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Method Development and Validation of UV-Spectrophotometric Estimation of Hydroxychloroquine Sulphate in Bulk and Pharmaceutical Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. Author TM contributed in preparation primary content. She performed extensive literature survey and compile the content. Author TM contributed in preparation of figures and table. Author SR contributed in checking of manuscript and correction of grammatical mistake, preparation of figure and finalization of manuscript and in its correction. Author KG contributed in finalization of content, preparation of concrete manuscript and in schematic presentation of content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The devised method for estimating hydroxychloroquine sulphate in bulk and pharmaceutical dose forms (tablets) is the subject of this work. The method can therefore be applied to regular quantitative analysis and stability. The following describes the planned work's goal and scope:
• To create an appropriate spectrophotometric method for tablet-based hydroxychloroquine sulphate estimation.
• Carry out the method's validation.

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Methodology: These techniques include the area under curve (AUC) method-I and the A1% solution method based on absorbance measurement, or method-II, both of which are chosen at wavelengths of 329.4 nm using a UV-visible spectrophotometer with a 1 cm matched quartz cell and 0.01N acetic acid with water as a solvent.
Results: Plots of the hydroxychloroquine sulphate absorption spectra in 0.01N acetic acid were made. 329.4 nm was found to be the average maximum. With correlation coefficients of 0.9992 and 0.999 (r2>0.999) for AUC and 1%, respectively, and a linear connection between the absorbance and drug concentration in concentration ranges of 5-35 g/mL, it is clear that the method is linear. The percentage of medication calculated by various methods was close to 100.12, and 99.41% of the results were consistent with the label claim of the commercially available tablet formulation. As per ICH requirements, the validation parameter research was completed.
Conclusion: The described UV methods offer good accuracy and precision with reduced limits of detection and quantification. They are straightforward, trustworthy, and highly selective. These disclosed methods are ideal for routine quantitative analysis in pharmaceutical dosage forms due to the decreased time required for analysis of hydroxychloroquine sulphate. The time-saving, low-cost alternative to the expensive high-performance liquid chromatographic technology is the applied spectrophotometric approaches.

Keywords: Hydroxychloroquine sulphate; UV-Visible spectroscopy; validation; calibration curve; absorbance; ICH; etc.

ABBREVIATIONS

HQC - Hydroxychloroquine sulphate
CQ - Chloroquine
HCl - Hydrochloric acid
NaOH - Sodium hydroxide
H2O2 - Hydrogen Peroxide
A1% - Absorbance value of solution
AUC - Area under curve
LOD - Limit of detection
LOQ - Limit of quantification
SD - Standard deviation
%RSD - Relative standard deviation
ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

1. INTRODUCTION

The compound hydroxychloroquine sulphate (HCQS), with the chemical formula C18H26ClN3O.H2SO4 (Fig. 1), is crystalline in nature, solid, and has the CAS number 747-36-4 [1-2]. In order to lessen the toxicity of the parent drug, chloroquine, the hydroxy group was added in 1946, leading to the creation of the first molecule of this substance. According to laboratory research on animals, chloroquine is more hazardous than hydroxychloroquine sulphate. HCQS is a member of the extensive class of 4-amino quinolones with antimalarial action. HCQS was primarily developed as an antimalarial medication, but it also possesses a number of other pharmacological properties. Due to its well-known anti-inflammatory properties, it has been successful in treating lupus erythematosus and rheumatoid arthritis [3-4]. Additionally, its efficacy in treating photoallergic reactions has been demonstrated. One of the N-ethyl substituents of CQ that has been hydroxylated is present in an analogue of the compound termed HCQ. It is preferred over CQ when high dosages of drug are required to treat malaria because HCQ has less ocular effects. For Cutaneous Lupus Erythematosus (CLE), hydroxychloroquine sulphate is typically regarded as the first-line systemic anti-malarial medication [5–6]. The analysis of drugs has undergone quality control in the current study. In this study, two different types of methods—A 1% value of absorbance (Method I) and area under curve (Method II)—were created to study the medication hydroxychloroquine sulphate. Once a method has been developed, method validation must be carried out. In order to make sure that the precise requirements for the intended application are met, method validation entails conducting extensive analyses and offering objective proof. The method's suitability for routine application is primarily determined by determining whether it consistently produces trustworthy findings. Evaluation of the analytical process's capacity to produce reliable, consistent results is a key component of validation [7-19].

