Original Research Article

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

ABSTRACT

Aims: This work deals with the developed method for the estimation of the hydroxychloroquine sulphate in bulk and pharmaceutical dosages form (tablets). Hence, the method can be used for routine quantitative analysis and also stability. The aim and scope of the proposed work are as under:

• To develop suitable spectrophotometric method for estimation of hydroxychloroquine sulphate from tablet.

• Perform the validation for the method.

Methodology: These methods include area under curve (AUC), A1% solution method based on measurement of absorbance at selected wavelengths 329.4nm using UV-visible spectrophotometer with 1 cm matched quartz cell and 0.01N acetic acid with water as a solvent. Developed method obeyed Beer's-lambert's law in the concentration range of 5-35µg/mL, with correlation coefficient value, r2>0.999.

Results: The absorption spectra of Hydroxychloroquine sulphate in 0.01N acetic acid was plotted. The average λ max was found to be 329.4nm. A linear relationship was found between the absorbance and the concentration of the drug at the concentration range of 5-35 µg/mL with the regression equation y = 0.049x + 0.0014 with the correlation coefficient of 0.9992 and 0.999 (r2>0.999) for A 1% and AUC respectively, which reveals good agreement with the linearity of the method. The percent amount of drug estimated by these methods was nearly 100.12 and 99.41% found to be in good agreement with label claim of marketed tablet formulation. The recovery study was carried out at three different levels 80, 100 and 120 %.

Conclusion: The proposed UV methods are simple, reliable and highly-selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Due to the shorter duration of analysis for hydroxychloroquine sulphate make these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms. The applied spectrophotometric methods are time-saving, and also considered a cheap substitute for the overpriced high-performance liquid chromatographic technique.

Keywords: Hydroxychloroquine Sulphate, UV-Visible spectroscopy, Validation, Calibration curve, Absorbance, HQCS, ICH.

1. INTRODUCTION

Hydroxychloroquine sulphate (HCQS) having chemical formula $C_{18}H_{26}ClN_3O.H_2SO_4$ (Fig.1) is solid in nature and crystalline form and having CAS No. 747-36-4[1-2]. The first molecule of this compound was primarily synthesized in 1946 by the addition of the hydroxy group to the parent compound, chloroquine to reduce toxicity[2-4]. Animal studies on the chloroquine in laboratories, chloroquine creates more toxicity as compares to hydroxychloroquine sulphate. HCQS is classified in the large series of 4-amino quinolones, having antimalarial activity. Although HCQS was mainly created as an antimalarial drug, it also has a number of additional pharmacological qualities, because of its well-known anti-inflammatory effects, it has been effective in treating lupus erythematosus and rheumatoid arthritis[3-4]. Additionally, its viability in the management of photoallergic reactions is established. An analogue of CQ called HCQ has one of the N-ethyl substituents of CQ that has been hydroxylated. Because HCQ has less ocular toxicity than CQ, it is favoured over CQ when high dosages of

medication are needed to treat malaria. In Oral anti-malarials agents, hydroxychloroquine sulphate mostly considered as the first-line systemic treatment for Cutaneous Lupus Erythematosus (CLE). In this current study an effort has by used in quality control for the analysis of drug[5-7]. In this paper, two types of methods was developed on hydroxychloroquine sulphate drug are A 1% value of absorbance (Method I) and area under curve (Method II).

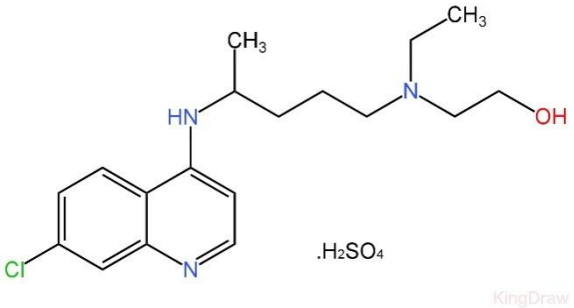

Figure 1 — Chemical structure of Hydroxychloroquine Sulphate ($C_{18}H_{26}ClN_3O.H_2SO_4$)

2. EXPERIMENTAL SECTION

A double beam UV-vis spectrophotometer (Jasco V-630 and Shimadzu-1700 double beam) connected to computer that was located with spectral band width of 1 nm wavelength array of ± 0.3 nm with a pair of 1 cm matched quartz cells. All weights were taken on electronic balance.

