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RESEARCH ARTICLE

Dependable Spectrophotometric analytical determination of Amantadine in pharmaceutical formulation

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ABSTRACT

A simple, rapid, sensitive and accurate UV-spectrophotometric method has been developed for the estimation of Amantadine (AMT) in pharmaceutical formulation. The method was developed by using 0.1 N NaOH as a solvent and absorbance was measured at 780 nm. The proposed method is economical and sensitive for the estimation of AMT in bulk and tablet dosage form. The proposed methods spectrophotometric analysis was linear and in the range of 8- 40 µg/ ml at 780 nm with correlation coefficients (R2) of 0.9955. LOD and LOQ were found to be 3.576643 and 5.787704 respectively [SD: 0.474474135; SE: 0.212191284]. From the drug content analysis obtained, we could confirm the recovery to be 99.40% with a relative error less than or equal to 0.91 [SD: 0.0493; SE: 0.02848]. It is evident from these results that this method is applicable for the analysis of the drug in its bulk and tablet forms with comparable analytical performance.

INTRODUCTION

The primary pathogenic agents responsible for serious illnesses in humans and other living organisms are viruses. Since they are among the most prevalent microbes, antiviral medications have been developed to both prevent and treat viral infections. Given that they are prescribed and used for a variety of illnesses and purposes, antiviral medications are a necessary class of pharmaceuticals [1]. Consequently, the development of precise and accurate analytical procedures is essential for the detection of antiviral medicines in a variety of matrices. Because chromatographic techniques enable the simultaneous measurement of antivirals, they are widely utilized for quantitative purposes. Because the analysis may be completed rapidly and without the requirement for pre-treatment, electrochemical approaches have also become more and more important. Spectrophotometric and spectrofluorimetric techniques are employed due to their ease of use, low cost, and shorter turnaround times[2].

A small sample size is necessary for accurate findings when spectrophotometers are used to analyze pharmaceutical active substances. Results of dissolving tests for oral medicine doses can be analyzed using spectrophotometers. They can also verify a drug's composition, purity, and components. Spectrophotometers can gather the extremely precise colour measurements required to guarantee the product's quality and purity[3]. For pharmaceuticals, strict quality control is essential. Spectrophotometers can produce data that validates the business's procedures by demonstrating compliance and any associated risks. Pharmaceutical businesses are required by regulatory agencies to show evidence of their quality control efforts[3].

The main aim of quality control is to contribute to the safety of drug therapy by assuring that the active component is present in the pharmaceutical product in the level specified on the label and also limited levels of impurities (associated organic impurities and degradation products, inorganic impurities and residual solvents, etc.) are present in the bulk drug material and formulations made from it[4]. In addition to this, quality control aims in checking whether, the formulation's active ingredient's bioavailability is under control. In contemporary pharmacopoeias, UV spectrophotometry has two roles. Identification of bulk pharmaceuticals and the active components in their formulations has a less significant function[5].

Amantadine belongs to the class of adamantanes, which includes medications used as an antiviral and antiparkinson's[5]. It functions as a dopaminergic agent, analgesic, NMDA receptor antagonist, antiviral, antiparkinson, and non-narcotic analgesic. It belongs to the class of adamantanes and is a primary aliphatic amine. It is an adamantan-1-aminium's conjugate base. It comes from an adamantane hydride[6]. Amantadine hydrochloride (AMD), is an antiviral medication used to treat herpes zoster and prevent influenza type A virus infection. It can also be used to treat symptoms when given early in the infection. Due to its modest anti-Parkinsonian action, it has been used to treat Parkinsonism, mostly in its early stages and with minor symptoms. The hydrochloride salt of AMD is often administered orally[7].

The spectroscopic method for assay of Amantadine is not official in any pharmacopoeia[8, 9]. A few high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) techniques have been suggested for analysis of the formulation[10]. HPLC is the most widely used technique for the estimation of Amantadine in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine. The suggested HPTLC and HPLC methods for assay of AMT are expensive and need complex and sophisticated instrumentation[11].

