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Development and Validation of Simple and Rapid UV Spectroscopic Method for estimation of Clonidine Hydrochloride in Tablet Dosage Form

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Abstract

A new simple, specific, linear, precise, accurate and robust UV Spectroscopic method was developed for the determination of Clonidine Hydrochloride in tablet dosage form. Solution was scanned over UV-visible range for its wavelength of maximum absorbance. The wavelength of maximum absorbance for Clonidine Hydrochloride was found to be 271 nm. The method was validated as per ICH guidelines. Linearity range was observed in concentration of 40.02 – 120.06 µg/mL for Clonidine Hydrochloride. The mean percentage recovery of Clonidine Hydrochloride was found to be 99.1%. The correlation coefficient was close to 1. Developed method was found to be robust for the intended use. A simple, precise and cost-effective UV spectroscopic method is very beneficial for routine analysis in pharmaceutical industry.

Keywords

Clonidine Hydrochloride, UV Spectroscopy, Validation

Introduction

Spectroscopy is the study of the interaction between matter and electromagnetic radiation. Historically, spectroscopy originated through the study of visible light dispersed according to its wavelength, by a prism. Later the concept was expanded greatly to include any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopy and spectrography are terms used to refer to the measurement of radiation intensity as a function of wavelength [1,2].

Clonidine Hydrochloride [CLND] chemical name is 2-(2,6-Dichloroanilino)-2 imidazoline hydrochloride. Refer figure B.1. It is a sympatholytic drug used to treat disease of attention deficit/ hyperactivity, anxiety disorders, menopausal flushing, migraine, diarrhoea, certain pain conditions and hypertension [3]. This causes dilation of the peripheral blood vessels or lessened cardiac output and thus lowered blood pressure [4,5]. It is a key α2 agonist drug that prevents the sympathetic nervous system's stimulation. Clonidine Hydrochloride can be used in the treatment of Tourette syndrome [6].

Many analytical methods are reported in literature for the determination of Clonidine Hydrochloride that are Spectrophotometric [7,8], HPLC [9-13], LC-MS [14], Colorimetric [15], Capillary Electrophoresis [16].

Clonidine is a white crystalline powder. It has a boiling point of 305oC. It soluble in water and ethanol, rather difficult to dissolve in dehydrated alcohol, very difficult to dissolve in chloroform, and practically insoluble in ether. The partition coefficient value in octanol/water is 1.59, and the clonidine pKa value at room temperature is 8.3 [17].

Clonidine is used alone or together with other medicines to treat high blood pressure (hypertension). High blood pressure adds to the workload of the heart and arteries. If it continues for a long time, the heart and arteries may not function properly. This can damage the blood vessels of the brain, heart, and kidneys, resulting in a stroke, heart failure, or kidney failure. High blood pressure may also increase the risk for heart attacks. These problems may be less likely to occur if the blood pressure is controlled.

Clonidine belongs to the class of medicines called antihypertensives. It works in the brain to change some of the nerve impulses. As a result, the blood vessels relax and blood passes through them more easily, which lowers blood pressure. When the blood pressure is lowered, the amount of blood and oxygen going to the heart is increased.

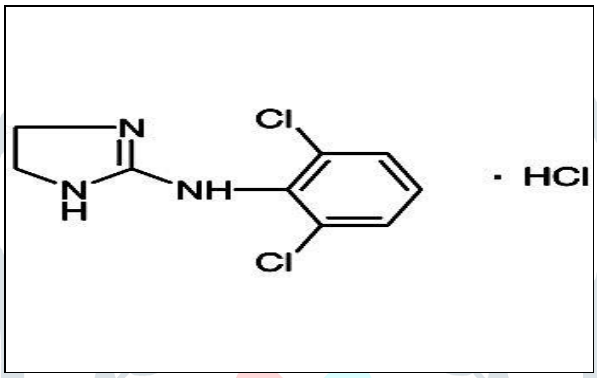

Fig. B. 1 Clonidine Hydrochloride

1. Material and Methods

1.1 Method Development:

To develop a rugged and suitable spectrophotometric method for the quantitative determination of Clonidine Hydrochloride, the analytical condition were selected after testing the different parameters such as diluents, buffer, buffer concentration and other conditions

Various compositions of diluents were tried for achieving the results. Diluent water which was degassed in an ultrasonic bath gave the best results hence water as diluent was finalized.