2.1. MATERIALS AND METHODS

2.1.1.1. Chemicals and Reagents

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

2.1.1.2. Instruments:

UV-Spectrophotometer: Jasco V-630 and Shimadzu-1700 double beam

Sonicator: PCi Mumbai, Model No.3.5L 100H

Weighing balance: Shimadzu AUX220 and Analytical Balance

2.1.1.3. Solubility

Hydroxychloroquine Sulphate soluble in acids like HCL, Acetic acid, freely soluble in water, slightly soluble in methanol and insoluble in alcohols, ethers, chloroform.

2.1.2.1. Preparation of standard solutions

Accurately ~10 mg of HCQS was weighed and dissolved in 5 mL 0.01N acetic acid then transferred to a 100 mL volumetric flask, sonicated for 15 min and volume was made up to the mark with 0.01N acetic acid to make a stock solution with the concentration of 100 µg/mL.

2.1.2.2. Preparation of sample solution

The stock dilution (100 µg/mL) of marketed product was prepared in 0.01N acetic acid by weighing accurately tablet powder equivalent to 10 mg of hydroxychloroquine sulphate. The calibration curves were prepared by plotting graph between absorbance and concentration. A 1.0 mL portion of this stock was diluted to 10 mL with water (10 µg/mL of test solution).

2.1.2.3. Preparation of Calibration Curve

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Appropriate dilutions of standard stock solution were made to get final concentration in the range of 5-35 µg/mL. Absorbance were measured of each prepared solution at selected 329.4nm wavelengths. The calibration curve was plotted between concentration vs. absorbance, having correlation coefficient 0.999(Figure 2). The spectra and overlay of API hydroxychloroquine sulphate and marketed hydroxychloroquine sulphate are shown in figure 2 (a& b) and in figure 4 and 5, respectively.

2.1.2.4. Determination of λ max

The working standard solution of 10 µg/mL was prepared and scanned in the UV range 400–200 nm; hydroxychloroquine sulphate shows a maximum absorbance at 329.4 nm (Figure 3 (a) & 9b)).

2.1.2.5. Determination of A1% value[8-10].

When beam of light is passed through a transparent cell containing a solution of anabsorbing substance, reduction of the intensity of light may occur.

Mathematically, **Beer's Lambert law** is expressed as

$$A = \varepsilon bc$$

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).

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

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λmax), where small error in setting the wavelength scale has little effect on measured absorbance.

2.2. METHOD VALIDATION[11-13]

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

2.2.1. Linearity and range

The linearity was determined by analyzing 5-6 independent levels of concentration in the range of 5-35 µg/mL. Absorbance of each solution against method was recorded at 329.4 nm. The calibration curve of absorbance versus concentration was plotted a correlation coefficient and regression line equation for hydroxychloroquine sulphate was determined. The determinations were done in triplicate and the average result was considered.

2.2.2. Accuracy (recovery test)

The accuracy of the method was the closeness of the measured value to the true value of sample. Accuracy of the method was studied by recovery experiment of known purity active substance. The recovery was performed by preparing of concentrations 80, 100 and 120 µg/mL of HCQS standard

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solution. Three samples were prepared for each recovery level. The solutions were then analyzed, and percentage recoveries were calculated from calibration curve.

2.2.3. Precision and stability

The precision of the method (intra-day and inter-day) was evaluated by carrying out one independent assay of 10 µg/mL test sample of drug, of the method that was evaluated by same analyst, system in different days in same laboratory. To assess the stability of the drug, the stability study was performed maintaining the drug working solution in the 0.01N acetic acid up to 48 h at 2-8°C temperature that was protected from light, looking for the decrease of absorbance compared with those of freshly prepared solutions.

2.2.4. Ruggedness:

Ruggedness of proposed methods was performed to examine effect of non-procedure related factors such as instruments and analysts. For this study, hydroxychloroquine sulphate (20 µg/mL) was analyzed by proposed methods using two different analyst and two different UV-spectrophotometers (Jasco V-630 and Shimadzu-1700) restraining similar operational and environmental conditions.

2.2.5. Robustness:

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for the routine analysis. The robustness was determined by analyzing the same samples under a variety of conditions of the method parameters, such as temperature condition, changes in concentrations, etc.

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:

$$LOD = 3.3 * SD/slope\ of\ calibration\ curve$$

$$LOQ = 10 * SD/slope\ of\ calibration\ curve$$

Where,

SD = Standard deviation of intercepts

2.2.7. Force Degradation Study

Force degradation or stress testing is undertaken to demonstrate specificity when developing UV spectroscopic methods, particularly when little information is available about potential degradation pathway and degradation product. These studies also provide the information about the degradation pathway and degradation product that could be form during the storage. The force degradation mechanism can be assessed in a systematic way by exposure to stress conditions of heat, humidity, photo-stress (UV and VIS), oxidative conditions and aqueous conditions across a broad pH range[9]. The forced degradation studies were carried out in accordance to the ICH guidelines, to produce the possible relevant degradant and test its 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) [Figure 7 (a-e)].