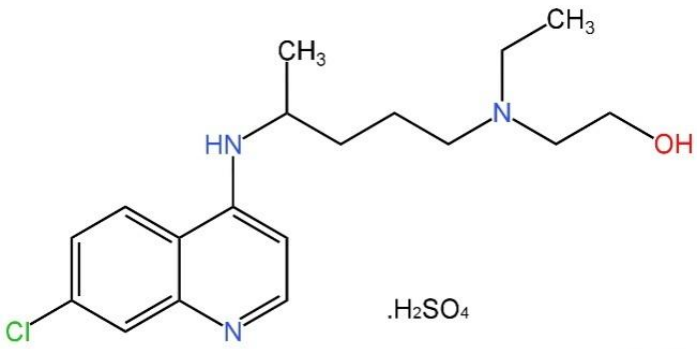

Fig. 1. Chemical structure of Hydroxychloroquine Sulphate ($C_{18}H_{26}ClN_3O.H_2SO_4$)

Regulatory agencies receive unbiased data from the validation process that attests to the effectiveness of procedures and systems. Various factors, such as the analytical curve, linearity, limit of detection, limit of quantification, accuracy, precision, and robustness, are often included in the validation of analytical methods [20-21]. The quantification of hydroxychloroquine sulphate has been studied using a variety of analytical methods, including gas chromatography [22-23], fluorescence spectroscopy [24-25], chemiluminescence [26], plasmon resonance light scattering [27], planar chromatography [28], Raman spectroscopy [29], high-pressure liquid chromatography (HPLC) [30-35], capillary zone electrophoresis [36-37], micellar electrokinetic chromatography [38] and UV spectroscopy [39-41] In this study, a novel, alternative, and fundamental analytical method for hydroxychloroquine sulphate measurement is developed and validated. The procedure is developed in accordance with ICH (International Conference on Harmonisation) principles, guaranteeing that it complies with accepted business practises. The study demonstrates how putting this technology to regular use in the pharmaceutical business is feasible, dependable, safe, and cost-effective [42–44].

2. EXPERIMENTAL SECTION

A computer was connected to a double-beam UV-vis spectrophotometer (Jasco V-630 and Shimadzu-1700 double beam) that had a 1 nm wavelength array and a pair of quartz cells that were spaced apart by 1 cm. Every weight was measured using an electronic balance.

2.1 Materials and Methods

2.1.1 Chemicals and reagents

Pharmaceutical grade Hydroxychloroquine Sulphate standard was obtained as generous gift from Wallace Pharmaceuticals Pvt Ltd, Mumbai, Maharashtra, India.

2.1.2 Instruments

The Shimadzu -1700 double beam UV spectrophotometer system (Shimadzu, Milford, MA, USA), a silicon photodiode detector, and the Jasco V-630 UV-spectrophotometer were used to develop the analytical method. Shimadzu UV Probe (Ver. 2.21), data analysis software, was used to assess the outcomes. For all parameters, high precision analytical balances of the AUX220 model (Shimadzu, Mumbai) and calibrated glassware were utilised.

2.1.3 Solubility

Hydroxychloroquine Sulphate is readily soluble in water, only slightly soluble in methanol, and insoluble in alcohols, ethers, and chloroform. It is soluble in acids like HCL and Acetic acid.

2.1.4 Preparation of standard solutions

A stock solution containing 10 mg of HCQS was precisely weighed, diluted in 5 mL of 0.01N acetic acid, transferred to a 100 mL volumetric flask, sonicated for 15 minutes, and then volume was brought up to the mark using 0.01N acetic acid.

2.1.5 Preparation of sample solution

By precisely measuring tablet powder equal to 10 mg of hydroxychloroquine sulphate, the stock dilution (100 g/mL) of the marketed product was made in 0.01N acetic acid. The calibration curves were created by graphing the relationship between concentration and absorbance. This stock was diluted with water (10 g/mL of test solution) from 1.0 mL to 10 mL.

2.1.6 Determination of λ max

The greatest absorbance of hydroxychloroquine sulphate is observed at 329.4 nm (Fig. 2 (a) and 2 (b)) when the working standard solution of 10 g/mL is scanned in the UV range 400-200 nm.

2.1.7 Preparation of calibration curve

To achieve a final concentration in the range of 5-35 g/mL, appropriate dilutions of the standard stock solution were produced. Each produced solution's absorbance was evaluated at various 329.4nm wavelengths. The calibration curve, which had a correlation coefficient of 0.999 between concentration and absorbance, was plotted (Fig. 2). the spectra and overlay of commercially available and API hydroxychloroquine sulphate, respectively.

2.1.8 Determination of A1% value [8-10]

There may be a drop in light intensity when a beam of light passes through a transparent cell containing a solution of an absorbing substance.

Beer's Lambert law is represented mathematically as

$$\boldsymbol{A = \varepsilon bc}$$

Where,

A=absorbance or optical density
ε=absorptivity or extinction coefficient
b=path length of radiation through sample (cm)
c=concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c. When c is in gm/100 ml, then the constant is called A (1%, 1 cm).

$$\boldsymbol{A = A\frac{1\%}{1cm}bc}$$

The preparation of a solution in a transparent solvent and the measurement of its absorbance at an appropriate wavelength can be used to quantify therapeutic substances using a spectrophotometer. The typical wavelength choice is the maximum absorption wavelength (max), where a tiny variation in wavelength scale setting has little impact on observed absorbance.

2.2 Method Validation [45-51]

The method was validated according to International Conference on Harmonization (ICH) guidelines.