The reference solution was scanned with medium scanning speed for a UV range of UV Spectrophotometer, the ranging from 400-200 nm with a diluent as a blank. Refer figure B. 2 & figure B. 3 for blank & reference solution spectrum scan. After acquiring the spectrum, λmax was identified. Clopidogrel shows λmax at 271 hence λmax at 271 was finalized.

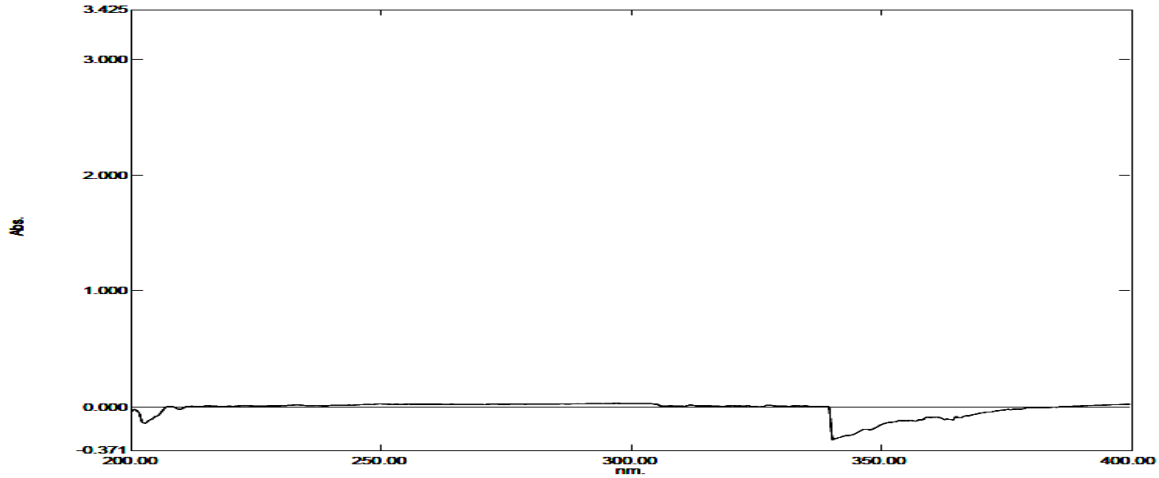

Fig. B. 2 Representative spectra of blank from 200 -400 nm

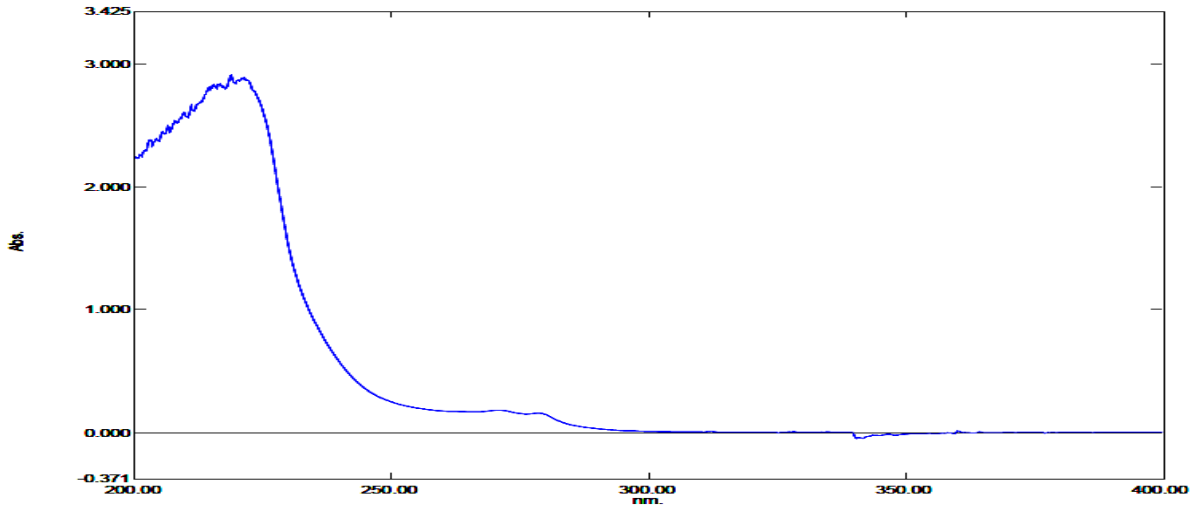

Fig. B. 3 Representative spectra of reference solution from 200 - 400 nm

1.1.1 Instrumentation

The Ultraviolet (UV) analysis was carried with UV 1800 (Shimadzu) UV Spectrophotometer. Quartz cell of 1.0 cm path length was used.

1.1.2 Reagents and chemicals

Clonidine Hydrochloride was taken from commercial source and tablets (containing 0.3 mg Clonidine) were obtained from Market. Demineralized water is used as a diluent.

1.1.3 Diluent

Demineralized water is used as a diluent.

1.1.4 Preparation of reference solution

20 mg of Clonidine Hydrochloride working standard was weighed & transferred in to 10 ml volumetric flask. 7 mL of diluent was added and shake the solution until dissolved. Volume was made up to the mark with diluent & mixed well. Further 2 mL was diluted to 50 ml with diluent. Solution was mixed well and absorbance was measured. (Concentration: 80 ppm of Clonidine Hydrochloride)

1.1.5 Preparation of Test solution

Intact 27 tablets were weighed (Each tablets containing 0.3 mg Clonidine Hydrochloride) and transferred into a 100 mL volumetric flask. 70 mL of diluent was added and sonicate for 15 minutes with intermittent shaking. Then solution was cooled and diluted up to the mark with diluent. Solution was mixed well and absorbance was measured. (Clonidine Hydrochloride other strength, sample size changed keeping final concentration 80 ppm of Clonidine Hydrochloride).