In our study, we analysed the purity and estimation of the drugs using the proposed method (UV spectroscopy). We analysed the test drugs from pharmaceutical companies (CIPLA) in its dosage form. Linearity of the test drugs was tested at the same concentrations and LOD (LOD = 3.3 * SD of intercept / slope) and LOQ (LOD = 10 * SD of intercept / slope) was estimated to check the efficacy of the method. We also performed precision tests using both interday and intraday precision experiments. This method was designed to analyse the precison of the method. Throughout the study, we compared the puritiy of the drugs, with standard drugs (HPLC grade purity >99%) purchased from SIGMA ALDRICH Ltd.

METHODS

Materials: Amantadine HCl in tablets form was obtained from Manufacturers (Care formulation labs Pvt Ltd, Mumbai). Standard drugs were obtained from Sigma Aldrich ltd and all the reagents and chemicals used in the study are procured from Sigma Aldrich and were of analytical grade. Sodium Hydroxide was procured from Qualigens.

Method Development: AMT (Pure and Tablet Powder), which is 100mg in exact weight, was dissolved in 100ml of 0.1N NaOH and further dilutions were made with 0.1 N NaOH. A series of standard solutions containing 8-40µg/ml of AMT was prepared in 0.1N NaOH and absorbance was measured at 780nm against reagent blank. The same process was used for recovery trials, which involved adding a known quantity of pure medication to the formulation that had already been examined. The amount of drug discovered was used to compute the percentage recovery.

Standard drug solution (10µg/ml): About 1mg of standard drug was weighed and made upto 100ml following sonication in a volumetric flask. Each ml contains 10µg. Drug was dissolved in 0.1N NaOH.

Sample preparation: Standard drug samples of varying drug concentrations (8-40µg/ml) were studied for spectrophotometric estimation. Respective weight of powder was weighed and made upto 100ml with 0.1N NaOH following sonication in a volumetric flask. Test drug samples were dissolved in 0.1N NaOH at varying concentrations of 5-25µg/ml. Amount of the test drug was estimated by referring to the calibration curve. Recovery studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, percentage recovery was calculated. Samples were studied in three independent experiments (n=3).

Method Validation: The process of creating concrete proof to give a high degree of assurance that a certain action will consistently deliver the anticipated outcome or a product that satisfies its set standards and quality attributes is known as method validation. The accuracy, intraday precision, linearity, and percent recovery of the analytical technique development [12] for ACV in pharmaceutical dosage forms and bulk samples were validated (USP, 2000).

Linearity Stock solutions of varying concentrations (8-40µg/ml) of that were appropriate for the suggested procedures were measured. It was discovered that the Beer-Lambert concentration ranges were 8-40µg/ml. Following working standards preparation, absorbance was recorded at 253nm.

Precision (intraday and inter day): Studies of intraday and interday variance provided proof of precision. By obtaining various solutions with the same concentration (5µg/ml and 20µg/ml), analyzing them three times daily, and recording the results, the intraday precision was ascertained. Solutions of the same concentration (5µg/ml and 20µg/ml) were made for the interday investigation, analyzed, and the results were provided as % RSD. We studied precision using two concentrations.

Drug Content Estimation in Formulations: By using this procedure, the amount of AMT in the commercial formulations was estimated. The average weight of 5 tablets was calculated, and it was then finely pulverized. We studied 200 and 400mg of tablet form. The specified amount of AMT (200 and 400mg) from a precisely weighed tablet powder was placed into a 100ml volumetric flask containing 0.1N NaOH and sonicated for 10min. The alkali solution was used to bring the volume up to the required level once the medicines had completely dissolved. The final product was filtered via a membrane filter with a 0.45µm pore size. To obtain a concentration of 20µg/ml AMT (200mg form), 1ml of the filtrate solution was transferred into a 100ml volumetric flask and the volume was made up with alkali solution. To obtain a concentration of 20µg/ml AMT (400mg form), 0.5ml of the filtrate solution was transferred into a 100ml volumetric flask and the volume was made up with alkali solution. The samples were then subjected to the suggested methods, and the amount of AMT was calculated using calibration curves using the two developed methods.