2.2.1 Linearity and range

Analysing five or six separate concentration levels in the range of 5-35 g/mL was done to evaluate the linearity. Each solution's absorbance was measured at 329.4 nm against the procedure. A regression line equation and correlation coefficient for hydroxychloroquine sulphate were calculated from the calibration curve of absorbance versus concentration. The results were calculated three times, with the average value taken into account.

2.2.2 Accuracy (recovery test)

The method's accuracy was determined by how closely the measured value matched the sample's actual value. By recovering an active material of known purity, the accuracy of the approach was investigated. For the recovery, HCQS standard solutions at concentrations of 80, 100, and 120 g/mL were prepared. For each stage of recovery, three samples were made. The solutions were then examined, and using the calibration curve, percentage recoveries were determined.

2.2.3 Precision and stability

By doing one independent assay of a drug test sample containing 10 g/mL of the technique that was evaluated by the same analyst, system, and lab on various days, the precision of the method (intra-day and inter-day) was assessed. In order to evaluate the drug's stability, a stability research that kept the drug's working solution in 0.01N acetic acid for up to 48 hours at 2-8°C temperature and protection from light was conducted. The study looked for a decrease in absorbance compared to that of newly created solutions.

2.2.4 Ruggedness

The ruggedness of the suggested procedures was tested to determine how non-procedure related elements like instruments and analysts would affect the results. In this investigation, hydroxychloroquine sulphate (20 g/mL) was analysed using the suggested procedures with the help of two separate analysts and two different UV spectrophotometers (Jasco V-630 and Shimadzu-1700) under identical operational and environmental restrictions.

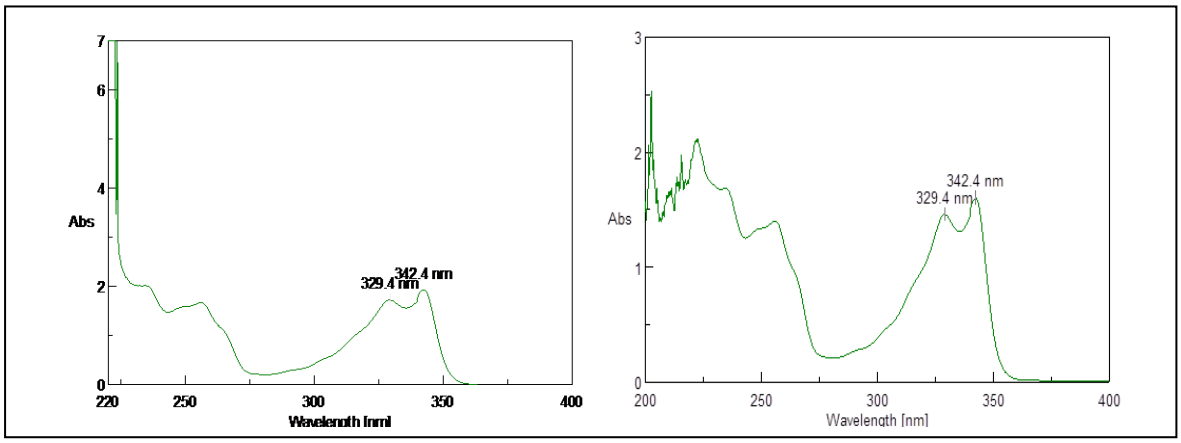

Fig. 2 (a & b). a) Spectrum of standard (API) Hydroxychloroquine Sulphate solution; b) spectrum of marketed Hydroxychloroquine sulphate solution

2.2.5 Robustness

An analytical procedure's robustness, which indicates how reliable it is for routine analysis, relates to its capacity to stay unaffected by modest, intentional modifications in technique parameters. Analysing the same samples under various technique parameter settings, such as temperature, concentration fluctuations, etc., allowed researchers to assess the robustness of the method.

2.2.6 LOD and LOQ (Limit of Detection and Limit of Quantification)

Calibration curve was repeated and the standard deviation (SD) of the intercept was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 X σ/S
LOQ = 10 X σ/S

Where,

σ = Standard Deviation of response
S = slope of the calibration curve of analyte

2.3 Force Degradation Study

When designing UV spectroscopic methods, force degradation or stress testing is carried out to show specificity, particularly when little information about probable degradation pathways and degradation products is known. These studies also reveal details on the possible storage-related degradation pathways and end products. Exposure to heat, humidity, photo-stress (UV and VIS), oxidative conditions, and aqueous conditions over a wide pH range allows for a thorough evaluation of the force degradation mechanism9. According to ICH recommendations, investigations on forced degradation were conducted in order to create potential relevant degradants and test their spectral behaviour. Intentional degradation was attempted to stress conditions of acid hydrolysis (0.1 N HCl), neutral (with water), base hydrolysis (using 0.1 N NaOH), oxidative degradation (using 3% H_2O_2) and thermal degradation (photosensitive degradation).

