precision was established by assaying six sample preparations under same conditions. Repeatability of sample application was assessed by spotting 3µl (15000ng/band Memantine HCl) of drug solution six times on a TLC plate, followed by development of plate and recording the peak area for six spots. Individual assay values, Mean assay value, %RSD was calculated.

Intra-Day and Inter-Day Precision (Intermediate Precision): Variation of results within the same day (intra-day),variation of results between days (inter-day) was analyzed .

Intra-day precision was determined by analyzing Memantine HCl for three times on the same day at 501 nm.

Inter day precision was determined by analyzing the drug daily for three days at 501 nm.

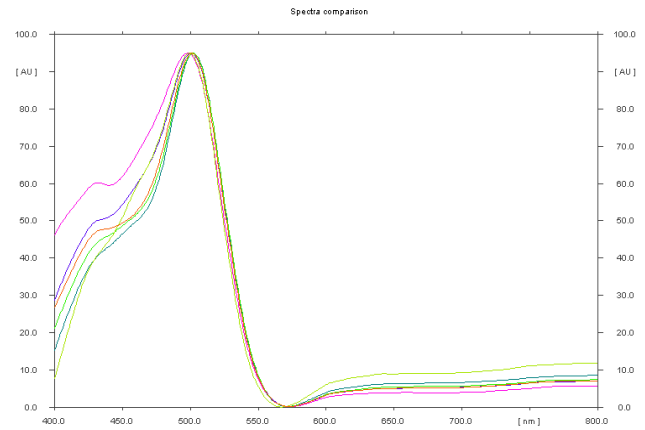

Figure 2: Overlain spectra of MEMHCl at 501 nm.

Sensitivity: The sensitivity of measurement of Memantine HCl by the use of proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ were calculated by equation. Based on the standard deviation of the response and the slope, LOD and LOQ were estimated using the formula:

LOD and LOQ

LOD and LOQ of the drug were derived by calculating the signal-to-noise (i.e.3.3 for LOD and 10 for LOQ) ratio using the following equations as per ICH guideline.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

where, σ=the standard deviation of the response.

S= slope of the calibration curve.

LOD and LOQ were determined from the standard deviations of the responses for six replicate determinations.

Robustness

The effect of small, deliberate variation of the analytical conditions on the peak are as of the drugs was examined. Change in chamber saturation time and change in volume of mobile phase were investigated and %RSD was assessed.

Results and Discussion

Optimization of the mobile phase

To develop the HPTLC method for the estimation of Memantine HCl, selection of the mobile phase was carried out on the basis of polarity. The mobile phase n-Hexane: Ethyl Acetate: Diethylamine (5:5:0.7v/*v*/*v*) mobile phase with a chamber saturation time of 25 min at ambient condition and solvent migration distance of 70 mm was selected as an optimum condition. These chromatographic conditions produced a well-defined, compact band of Memantine HCl with R_f 0.49 ± 0.02.

Selection of detection wavelength

The developed plate was subjected to densitometric measurements in scanning between 200–800 nm, and the overlaid spectrum was

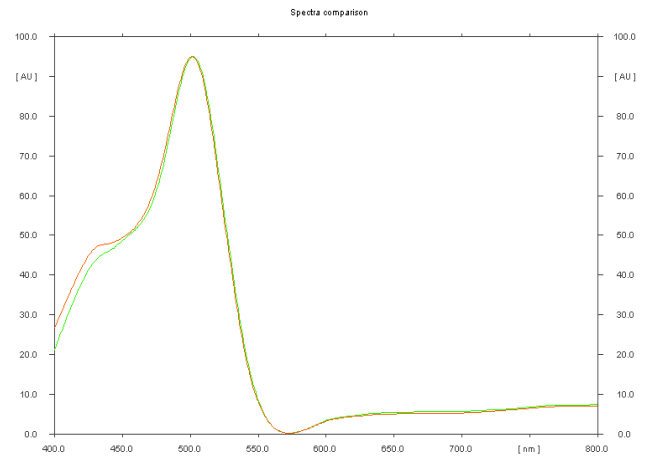

Figure 3: Overlain spectra of standard solution and dosage form solution.

Figure 4: Photograph of Developed TLC plate of MEMHCl 5000 µg/ml sample with 5000 – 30000 ng/band.

Table 1: Statistical data for MEMHCl by HPTLC Method.

PARAMETER	MEM HCl
Linearity [ng/band]	5000-30000 ng/band
Linearity Equation	y = 0.094x + 628.0
Slope	0.094
Intercept	628.0
Correlation Coefficient (R2)	0.995

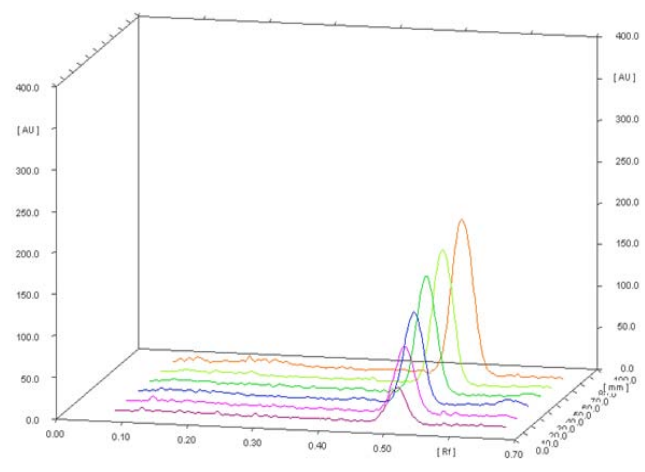

Figure 5: 3D representation of Densitogram for Calibration curve of MEMHCl.

Table 2: Recovery study for MEM HCl by HPTLC Method.