Recovery Studies: Analytical recovery studies were carried out by adding known quantities of test drug with concentration ranges of 2, 10, and 20 and 25µg/ml in order to further validate the accuracy of the method developed.

Statistical analysis: SPSS (faculty version) was used for the analysis. Regression analysis was performed to calculate the intercept along with LOD and LOQ. LOQ was calculated by using the formula (3.3 * SD of intercept / slope) and LOD was calculated with the formula (10 * SD of intercept / slope) [13]. ANOVA was used to assess the statistical significance between the samples.

RESULTS

Validation of the method: According to the International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, guidelines for the validation of analytical procedures, the newly developed method was validated in terms of precision, specificity, linearity, limit of detection (LOD) and limit of quantification (LOQ).

Absorbance and recovery: From our study, we found potent recovery from the samples by all the methods. Results of analysis were shown in the table. The percentage recovery for all the methods performed was in the range of 99-100% indicating of zero interference of the excipients (in the formulation). Results were shown in the table. Relative error % was found to be 1.1640, 0.7453, 1.1633 and 0.1960 for 5, 10, 20 and 25mg samples respectively. All of the proposed methods are found to be free from any interference from the excipients in the formulation. Absorbance values obtained at 253nm are shown in the table. The proposed methods spectrophotometric analysis was linear and in the range of 8- 40µg/ml at 780nm with correlation coefficients (R^2) of 0.9955 (Y=0.0265x-0.118). Results from the linearity and accuracy studies are summarized in table 1.

Table 1: Table showing the accuracy data along with SE, COV and relative error.

Sample in µg/ml	Calculated amount	SD	COV	SE	% recovery	Relative error
5	4.942	0.0574	1.1611	0.0331	98.8360	1.1640
10	9.925	0.0782	0.7880	0.0452	99.2547	0.7453
20	19.767	0.1144	0.5788	0.0661	98.8367	1.1633
25	24.951	0.5738	2.2997	0.3313	99.8040	0.1960

Linearity and accuracy: Regression analysis was done for method validation to see the significant effects at varying concentrations. LOD and LOQ were found to be 3.576643 and 5.787704 respectively [SD: 0.061156459; SE: 0.02735]. Intercept and slope were found to be 0.118 and 0.0265 respectively. The devised method was deemed permissible as the percentage recovery and percentage RSD were determined to be within acceptable levels for accuracy and precision, respectively. LOD and LOQ were also discovered to be within an acceptable range. The outcomes show that the developed approach works well for the medication acyclovir.

Interday validation: The Interday precision of the proposed method was also determined by analysing test drugs at two different concentration levels by the proposed procedure for 5 different days. The % recovery was found to be in the range of 98.33 to 99.83%. The low Relative error (<2) values showed that the proposed procedure exhibited excellent Interday precision. However, no significant observation was noted on the interday precision between the concentrations ($p<0.05$). F value were found to be too low than the F critical value ($p<0.05$).

Intraday validation: The Intraday precision of the proposed method was also determined by analysing test drugs at two different concentration levels by the proposed procedure at 5 different times. The % recovery was found to be in the range of 98.83 to 99.58%. The low Relative error (<2) values showed that the proposed procedure exhibited excellent Intraday precision. However, no significant observation was noted on the intraday precision between the concentrations ($p<0.05$). F value were found to be too low than the F critical value ($p<0.05$).

In precision studies, the recommended UV spectroscopic technique demonstrated adequate reproducibility when the RE was less than 2%. The percentage RE was found to be less than 2, indicating that the medication or sample solution is stable for a day and 3days signifies great accuracy of the procedure. The findings for the inter and intra-day precision studies were provided in table 2 and 3 respectively.

Table 2: Table showing the accuracy data along with SE, COV and relative error for the interday analysis.

