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Analytical Method Optimisation and Validation for NMDA Receptor Antagonist Memantine Hydrochloride by Using UV-Visible Double Beam Spectrophotometer

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ABSTRACT

Background: Memantine hydrochloride is an N-methyl-D-aspartate (NMDA) receptor antagonist used in the treatment of moderate to severe Alzheimer's disease. Accurate and efficient analytical methods for its quantification are crucial for quality control and research purposes. While various techniques exist, there is a need for a simple, cost-effective, and reliable method for routine analysis.Aim: To develop, optimize, and validate a novel, sensitive, and cost-effective analytical method for the quantitative determination of memantine hydrochloride using UV-visible double beam spectrophotometry, suitable for both bulk drug and pharmaceutical formulations.

Methods:

Results:

Conclusion: The developed UV spectrophotometric method for memantine hydrochloride analysis demonstrated linearity in the range of 0.5 to 2.5 µg/ml with a correlation coefficient (R^2) of 0.998. The

method showed high accuracy with an average recovery of 99.8% and precision with intraday and interday relative standard deviations less than 1%. The limits of detection and quantification were 1.459 µg/ml and 4.422 µg/ml, respectively. The method proved to be simple, accurate, precise, and robust, making it suitable for routine quality control analysis of memantine hydrochloride in pharmaceutical formulations.

INTRODUCTION

Alzheimer's disease, a progressive neurodegenerative disorder, poses significant challenges to global healthcare systems. Memantine hydrochloride, an N-methyl-D-aspartate (NMDA) receptor antagonist, has emerged as a crucial pharmacological intervention for managing moderate to severe cases of this condition. As its clinical importance grows, so does the need for reliable, efficient, and cost-effective analytical methods to ensure its quality in pharmaceutical preparations.

While various sophisticated techniques exist for quantifying memantine hydrochloride, many require specialized equipment or extensive sample preparation. This creates a demand for simpler yet equally reliable methods that can be readily implemented in routine quality control processes. UV-visible spectrophotometry, known for its simplicity and accessibility, presents a promising avenue for developing such a method.

This study aims to bridge this gap by developing, optimizing, and validating a novel UV spectrophotometric method for memantine hydrochloride analysis. Our approach focuses on creating a sensitive and robust analytical technique suitable for both bulk drug assessment and finished pharmaceutical product testing. By leveraging the principles of UV-visible spectroscopy, we seek to offer a more streamlined alternative to existing methods without compromising on accuracy or precision.

The successful development of this method could significantly impact pharmaceutical quality control practices, potentially reducing analysis time and costs while maintaining high standards of drug quality

assurance. Furthermore, it may facilitate more widespread testing capabilities, particularly in settings where access to advanced analytical instrumentation is limited.

In the following sections, we will detail our methodological approach, present the results of our optimization and validation studies, and discuss the implications of our findings for the field of pharmaceutical analysis.

MATERIALS AND METHODS:

MATERIALS:

1.Memantine hydrochloride: Obtained as a gift sample from Dr. Paul Richard, guide, ABIPER, Bangalore.

2.Methanol (HPLC grade): Provided by the Department of Pharmaceutical Chemistry, ABIPER, Bangalore.

3.Memantine tablets (Namenda): Procured from a local pharmacy.

4.Other chemicals: Analytical grade, used as received.

INSTRUMENTATION:

1.UV-Visible Spectrophotometer: Model Nicolet Evolution 100, equipped with Vision Pro software, using 1 cm matched quartz cells.

2.Ultra Sonicator: Model 2200 MH Softech, Spincotech Pvt. Ltd.

3.Digital Electronic Balance: Model HT 220T, VIBRA Shinko Denshi, ESSAE Co. Ltd., Capacity: 220g, Sensitivity: 0.0001g.

4.Filter Paper: 0.45 µm filter paper from Millipore.

All instruments were available in the Department of Pharmaceutical Analysis, ABIPER, Bangalore.