1.2 Method Validation:

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements

for the intended application of the method. They were tested using the optimized spectroscopic conditions and instruments.

1.2.1 Specificity

In the work, a solution containing a mixture of the tablet excipients were prepared using the sample preparation procedure to evaluate possible interfering peaks. Maxima /spectral pattern of test solution should match with that of reference solution.

1.2.2 Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity of Clonidine Hydrochloride was established by analyzing serial dilutions of a stock solution of the working standard. Five concentrations such as 40.02, 64.03, 80.04, 96.05 & 120.06 ppm for Clonidine Hydrochloride were prepared and analyzed as per table A. 1. Correlation coefficient & %Y-axis intercept were calculated. The interval between upper and lower concentration limit with acceptable linearity was reported to be the range of the proposed UV method.

Table A.1 Linearity Concentration Levels of Clonidine Hydrochloride

% level	Volume of stock solution (2001 ppm) (mL)	Diluted to (mL)	Final concentration in ppm
50%	1.0	50	40.02
80%	1.6	50	64.03
100%	2.0	50	80.04
120%	2.4	50	96.05
150%	3.0	50	120.06

1.2.3 Accuracy

The accuracy of the method was determined by recovery experiments known concentrations of working standard was added to the fixed concentration of the pre-analyzed Tablet sample. Percent recovery was calculated by comparing the area with pre-analyzed sample. Three different solutions of Clonidine Hydrochloride were prepared in triplicate at level of 50%, 100% and 150% of its predefined concentration (40.16, 80.32, 120.48 µg/mL) and the percentage mean and individual recovery was calculated. Refer Table A. 2 for accuracy solution preparation.