Sample (5µg /ml)	Calculated amount	SD	COV	SE	% recovery	Relative error
Day 1	4.967	0.0096	0.1942	0.0056	99.337	0.662
Day 2	4.992	0.0117	0.2348	0.0068	99.838	0.161
Day 3	4.967	0.0092	0.1845	0.0053	99.337	0.662
Day 4	4.942	0.0227	0.4589	0.0131	98.837	1.163
Day 5	4.979	0.0112	0.2257	0.0065	99.588	0.411
Sample (20µg /ml)	**Calculated amount**	**SD**	**COV**	**SE**	**% recovery**	**Relative error**
Day 1	19.70503	0.00964	0.04894	0.00557	98.52515	1.4749
Day 2	19.66746	0.00751	0.03816	0.00433	98.33732	1.6627
Day 3	19.66746	0.00404	0.02055	0.00233	98.33732	1.6627
Day 4	19.69251	0.00693	0.03518	0.004	98.46254	1.5375
Day 5	19.67999	0.01217	0.06182	0.00702	98.39993	1.6001

Table 3: Table showing the accuracy data along with SE, COV and relative error for the intraday analysis.

Sample (5µg /ml)	Calculated amount	SD	COV	SE	% recovery	Relative error
Day 1	4.9794	0.0057	0.1142	0.0033	99.5883	0.4117
Day 2	4.9544	0.0038	0.0764	0.0022	99.0874	0.9126
Day 3	4.9794	0.0131	0.2621	0.0075	99.5883	0.4117
Day 4	4.9419	0.0035	0.0711	0.002	98.837	1.163
Day 5	4.9419	0.0068	0.1377	0.0039	98.837	1.163
Sample (20µg /ml)	**Calculated amount**	**SD**	**COV**	**SE**	**% recovery**	**Relative error**
Day 1	19.7927	0.00586	0.0296	0.00338	98.963	1.0365
Day 2	19.7301	0.01206	0.0611	0.00696	98.65	1.3496
Day 3	19.7426	0.00361	0.01826	0.00208	98.712	1.287
Day 4	19.7927	0.01185	0.05985	0.00684	98.963	1.0365
Day 5	19.7676	0.01762	0.08912	0.01017	98.838	1.1618

Drug Content Estimation in Formulations: From the drug content analysis obtained, we could confirm the recovery to be 99.40% with a relative error less than or equal to 0.91 [SD: 0.0493; SE: 0.02848]. It is evident from these results that this method is applicable for the analysis of the drug in its bulk and tablet forms with comparable analytical performance. Results from the drug content estimation studies are summarized in Table 4.

Table 4: Table showing the accuracy data along with SE, COV and relative error for the drug content estimation.

Sample (20µg /ml)	Calculated amount	SD	COV	SE	% recovery	Relative error
200mg	19.8803	0.0493	0.2481	0.02848	99.4016	0.5983
400mg	19.8177	0.0624	0.3141	0.03606	99.0886	0.9113

Selectivity: UV spectra of the standard drugs and test drugs were compared with those for ascertaining the selectivity of the method. The λmax remained unchanged in both the standard as well as test showing the selectivity of method for the studied drugs in comparison to those of the standards. AMT exhibited its maximum absorption at 780nm and obeyed Beer's law 8-40µg/ml. Standard showed maximum peak (Figure). Sample was dissolved in 0.1N NaOH. Both showed absorbance at 780nm but the standard showed high quality absorbance than the sample.

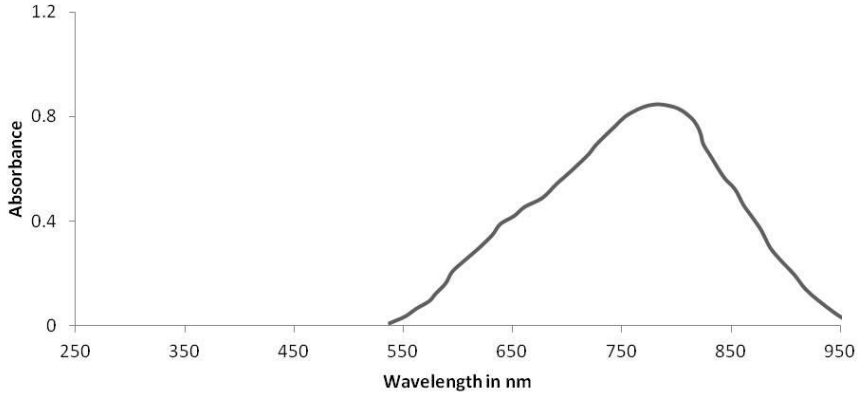

Figure 1: UV spectra showing the peaks of the standard (20µg/ml). Peak absorbance was seen at 780nm. Standard shows maximum peak than the sample dissolved in 0.1N NaOH.