Conc. of Sample taken (ng/band)	Level	Conc. of pure API spiked (ng/band)	Total conc. (ng/ band)	Mean total conc. Found (n=3) [ng/ band]±SD	%Recovery Mean (n=3)±SD	%RSD
MEM HCl 10000	0%	0	10000	1563.13 ± 3.8682	99.54 ± 0.4565	0.46
	80%	8000	18000	2312.43 ± 7.7783	99.3 ± 0.2064	0.21
	100%	10000	20000	2493.83 ± 5.3780	99.26 ± 0.2137	0.22
	120%	12000	22000	2680.76 ± 4.4275	99.47 ± 0.4119	0.41

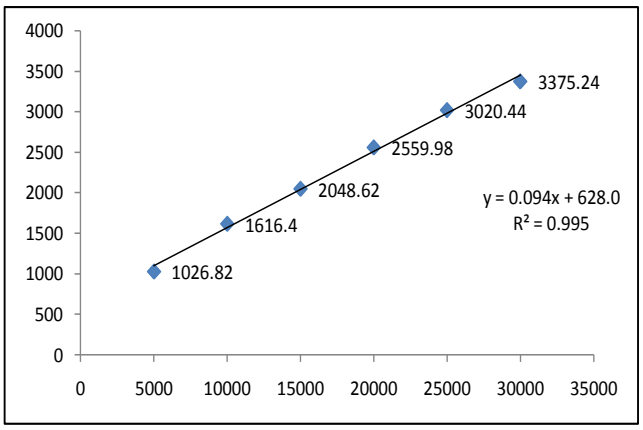

Figure 6: Calibration curve of Memantine HCl.

Table 3: Intraday and Interday precision data for MEM HCl by HPTLC Method.

Conc. (ng/band)	MEM HCl	
	Area Mean (n=3) ± SD	%RSD
Intra – day Precision		
10000	1622.20±4.0447	0.25
20000	2553.133±4.3061	0.17
30000	3372.833±6.7678	0.2
Inter – day Precision		
10000	1623.16±3.9068	0.24
20000	2549.36±5.6695	0.22
30000	3371.6±7.7252	0.23

Table 4: Repeatability data for MEM HCl by HPTLC Method.

Sr.No.	Area Of MEM HCl (15000ng/band)
1	2036.1
2	2043.5
3	2048.3
4	2042.3
5	2038.2
6	2053.4
Avg.	2043.633
SD	6.406767
%RSD	0.31

recorded using CAMAG TLC Scanner 3. The overlaid spectra showed that drug absorb appreciably at 501 nm. (Figure 2).

Validation

Specificity: The peak purity of MEM HCl was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9994 and r (M, E) =0.9992 Good match was obtained between standard and sample spectra of MEM HCl (Figure 3).

Linearity and Calibration Curves: The method was found to be linear in concentration ranges of 5000-10000 ng/ band of Memantine HCl (Figure 4). Three dimensional overlay of HPTLC densitogram of Calibration bands of Memantine HCl was obtained in above concentration ranges. The regression data showed a good linear relationship over the concentration range studied, demonstrating the suitability of the method for analysis (Table 1, Figure 5, Figure 6).

Accuracy: Accuracy of an analytical method is the closeness of test results to the true value (100%). It was determined by the application of analytical procedure to recovery studies, where a known amount of standard is spiked into pre-analyzed sample solutions. % Recoveries was found to be 99.41-100.42% for Memantine HCl. Values demonstrated the accuracy of the method was found to be 99.26% - 99.54% (Table 2).

Analysis of formulation

The formulation was analyzed using the proposed method which gave percentage recovery of more than 98.0% for Memantine HCl. A single band at R_f 0.49 ± 0.02 was observed in the chromatogram for Memantine HCl, and no interference from the excipients present in the formulation was observed.

Precision

Intra-day precision refers to the use of an analytical procedure within a laboratory over a short period of time by the same operator with the same equipment, whereas inter-day precision involves estimation of variations in analysis when a method is used within a laboratory on different days. Repeatability of the scanning device and injection was studied by applying and analyzing Memantine HCl samples (15000 ng/ band) 6 times. The RSD values obtained were less than 2%, which was under the acceptance criteria of ICH method validation guideline (<2%). The results indicated that the method is repeatable and reproducible (Table 3, Table 4).

Limit of detection and limit of quantification: Under the experimental conditions used, the lowest amounts of drug that could be detected (LOD) for Memantine HCl was found to be 80.07 ng/ band. The limit of quantification (LOQ) for Memantine HCl was found to be 242.637 ng/ band. This indicates that the nanogram quantity of drug can be estimated accurately and precisely which means the method is sensitive.

Robustness: The % RSD values less than 2% were obtained after introducing small, deliberate changes in examined. Change in chamber saturation time and change in volume of mobile phase were investigated and %RSD was assessed. Parameter of the developed HPTLC method, confirming its robustness (Table 5).

Table 5: Robustness for MEM HCl by HPTLC Method.

Conc. Of Sampe taken [ng/band]	Parameters	Level	Mean Peak Area ± SD (n=3)	%RSD
MEM HCl 15000	Chamber saturation time	20	2026.2 ± 7.9793	0.39
		30	2033.1 ± 4.6292	0.23
	Volume of mobile phase	+0.1ml	2029.2 ± 6.0058	0.3
		-0.1ml	2026.2 ± 5.9573	029

Conclusion

Proposed study describes HPTLC method for the estimation of Memantine HCl. The method was validated and found to be sensitive, accurate and precise with low level of LOD and LOQ. Statistical analysis proved that method was repeatable and selective for the analysis of Memantine HCl without any interference from the excipients. The method was successfully used for estimation of Memantine HCl from bulk and pharmaceutical dosage form determination of drug in its formulation.

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