2.3.1 Acid degradation

A study on acid breakdown was carried out by adding 2 ml of stock solution to a 10 ml volumetric flask. Two cc of 0.1 N HCl solutions were added, thoroughly mixed, and maintained at RT for 6 hours. The volume was then adjusted with diluent to achieve hydroxychloroquine sulphate concentration of 20 g/ml.

2.3.2 Neutral degradation

The volumetric flask was filled with 10 ml of water and 2 ml of the stock solution to conduct a neutral decomposition investigation.

2.3.3 Base degradation

Transferring 2 ml of the stock solution into a 10 ml volumetric flask allowed for the basic decomposition research to be completed. Two ml of the 0.1 N NaOH solution were added, thoroughly mixed, and left for 6 hours. To get 20 g/ml of hydroxychloroquine sulphate, the volume was then adjusted with diluent.

2.3.4 Oxidative degradation

Transferring 1 ml of the stock solution into a 10 ml volumetric flask was done to conduct the

oxidative breakdown research. Three hours were given for the two ml of 3% H2O2 solutions to mix well. Following that, diluent was used to modify the volume to achieve hydroxychloroquine sulphate concentration of 20 g/ml.

2.3.5 Photosensitive degradation

A study on photosensitive deterioration was conducted using powder that had been weighed, combined with water, and then exposed to UV light or fluorescent light at 40 to 50 degrees Celsius for six hours. Records of the dilution spectra were made.

3. RESULTS AND DISCUSSION

It was discovered that hydroxychloroquine sulphate was highly soluble in 0.01N acetic acid and stable in acetic acid-water. Working standard solutions of the necessary concentration were made using these solvents throughout the experiment. In the concentration range of 5-35 g/mL, all proposed approaches adhered to Beer's-Lambert's law with correlation coefficient values under 1. An estimation of hydroxychloroquine sulphate tablets was carried out at working concentration to evaluate the applicability of the described methodologies to the pharmaceutical formulation. Plots were made of the HCQS absorption spectra in 0.01N acetic acid. The three levels used for the recovery study were 80, 100, and 120%. According to ICH criteria, all developed procedures underwent validation.

3.1 Calibration of HCQ

A 329.4nm average maximum was discovered. With the regression equation y = 0.049x + 0.0014 and the correlation coefficient of 0.999 (r2>0.999), which reveals good agreement with the method's linearity, a linear relationship between the absorbance and the drug concentration was discovered at the concentration range of 5-35 g/mL (Fig. 2 and Table 1).

Table 1. Calibration of HCQ

Sr. No.	Conc.(µg/ml)	Absorbance (329.4nm)	Method I	Method II
1	5	0.2457	491.4	0.2993
2	10	0.4898	489.8	0.4113
3	15	0.7354	490.3	0.5006
4	20	0.9812	490.6	0.6129
5	25	1.2289	491.6	0.7056
6	30	1.4767	492.2	0.7923
7	35	1.7087	488.2	0.8994
		Mean	490.58	0.6030
		±SD	1.3378	0.1977
		%RSD	0.2727	0.2375

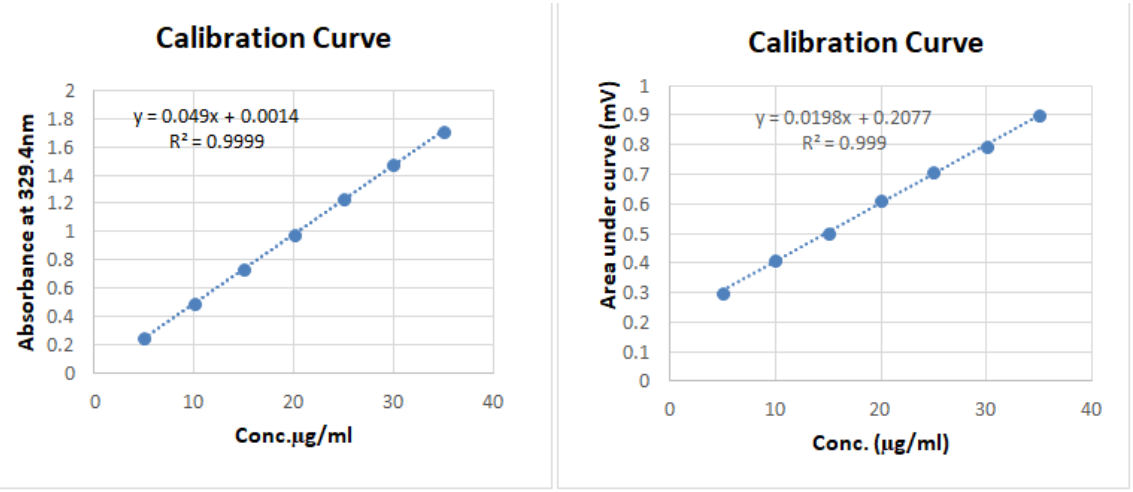

Fig. 3 (a & b). (a) Calibration curves for Method I & (b) Calibration curve for Method II

3.2 Analysis of Marketed Formulation

This procedure resulted in a 100.12% estimate of the amount of hydroxychloroquine sulphate present in the tablet formulation. The percentage amount calculated from the tablet formulation shows that there was no excipient influence (Table 2).