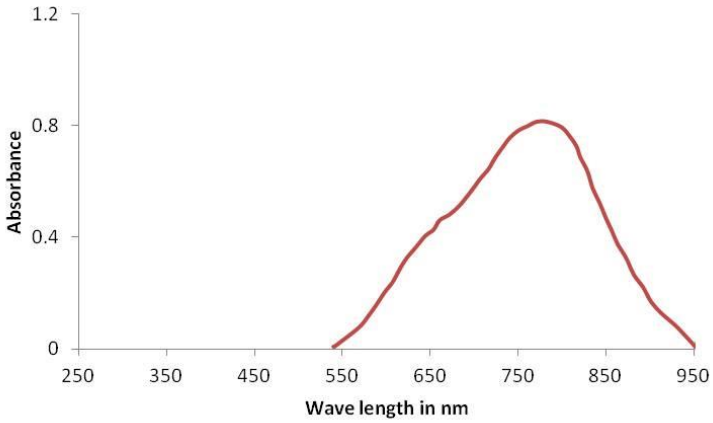

Figure 2: UV spectra showing the peaks of the test sample (20µg/ml). Peak absorbance was seen at 780nm. Standard shows maximum peak than the sample dissolved in 0.1N NaOH.

It is evident from the aforementioned results that the proposed methods gave satisfactory results with AMT in bulk. Thus, its capsules were subjected to the analysis for their contents for AMT by the proposed methods and the official method. The label claims, as percentages, ranged from 99.38 to 99.83% % (Table 3). These results were compared with those obtained from the official method by statistical analysis with respect to the accuracy (t-test) and precision (F-test). No significant differences were found between the calculated and theoretical values of t- and F-tests at 95 % confidence level proving similar accuracy and precision in the analysis of AMT in its capsules. It is evident from these results that all the proposed methods are applicable to the analysis of the drug in its bulk and capsule forms with comparable analytical performance.

Discussion: The results of this study show that the designed and validated method was able to accomplish efficient identification and selectivity in a shorter duration. With a correlation coefficient of 0.9927, the linear response of the spectrophotometric technique was obtained in the concentration range of 5–25µg/ml. The proposed and validated approach was found to be linear, accurate, and robust against the wide concentration of Amantadine, which may aid in both qualitative and quantitative validation. A systematic risk analysis was carried out and the target analytical profile was developed to determine the critical method attributes influencing the critical quality attributes. The most crucial aspects of quality were solvent suitability, resolution, specificity, and spectrum. The results obtained indicate that AMT could be successfully estimated using this analytical approach without interference from excipients. In comparison to HPLC and UPLC methods, this approach is much more cost-effective and only requires a standard solvent and basic laboratory supplies. The created technique is practical and efficient for both routine acyclovir analysis in the drug formulation and quality control[14].

Conclusion: It was discovered that the suggested approach was straightforward, exact, repeatable, and yielded a respectable analyte recovery, making it suitable for use in the examination of both pharmaceutical capsule formulations and bulk dosages of AMT. The other benefits of these techniques for routine analysis are their low cost and short analytical time. Its benefits over other current techniques are affordability, simplicity, and speed. It is possible to accurately quantify acyclovir in tablet form using the current UV spectrophotometric technique, and excipient interference does not occur throughout the investigation. The procedure has been verified in accordance with the recommendations of the International Conference on Harmonization (ICH), and for these dosage forms, it ought to be employed as a standard quality control analysis, or assay.

Conflicts of Interest: The authors declare no conflict of interest.
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Ethics Statements: The study involves no animal or clinical studies. Hence not applicable for our study.

Author Contribution: The authors confirm contribution to the paper as follows: Author 1: Study conception and design, data collection: Author 2: Analysis and interpretation of results: Author 3: Draft manuscript preparation: All authors reviewed the results and approved the final version of the manuscript.

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