2.2.7.1 Acid degradation

Acid decomposition study was performed by Transferring 2 ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and kept for 6 hrs

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at RT. Then the volume was adjusted with diluent to get 20 µg/ml for hydroxychloroquine sulphate. (figure 7b).

2.2.7.2 Neutral degradation

Neutral decomposition study was performed by transferring 2ml of stock solution in to 10 ml of water in to the volumetric flask (figure 7a).

2.2.7.3 Base degradation

Basic decomposition study was performed by Transferring 2 ml of stock solution in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and kept for 6 hrs. Then the volume was adjusted with diluent to get 20µg/ml for hydroxychloroquine sulphate. (figure 7c).

2.2.7.4 Oxidative degradation

Oxidative decomposition study was performed by Transferring 1ml of stock solution in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and kept for 3 hrs. Then the volume was adjusted with diluent to get 20µg/ml for hydroxychloroquine sulphate (figure 7d).

2.2.7.5 Photosensitive degradation

Photosensitive degradation study was performed by weigh powderand mixed with water and than exposed to sun light in presence of short/ long wavelength of UV-radiation or fluorescent light at 40-50°C for 6 hrs. the dilution spectra were recorded (figure 7e).

3. RESULTS AND DISCUSSION

Hydroxychloroquine sulphate was found to be highly soluble in 0.01N acetic acid and stable in acetic acid-water, using these solvents working standard solutions were prepared of desired concentration throughout experimentation. All developed methods obeyed Beer's-lambert's law in the concentration range of 5-35 µg/mL with correlation coefficient value less than 1. In order to test the appropriateness of the developed methods to the pharmaceutical formulation, an estimation of hydroxychloroquine sulphate tablets was performed at working concentration. The absorption spectra of HCQS in 0.01N acetic acid was plotted. The average λ max was found to be 329.4nm. A linear relationship was found between the absorbance and the concentration of the drug at the concentration range of 5-35 µg/mL with the regression equation y = 0.049x + 0.0014 with the correlation coefficient of 0.999 (r2>0.999) which reveals good agreement with the linearity of the method (figure 2 and table 1). The recovery study was carried out at three different levels 80, 100 and 120 %. All developed methods were validated as per ICH guidelines.

3.2. Analysis of marketed Tablets

The percentage amounts of hydroxychloroquine sulphate estimated from tablet formulation using this method was found to be 100.12%. The % amount estimated from tablet formulation indicates that there was no interference from excipients present in it (Table 2).

3.2. METHOD VALIDATION

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

3.2.1. Linearity and range

From the linear regression data, it is clear that the calibration curves showed good linear relationship over the concentration range of 98-101% of label claim. The calibration curves is shown in Figure 3 (a) & (b) and overlay of these concentrations are shown in figure 4 & 5.

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3.2.2. Accuracy (Recovery Study)

The 20 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The absorbance of each solution peak was measured at 329.4 nm. The solutions are re-analyzed by proposed methods; results of recovery studies are reported in Table 2. The % RSD value is found to be less than 2 indicate that the methods is accurate (Table 3).

3.2.3. Precision

The precision of the method is expressed in terms of % RSD. The obtained results shown reproducibility of the assay. The % RSD values is found within limit, so this indicates that the methods is precise (Table 4).

3.2.4. Robustness

From the robustness study data, the results were represented in the table 4, the study is performed by changing the temperature conditions and by changing the concentrations(µg/ml) (Table 5).

3.2.5. Ruggedness

The results of ruggedness study is found in the acceptable range with % RSD values less than 2 by all the methods as shown in Table 5. The results showed no statistical differences between different operators and instruments suggesting that the developed methods is rugged in Table 6 and recorded spectra is shown in figure 6(a-b).

3.2.6. LOD and LOQ

The LOD is found 0.24 µg/ml where lower limit of quantification (LOQ) is found 0.84 µg/ml. The lower values of LOD and LOQ with high precision prove good sensitivity. These values indicate that the method is very sensitive with respect to the detection and quantification of the drug without major interference from the equipment (Table 7).

3.2.7. Forced Degradation Studies

To evaluate the stability indicating preparation of the developed UV method, forced degradation studies were carried out in accordance to the ICH guidelines, to produce the possible relevant degradant and test its 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) [Figure 7 (a-e)] (Table 8).