3.3 Method Validation

Developed methods were validated for linearity, accuracy, precision, ruggedness, and sensitivity as per the ICH guidelines.

3.3.1 Linearity and range

According to the findings from the linear regression, the calibration curves clearly displayed a solid linear relationship over the concentration range of 98–101% of the label claim. Figs. 3(a) and 3(b) illustrate the calibration curves, and Figs. 4 and 5 show the overlay of these concentrations.

3.3.2 Accuracy (Recovery Study)

Three distinct flasks labelled A, B, and C were used to administer the 20 g/ml drug solution. It was spiked with 80%, 100%, and 120% of the reference solution and diluted to a maximum of 10ml. Each solution peak's absorbance was calculated at a wavelength of 329.4 nm. The solutions are re-analyzed using the suggested techniques, and the outcomes of recovery investigations are shown in Table 2. The fact that the % RSD value is less than 2 shows that the approach is accurate (Table 3).

Table 2. Assay of marketed formulation

Sr. No.	Wt. of sample taken(mg)	Amt. of estimation in avg. wt. of table (mg)	Observed value of AUC (mV)	Amount of estimation of drug (mg)	% Assay* Method I	Method II
1	315.1	15.44	0.6114	10.03	100.53	100.05
2	311.4	15.45	0.6064	9.94	99.53	99.16
3	312.1	15.49	0.6099	10.08	100.74	99.48
4	302.4	15.45	0.6082	10.04	100.55	99.20
5	311.1	15.53	0.6080	10.00	99.67	98.92
6	310.1	15.52	0.6120	10.00	99.67	99.63
				Mean	100.12	99.41
				SD	0.707	0.3678
				%RSD	0.744	0.4053