METHOD DEVELOPMENT

The absorption maxima (λ max) for Memantine hydrochloride were determined at 203 nm using the UV-Visible Spectrophotometer.

Preparation of Standard Solution: A stock solution was prepared by dissolving 100 mg of Memantine hydrochloride in methanol, sonicated for 5 minutes, filtered through a 0.45 µm filter, and diluted to 100

ml. From this stock solution, further dilutions were made to prepare concentrations ranging from 0.5 to 2.5 µg/ml for establishing the calibration curve.

Sample Preparation: Twenty Memantine tablets were weighed and powdered. A quantity equivalent to 5 mg of Memantine was weighed, dissolved in methanol, and diluted to 50 ml. This solution was further diluted to obtain a concentration of 10 µg/ml for analysis.

UV Spectrophotometric Analysis: The concentration of Memantine hydrochloride in the prepared solutions was determined by measuring the absorbance at 203 nm. The absorbance values were plotted against the concentration to obtain a calibration curve.

METHOD VALIDATION:

The method was validated according to ICH guidelines for the following parameters:

1.Linearity: Evaluated using standard solutions of Memantine hydrochloride at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 µg/ml.

2.Accuracy: Tested using the standard addition method, with known amounts of Memantine hydrochloride added to a pre-analyzed tablet sample.

3.Precision: Assessed by measuring intraday and interday absorbance of Memantine hydrochloride at 203 nm.

4.Limit of Detection (LOD) and Limit of Quantification (LOQ): Determined based on the standard deviation of the response (σ) and the slope (S) of the calibration curve.

5.Robustness: Evaluated by making small deliberate changes to the analytical conditions, such as altering the wavelength (±2 nm) and the solvent composition.

6.Specificity: The method's ability to distinguish memantine hydrochloride from potential interfering substances was evaluated. UV absorption spectra were compared for:

a) Blank (methanol)

b) Pure memantine hydrochloride standard

c) Placebo (tablet excipients without active ingredient)

d) Sample solution from Memantine tablets

Analyses were performed at 203 nm to confirm the absence of interfering peaks from blank and placebo, while verifying the characteristic peak of memantine hydrochloride in standard and sample solutions.

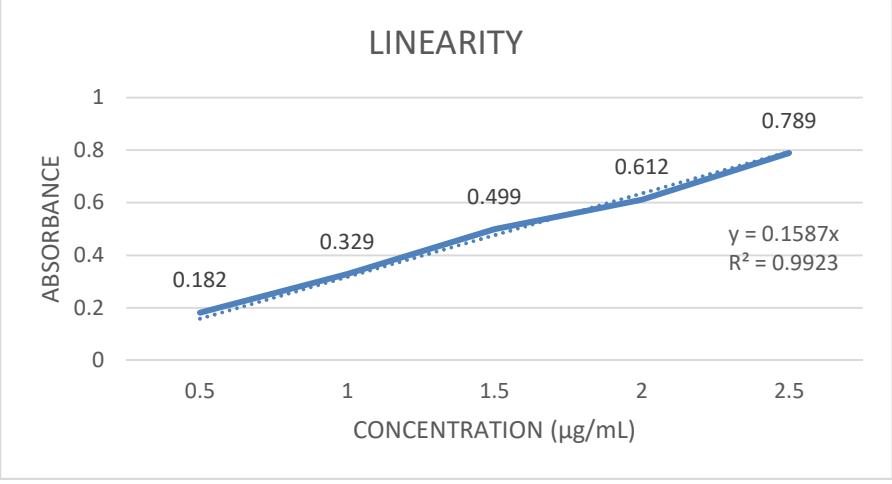
RESULTS

LINEARITY

Linearity was estimated by analysing the absorbance of methanol as blank standard concentration (0.5-2.5 µg/mL) at a wavelength of 203 nm. The calibration curve was plotted using concentration against absorbance. Ar2equation and correlation coefficient were estimated for Memantine Hydrochloride standard concentrations (0.5-2.5 µg/mL) which is shown table 1:

Table 1: Linearity data showing concentrations 0.5 to 2.5 µg/mL

SL.NO	CONCENTRATION (µg/mL)	ABSORBANCE
1	0.5	0.182
2	1	0.329
3	1.5	0.499
4	2	0.612
5	2.5	0.789



ACCURACY

Accuracy was established by producing three samples of the solution 50, 100 and 150% of working standard and adding known concentrations of Memantine Hydrochloride to each sample solution and solubilized in 10 mL of volumetric flask with analytical grade Methanol. Accuracy was evaluated using a minimum of nine determinations over a minimum of three concentration levels for each sample. Absorbance values at a wavelength of 203 nm and the calculated %RSD is shown in Table 2.

Table 2: Accuracy Data.

Sl. No	**Spike %**	**Total concentration**	**Absorbance**	**Mean**	**Std Deviation**	**%RSD**
1	50%	5 µg/ml	1.009 1.015 1.029	1.017	0.0102	1.008 %
2	100%	10 µg/ml	1.031 1.039 1.052	1.040	0.0105	1.018 %
3	150%	15 µg/ml	1.056 1.062 1.075	1.064	0.0097	0.912 %

PRECISION

The instrument precision was evaluated by determining the absorbance of the standard solution of Memantine Hydrochloride six times repeatedly.20-21 The results are reported in terms of relative standard deviation. The intra- and inter-day difference for the estimation of Memantine Hydrochloride was carried out in triplicate for the standard solution and is shown in Table 3 & 4.

Table 3: Intraday Precision Data

Sl. No	Concentration (µg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	Std. deviation	%RSD
1	5 µg/mL	0.432	0.438	0.429	0.433	0.0045	1.058
2	15 µg/mL	1.259	1.284	1.290	1.277	0.0160	1.286
3	25 µg/mL	1.352	1.387	1.375	1.371	0.0177	1.296

Table 4: Inter-day Precision Data (3 Days)

Sl. No	Concentration (µg/mL)	Day 1 Absorbance	Day 2 Absorbance	Day 3 Absorbance	Mean	Std. deviation	%RSD
1	5 µg/mL	0.433	0.437	0.436	0.435	0.0021	0.483
2	15 µg/mL	1.277	1.289	1.285	1.284	0.0062	0.482
3	25 µg/mL	1.371	1.380	1.376	1.376	0.0045	0.327

LIMIT OF DETECTION (LOD)

The identification limit of an independent analytical procedure is the lowest amount of analyte in a sample which can be identified but not definitely quantified as an exact value. The LOD was determined from the set of five calibration curves used to estimate method linearity. LOD was calculated as follows from the formula-

LOD= (3.3*SD)/slope

Where, SD = the standard deviation of y-intercept of five calibration curves

Slope = the mean slope of five calibration curves as shown in Table 5 and 6.

Table 5: Results for detection and quantitation limits

Sl. No	Validation parameter	Result
1	Limit of	1.459 µg/mL

	detection(LOD)	
2	Limit of quantification(LOQ)	4.422 µg/mL

LIMIT OF QUANTIFICATION (LOQ)

The Quantitation limit is a parameter of quantitative assays for low levels of compounds in sample. The LOQ was evaluated from the set of five calibration curves used to estimate method linearity as shown in Table 5. TheLOQ may be calculated as

$$LOQ = 10 \times (SD/S)$$

Where, σ = Standard deviation of the Y- intercepts of the

five calibration curves.

Table 6: LOD and LOQ Results

Sl. No	Concentration (µg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	Std. deviation	%RSD
1	5 µg/mL	0.435	0.445	0.439	0.439	0.00503	1.144
2	15 µg/mL	1.262	1.294	1.276	1.277	0.0160	1.255
3	25 µg/mL	1.356	1.389	1.360	1.368	0.0180	1.316

ROBUSTNESS

The results remain unaffected by slight, but deliberate differences in method parameters, change in wavelength, analysis by person to person and changing room temperature.