Table A. 2 Accuracy concentration levels preparation of Assay of Clonidine Hydrochloride

% level	Weight of placebo (in mg)	Volume of Clonidine Hydrochloride stock solution added (2008 ppm) (in mL)	Diluted to (mL)	Final concentration of Clonidine Hydrochloride in ppm
50%	2150.05	1.0	50	40.16
	2149.58			40.16
	2148.28			40.16
100%	2149.78	2.0	50	80.32
	2151.34			80.32
	2150.98			80.32
150%	2156.79	3.0	50	120.48
	2152.15			120.48
	2149.85			120.48

1.2.4 Method Precision (Repeatability)

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under prescribed conditions. Repeatability of the method was checked by carrying out six independent assays of Clonidine Hydrochloride at 81 µg/mL concentration. The mean area and % relative standard deviation (RSD) was calculated. % RSD should be ≤ 2 %.

1.2.5 Intermediate Precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. The data of the 1st day was taken from the analysis of "Repeatability". The second set of experiments was performed by a different analyst or on different instrument. The standard deviation, relative standard deviation and mean value difference was calculated from the results obtained on each day.

1.2.6 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was observed that the variations like sonication time & change in wavelength etc.

1.2.7 Solution Stability

The reference solution and test solution absorbance should be measured immediately after preparation and then after 24 hours at room temperature. Following this store these solutions at room temperature.

2. Results and Discussion

The proposed method for determination of Clonidine Hydrochloride showed molar absorptivity of 549.3112 L mol^{-1} cm^{-1}. Linear regression of absorbance on concentration gave the equation y = 0.0021x -0.0054 with a correlation coefficient (r) of 0.9969. The optical characteristics such as Beer's law limit and Sandell's sensitivity were calculated and are summarized in Table A.3.

The objective of the method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. Clonidine Hydrochloride showed maximum absorbance at 271 nm.

Table A. 3 Optical characteristics of Clonidine Hydrochloride

Parameter	**Results**
λmax	271 nm
Beer's law limit	40.02 – 120.06 µg/mL
Molar absorptivity	549.3112
Sandell's sensitivity (µg cm-2 / 0.001 absorbance unit)	0.4666
Regression equation (Y = a + bC)	Y=-3.2046 + 0.00214C
Slope (b)	0.00214
Intercept (a)	-3.2046
Correlation coefficient (R)	0.9969
% Range of error (Confidence limits) 0.05 Level 0.01 Level	 0.1357 0.1950

2.1 Specificity

By comparing the spectra of reference solution & test solution it was observed that maxima /spectral pattern of test solution were matching with that of reference solution. Refer figures B. 4 – B. 7 for UV scans of blank, placebo solution, reference solution and test solution respectively.