**Each observation value is the mean of six reading*

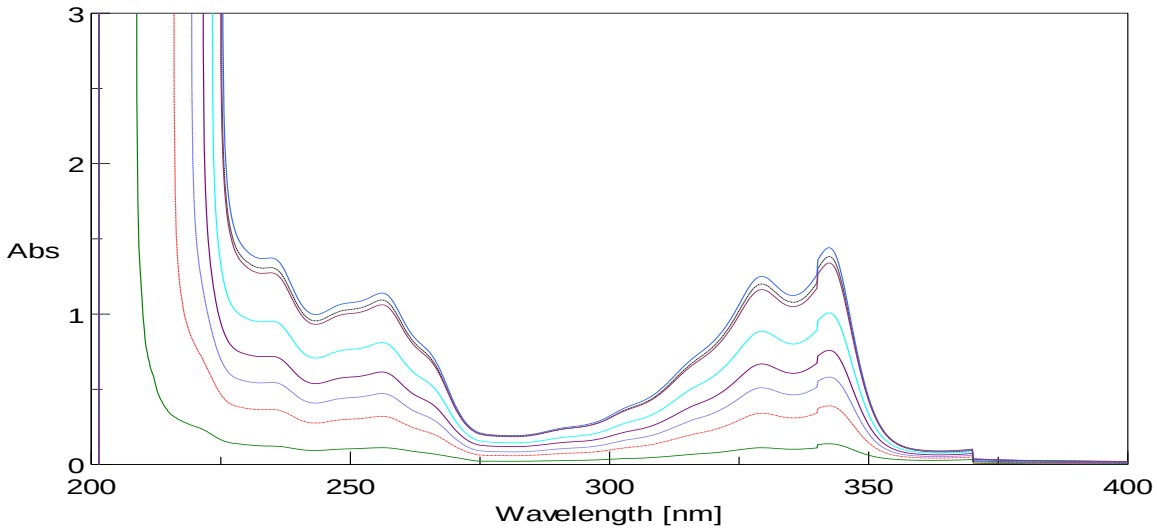

Fig. 4. Overlay of API Sample of Hydroxychloroquine Sulphate

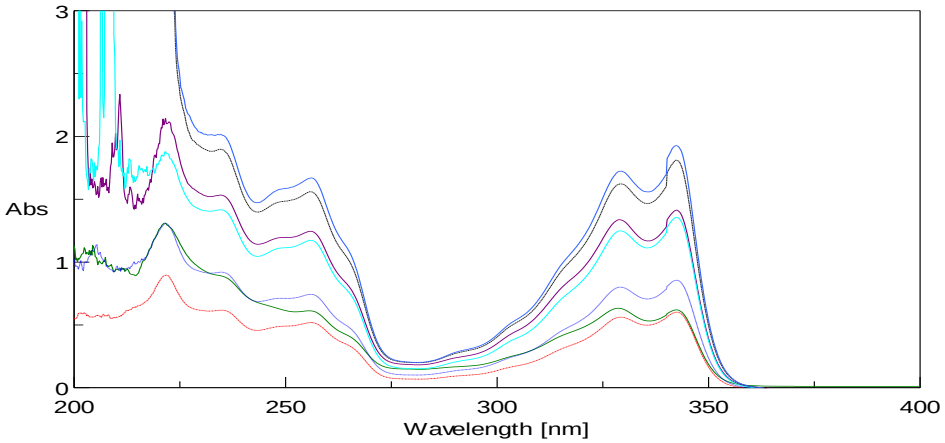

Fig. 5. Overlay of Marketed Sample of Hydroxychloroquine Sulphate

Table 3. Recovery study

Sr. No.	Level of drug taken %	Amount of estimation (mg)	Total amt. estimated (mg)	% Recovery*	
				Method I	Method II
1	80	10.16	7.85	100.62	98.03
2	100	9.83	10.01	99.24	99.89
3	120	10.07	12.15	100.57	101.19
			Mean	100.14	99.70
			±SD	0.7826	1.2984
			%RSD	0.7814	1.5950

**Each observation value is the mean of six reading*

3.3.3 Precision

The method's accuracy is described in terms of percent RSD. The data obtained demonstrated the assay's repeatability. Since the % RSD values were determined to be within the acceptable range, the approaches appear to be accurate (Table 4).

3.3.4 Robustness

The investigation is carried out by adjusting the temperature conditions and by changing the concentrations (g/ml), as shown in Table 5 (based on the robustness study data).

3.3.5 Ruggedness

As indicated in Table 5, the findings of the ruggedness investigation were within an acceptable range with % RSD values less than 2. No statistically significant discrepancies across operators or instruments were found in the results, indicating the robustness of the devised procedures (Table 6); the recorded spectra are displayed in Fig. 6(a) and 6(b).

Table 4. Precision study

Sr. No.	Condition	Wt. of sample (mg)	Amt. of drug estimated (mg)	% Lable Claim*	
				Method I	Method II
1	Intra-day	15.45	9.99	100.05	100.38
2	Inter-day	15.45	9.87	98.87	99.49
3	Analyst 1	15.32	9.95	100.50	101.02
4	Analyst 2	15.48	9.98	99.75	99.29
5	Instrument 1	15.42	9.96	100.00	100.53
6	Instrument 2	15.32	9.95	100.51	100.89
			Mean	99.95	100.26
			±SD	0.6031	0.6569
			%RSD	0.6034	0.7177