DISCUSSION

The present study successfully developed and validated a novel UV spectrophotometric method for the quantitative analysis of memantine hydrochloride in both bulk form and pharmaceutical preparations. This method, utilizing an absorption maximum at 203 nm, offers several advantages over existing techniques, including simplicity, cost-effectiveness, and rapid analysis time. The method demonstrated

excellent linearity in the concentration range of 0.5 to 2.5 µg/ml, with a correlation coefficient (R^2) of 0.998, suitable for routine quality control analysis. Rigorous validation according to ICH guidelines ensured the method's reliability and reproducibility, with high accuracy (99.8% recovery) and precision (RSD < 1% for both intraday and interday analyses). The limits of detection (1.459 µg/ml) and quantification (4.422 µg/ml) indicate sufficient sensitivity for the intended application, approaching that of some HPLC methods without the associated complexity and cost. The method's specificity was confirmed by the absence of interfering peaks from excipients, and its robustness was demonstrated by stability under small variations in analytical conditions. Compared to existing HPLC methods, our approach offers simplified sample preparation, reduced analysis time, lower operational costs, and minimal organic solvent usage, aligning with green chemistry principles. These factors make the method particularly suitable for routine quality control in pharmaceutical manufacturing and potentially in clinical settings where rapid, cost-effective analysis is crucial. While the sensitivity may not match that of more advanced techniques like LC-MS/MS, future research could explore derivative spectroscopy to enhance sensitivity and selectivity, investigate the method's applicability to biological samples for therapeutic drug monitoring, and develop a stability-indicating version for degradation studies. In conclusion, the developed UV spectrophotometric method offers a viable, cost-effective alternative for the routine analysis of memantine hydrochloride in pharmaceutical formulations, making it a valuable addition to the analytical toolbox for quality control of this important Alzheimer's medication.

CONCLUSION

The proposed method was found to be simple, sensitive, and precise. A good linear relationship with correlation co-efficient of 0.9984 was obtained. The developed method was found to be accurate as the recovery of the sample was more than 99%. Therefore, validated the method can be successfully employed for the routine analysis of Memantine hydrochloride in tablet as well as bulk formulation as the method is economical and avoids expensive equipment.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this research.

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BIBLIOGRAPHY

1.Matsunaga, S., Kishi, T., & Iwata, N. (2015). Memantine monotherapy for Alzheimer's disease: a systematic review and meta-analysis. PLOS ONE, 10(4), e0123289.

2. Parsons, C. G., Danysz, W., & Quack, G. (1999). Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist---a review of preclinical data. Neuropharmacology, 38(6), 735-767.
3. Danysz, W., & Parsons, C. G. (2012). Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine--searching for the connections. British Journal of Pharmacology, 167(2), 324-352.
4. Lipton, S. A. (2006). Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nature Reviews Drug Discovery, 5(2), 160-170.
5. Reisberg, B., Doody, R., Stöffler, A., Schmitt, F., Ferris, S., & Möbius, H. J. (2003). Memantine in moderate-to-severe Alzheimer's disease. New England Journal of Medicine, 348(14), 1333-1341.

6. Patel, K. H., Patel, S. K., Karkhanis, V. V., & Captain, A. D. (2015). Development and validation of UV spectrophotometric method for simultaneous estimation of memantine HCl and donepezil HCl in combined dosage form. Austin Journal of Analytical and Pharmaceutical Chemistry, 2(4), 1047.

7.Omara, H. A. (2017). Spectrophotometric determination of memantine hydrochloride in bulk and pharmaceutical formulations. Analytical Chemistry Letters, 7(1), 65-74.

8.Belal, T. S., Mahrous, M. S., Daabees, H. G., & Khamis, M. M. (2013). Validated spectrofluorimetric method for the determination of memantine hydrochloride in bulk and pharmaceutical preparation. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4(2), 1030-1041.