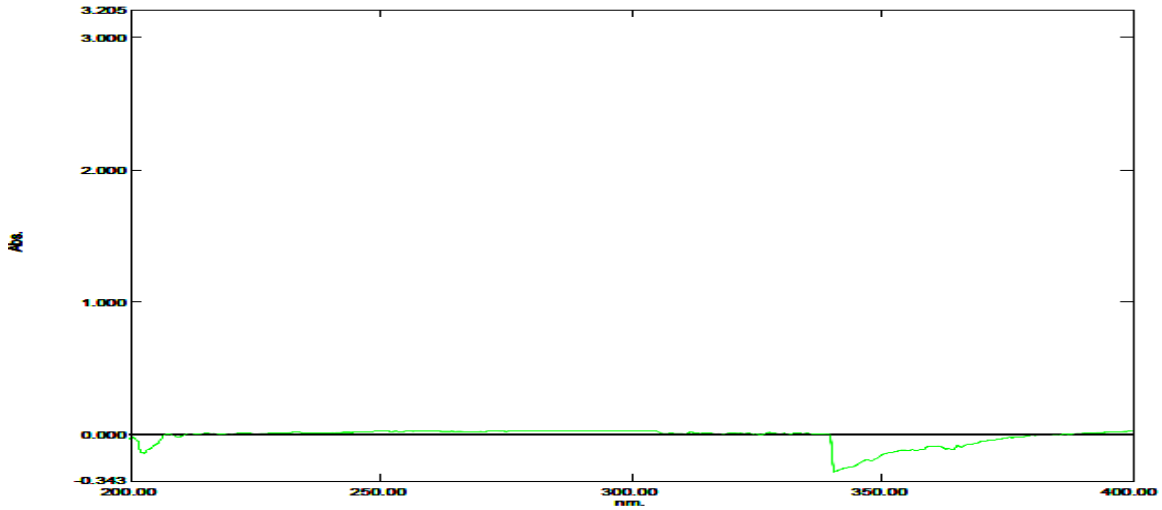

Fig. B. 4 Representative scans of blank

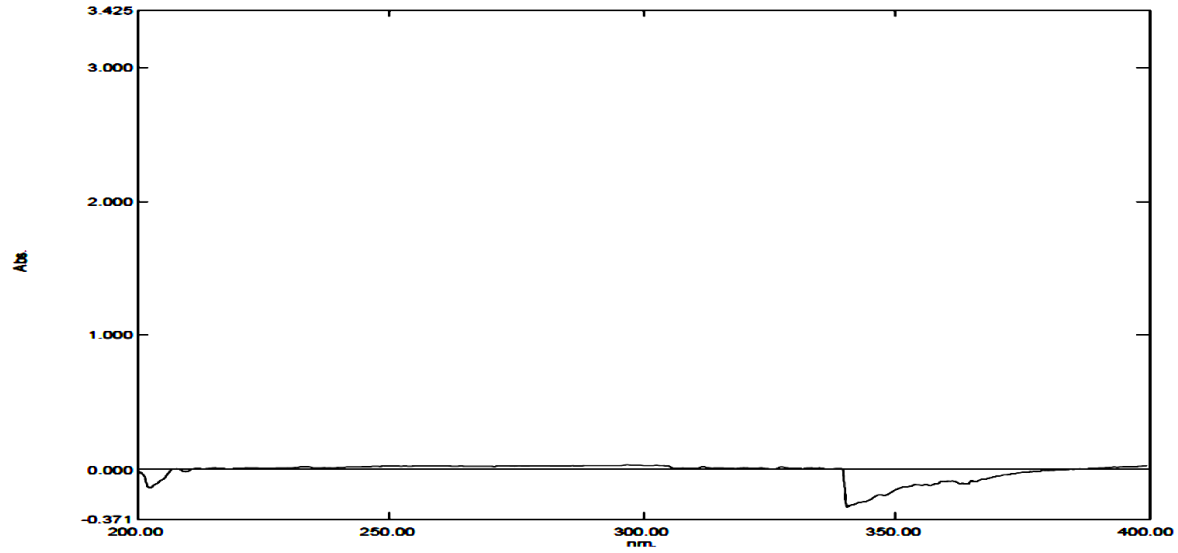

Fig. B. 5 Representative scans of placebo solution

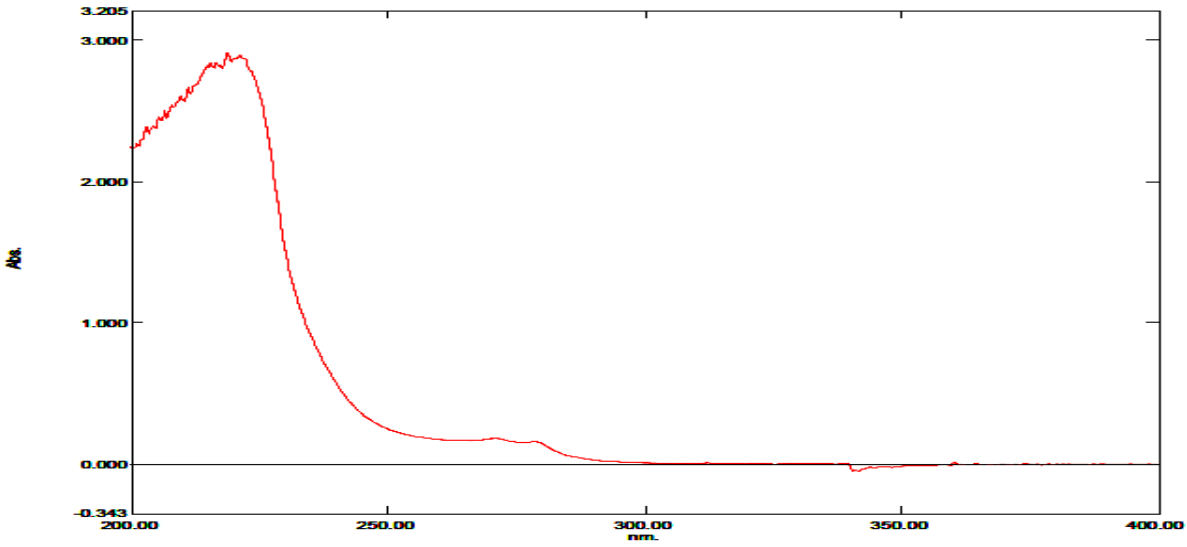

Fig. B. 6 Representative scans of reference solution

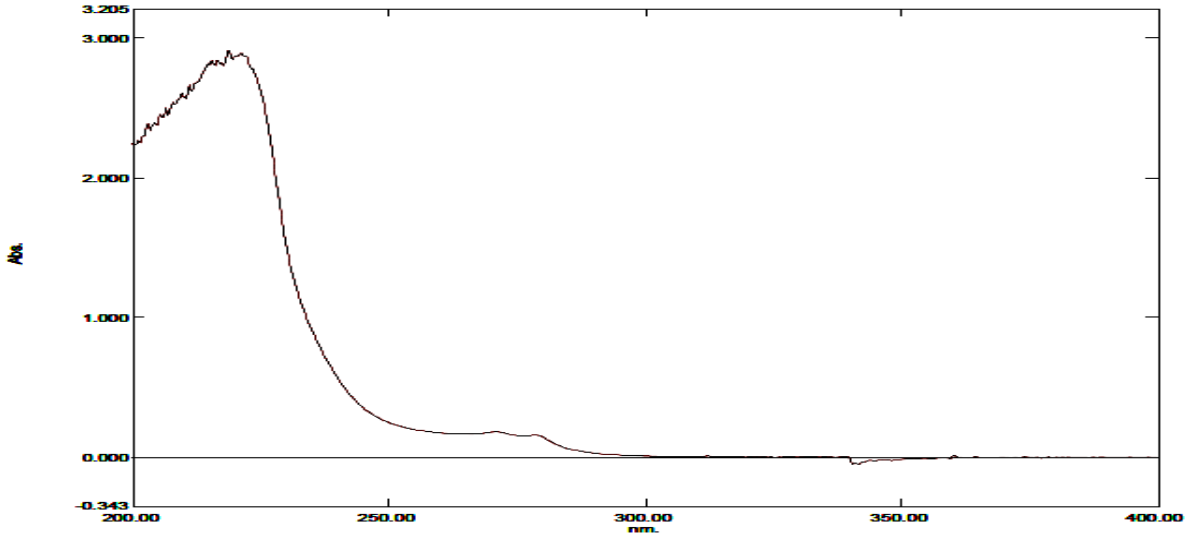

Fig. B. 7 Representative scans of test solution

2.2 Linearity & Range

Five concentrations such as 40.02, 64.03, 80.04, 96.05 & 120.06 µg/mL for Clonidine Hydrochloride were prepared and the linearity graph was plotted using absorbance verses concentration as shown in Figure B. 8. Graph of Residuals against concentration was also plotted as per shown in Figure B. 9. A linear relationship was obtained in the range of 50 to 150% (40.02 – 120.06 ppm for Clonidine Hydrochloride as Correlation coefficient R and %Y – axis intercept was within the acceptance criteria (refer table A. 4).

The method was considered to be linear in the range on 40.02 – 120.06 µg/mL for Clonidine Hydrochloride as Correlation coefficient & %Y-axis intercept should be within the limit.