**Each observation value is the mean of six reading*

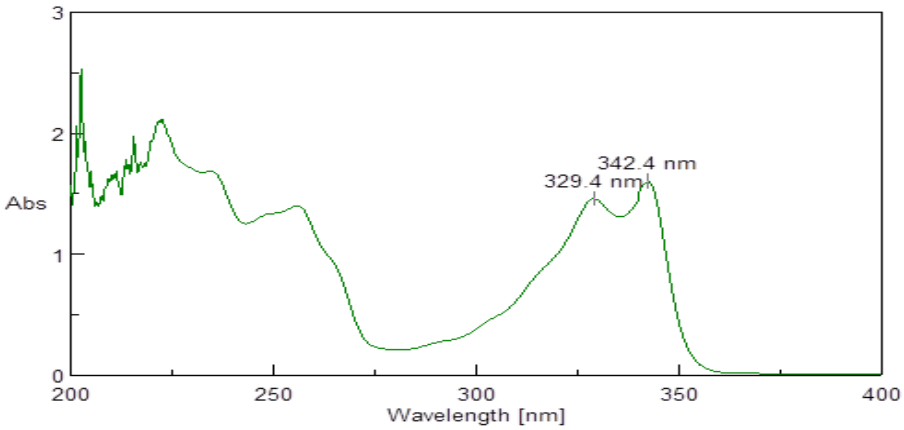

Fig. 6a. Spectrum of marketed HCQ on Jasco V-630 spectrophotometer

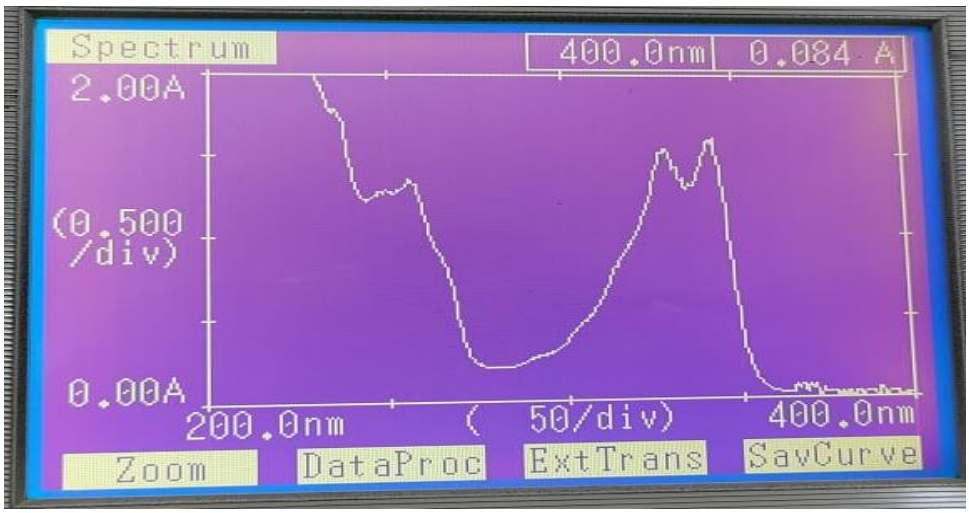

Fig. 6b. Spectrum of marketed HCQ on Shimadzu 1700 spectrophotometer

Table 5. Robustness study

Sr.No.	Conditions		Amt. est. (mg)	% Estimation	
				Method I	Method II
1	Changes in conc. µg/ml	18	10.08	100.52	97.84
		20	10.01	99.85	99.64
		22	10.01	99.80	101.39
2	Temperature condition (◦C)	50	9.95	99.97	99.74
		37	10.01	100.50	100.33
		25	9.72	97.64	100.05
			Mean	99.71	99.84
			±SD	0.9713	1.0595
			%RSD	1.0670	1.1627

**Each observation value is the mean of six reading*

3.3.6 LOD and LOQ

Lower limit of quantification (LOQ) is determined to be 0.84 g/ml whereas LOD is found to be 0.24 g/ml. Low LOD and LOQ values with high precision demonstrate good sensitivity. With respect to the detection and quantification of the drug without significant equipment interference, these data suggest that the approach is very sensitive (Table 7).

3.4 Forced Degradation Studies

According to ICH requirements, forced degradation tests were conducted to create a potential relevant degradant and examine its spectral behaviour in order to evaluate the stability suggesting preparation of the suggested UV technique. With the help of 0.1 N HCl, water, 0.1 N NaOH, 3% H2O2, oxidative degradation, and thermal degradation (photosensitive degradation), intentional degradation was performed to stress the conditions. [Fig. 7 (a-e)] (Table 8).

3.4.1 Acid degradation

The % acid degradation of sample after 3-4 hrs is found at approximately 39.45% and the recorded spectra is shown in Fig. 7b and Table 8.

Table 6. Ruggedness study

Sr. No.	Condition		Amount Estimated (mg)	% Estimation*	
				Method I	Method II
1	Different Analyst	Analyst 1	9.95	100.50	101.02
		Analyst 2	9.98	99.75	99.29
2	Intermediate Precision	Intra-day	9.99	100.05	100.38
		Inter-day	9.87	98.87	99.49
			Mean	99.79	100.05
			±SD	0.5958	0.6965
			%RSD	0.6893	0.8039

**Each observation value is the mean of three reading*

Table 7. LOD and LOQ study

LOD and LOQ	Method I	Method II
Limit of Detection (LOD) (µg mL−1)	0.2944	1.2546
Limit of Quantification (LOQ) (µg mL−1)	0.8922	3.8018

Table 8. Force degradation study

Forced Degradation parameter	% Recovery	% Degradation
Acid degradation	60.55	39.45
Base degradation	3.25	96.75
Neutral degradation	81.22	18.78
Oxidative degradation	24.36	75.64
Photosensitive degradation	2.08	97.92