Table A. 4 Observation table for linearity of Clonidine Hydrochloride

Parameter	Values	Acceptance Criteria
	Clonidine Hydrochloride	
Correlation coefficient R	0.9969	$\geq$ 0.99
%Y – axis intercept	-3.2046	$\leq \pm$ 5 %
Slope of regression line	0.00214	To be reported
Residual sum of squares	68.4111	To be reported

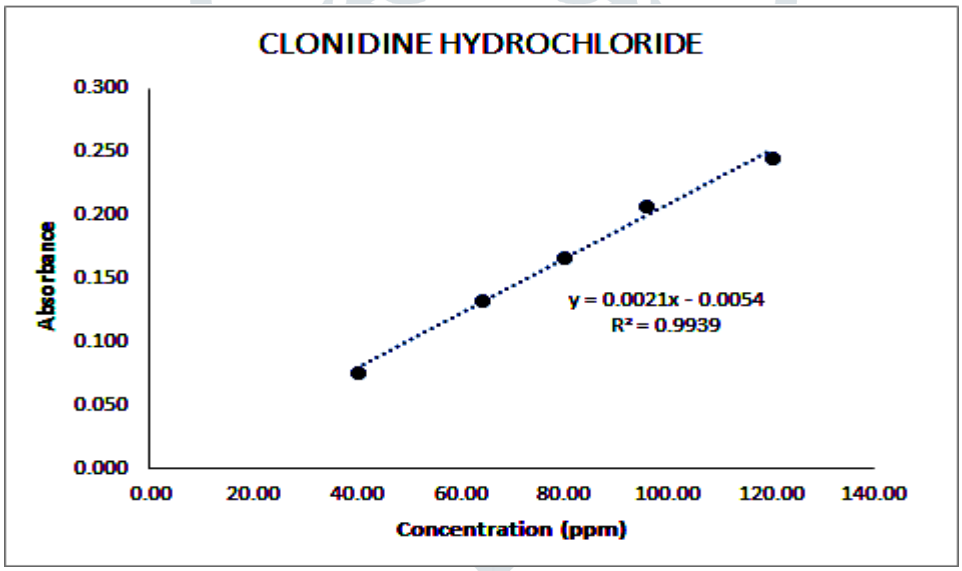

Fig. B. 8 Linearity plot of Clonidine Hydrochloride

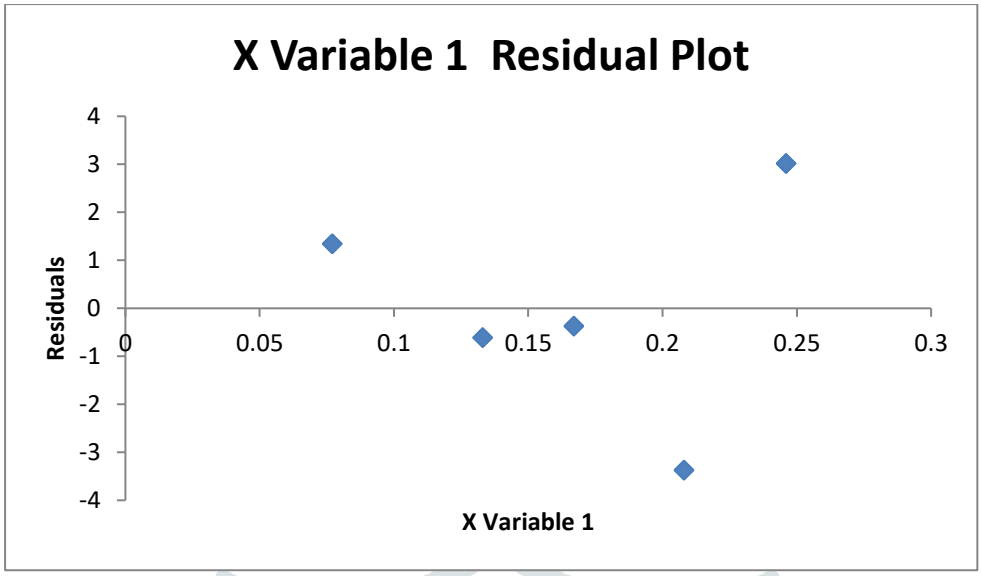

Fig. B. 9 Plot of Residuals against concentration for Clonidine Hydrochloride

2.3 Accuracy

The percentage recovery of Clonidine Hydrochloride was tabulated in tables A. 5. The method was considered to be accurate as the % individual recovery was within the acceptance criteria of 97-103 % and the % mean recovery was within the acceptance criteria of 98 – 102 %.

Table A. 5 Recoveries of Clonidine Hydrochloride at Different Concentration Levels

Level	% recovery of Clonidine Hydrochloride	Acceptance Criteria
50%	99.4	Individual Recovery = 97 % - 103 %
	99.4	
	98.3	
100%	97.8	
	97.8	
	98.3	
150%	100.6	
	100.2	
	99.8	
Mean	**99.1**	Mean Recovery 98 % – 102 %

2.4 Method Precision (Repeatability)

The exactness of the method as defined by precision and method was considered to be precised as since the relative standard deviation from 6 determinations was well within the acceptance limit of ≤ 2 %. Refer tables A. 6.

Table A. 6 Repeatability of Clonidine Hydrochloride

Sample No.	**% Assay of Clonidine Hydrochloride**
Sample 01	98.7
Sample 02	98.1
Sample 03	98.1
Sample 04	99.2
Sample 05	99.9
Sample 06	100.4
Mean	**99.1**
Standard Deviation **(STD Dev.)**	**0.95**
% RSD	**0.96**

2.5 Intermediate Precision

The intermediate precision of the assay method was established by comparison of two independent repeatability experiments on 2 different days. Refer tables A. 7 for % Assay of Clonidine Hydrochloride and tables A. 8 for comparison of two independent repeatability.

Table A. 7 Intermediate precision of Clonidine Hydrochloride

Sample No.	**% Assay of Clonidine Hydrochloride**
Sample 01	99.6
Sample 02	98.1
Sample 03	100.3
Sample 04	99.5
Sample 05	98.1
Sample 06	101.1
Mean	**99.5**
STD Dev.	**1.19**
% RSD	**1.20**

Table A. 8 Difference between two independent repeatability experiments for Assay of Clonidine Hydrochloride

Parameter	**Clonidine Hydrochloride**	
	1st day Repeatability	**2nd day Repeatability**
Number of determinations	6	6
Mean (%) Assay	99.1	99.5
RSD (%)	0.96	1.20
Mean value difference (%) **Acceptance Criteria:** < 2.0 % absolute	**0.4**	

2.6 Robustness

Method was found to be robust as system suitability criteria was achieved for all the robustness parameters tested. Deliberate change in parameter does not have any significant effect on the method performance, which demonstrated that the developed UV method was robust. The results were shown in tables A. 9.

Table A. 9 Robustness observations

Name of Test	**Method precision**	**Change in sonication time**		**Change in wavelength**	
		10 minutes	**20 minutes**	**269 nm**	**273 nm**
% Assay					
Clonidine Hydrochloride	99.1	99.4	100.4	99.3	100.1

2.7 Solution stability

Solution was preserved for 24 hours. After 24 hours absorbance was noted and it shows no critical change in % Assay. The results were shown in tables A. 10.

Table A. 10 Solution Stability of Clonidine Hydrochloride

Name of test	**Time**	**Absorbance**	**% Recovery**
% Assay			
Clonidine Hydrochloride	24 hours	0.172	98.5

3. Conclusions

In this present work a new simple, selective, linear, precise, accurate and robust UV method was developed and validated for the estimation of Clonidine Hydrochloride in pharmaceutical tablet dosage form in accordance with the ICH guidelines. The current work is worthwhile as developed UV spectroscopic method is selective, simple and rapid which can be very beneficial for the routine analysis of Clonidine Hydrochloride in pharmaceutical tablet dosage form.

4. Author Contributions

All authors contributed equally for preceding this research. The contribution of each author is mentioned below. DR. Sushama Raju Ambadekar is Research Guide and under her noble guidance the UV Spectroscopic method has been developed and validated as per ICH guidelines. She was involved in interpretation of data. Vijay Arjun Bagul analysed these data and necessary input were given by Vijay Arjun Bagul and Anand Radheshyam Tiwari towards the designing of the manuscript. The final manuscript was prepared and checked by Vijay Arjun Bagul and Jayesh Pandharinath Tamhanekar. All authors discussed the methodology and results and contributed to the final manuscript.

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