**Each observation value is the mean of three reading*

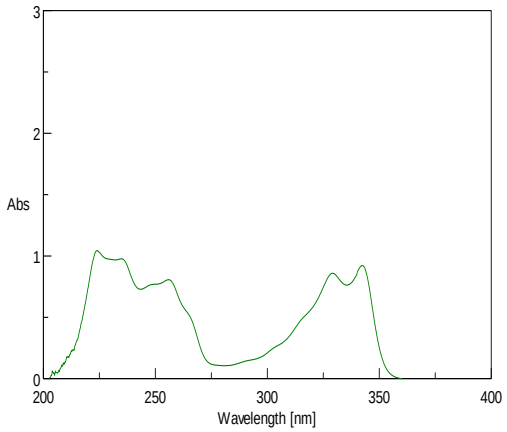

Fig. 7a. Degradation at Neutral Condition

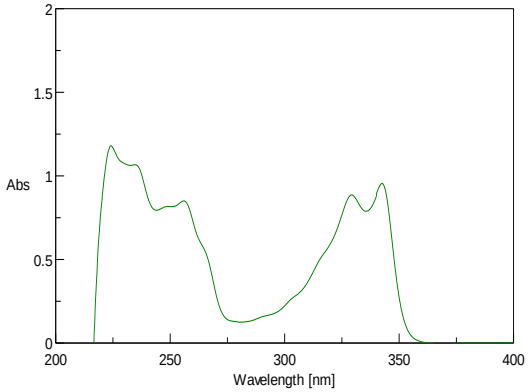

Fig. 7b. Degradation at Acidic Condition

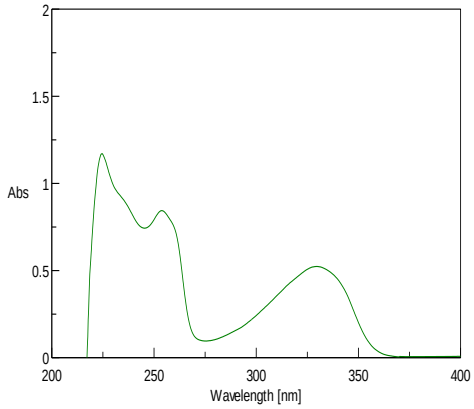

Fig. 7c. Degradation at Basic Condition

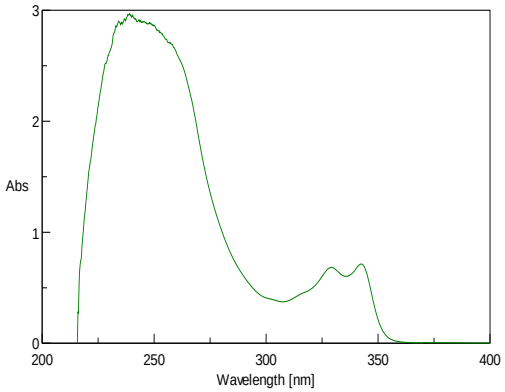

Fig. 7d. Degradation at Oxidative Condition

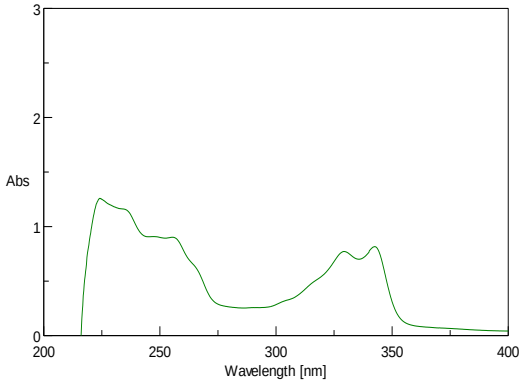

Fig. 7e. Degradation at Photosensitive Condition

Figure 7 (a-e) : Spectra of Degradation Studies at Specific Conditions showing Degradation in Drug Sample; a) Degradation at Neutral Condition; b) Degradation at Acidic Condition; c) Degradation at Basic Condition;d) Degradation at Oxidative Condition; e) Degradation at Photosensitive study.

3.4.2 Neutral degradation

The neutral degradation after 3-4 hrs is approximately up to 18.78% and the recorded spectra is shown in Fig. 7a and Table 8.

3.4.3 Base degradation

The degradation of sample after 3-4 hrs is recorded at approximately 96.75%. The recorded spectra is shown in Fig. 7c and Table 8.

3.4.4 Oxidative degradation

The result of degradation after 3-4 hrs is found at approximately 75.64%. The oxidative degradation spectra is shown in Fig. 7d and Table 8.

3.4.5 Photosensitive degradation

The results of degradation of sample after 5-6hrs of exposure is found approximately at 97.92%. The recorded spectra is shown in Fig. 7e and Table 8.

3.4.6 Application to marketed tablet study

The current analytical technique was used to evaluate hydroxychloroquine in pharmaceutical tablet form (HCQS-200, 200 mg; Plaquenil, 200 mg). The label claim% (recovery SD) for the suggested UV-spectroscopic methods for HCQ were 100 1.2 (method-I) & 99.70 1.5 (method-II), respectively. The results of the suggested and available spectroscopic methods have been assessed using the student's t-test and the variance ratio F-test. According to computed results, the assay for methods I and II was determined to be 100.12% and 99.41%, respectively. This demonstrates that method I is more effective (99.71%) than a way that has been previously reported, however method II is less significant than a standard method. There has been not a significant variation between the results obtained by calculation of the suggested UV-spectroscopic system and results obtained by spectroscopic methods, demonstrating the same precision and accuracy within the

evaluation of HCQ drug in its tablet dosage through the proposed technique.

4. CONCLUSION

The suggested method has various advantages. In the beginning, it uses water as the only diluent solution, reducing the need for additional chemicals. Second, direct dilution is used during the sample preparation phase, making it a quicker and more effective procedure. This strategy increases economic viability and saves time. Additionally, this approach encourages sustainability by minimising environmental effect by following the principles of green chemistry. Overall, these benefits make the suggested method an appealing and environmentally responsible choice. The approach is characterised by the following qualities, according to the validation process: linearity, efficacy, selectivity, precision, accuracy, and robustness across all examined parameters. Furthermore, because there were no appreciable discrepancies in the data produced from the two procedures, the methodology was deemed to be suitable for substituting the official method advised by the USP. In conclusion, it was determined that the employment of spectrophotometric methods utilising UV absorption was crucial for the testing and analysis of the medicine and dosage form containing hydroxychloroquine sulphate. It proved to be dependable and safe, establishing itself as a reliable method for this particular use.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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