3.2.7.1 Acid degradation

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

3.2.7.2 Neutral degradation

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

3.2.7.3 Base degradation

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

3.2.7.4 Oxidative degradation

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

3.2.7.5 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 figure 7e and table 8.

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CONCLUSION

The proposed UV methods are simple, reliable and highly-selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Due to the shorter duration of analysis for hydroxychloroquine sulphate make these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms. The recoveries achieved are good by both the methods that were selected for quantitative analysis.

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Abbreviations

HQCS- Hydroxychloroquine sulphate

HCQ- Hydroxychloroquine

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

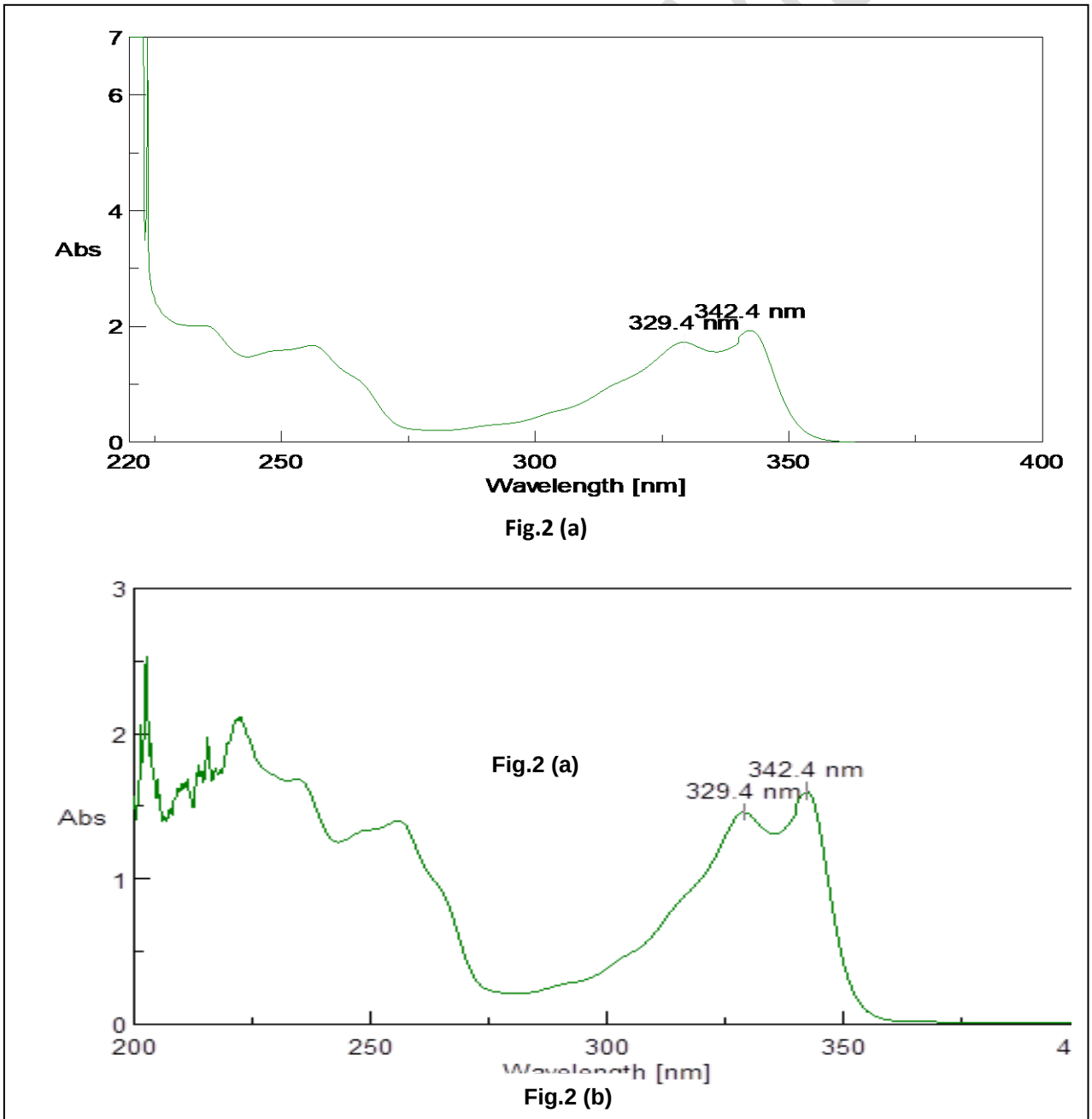

Figure 2 (a & b) – a) Spectrum of standard (API) Hydroxychloroquine Sulphate solution; b) spectrum of marketed Hydroxychloroquine sulphate solution.

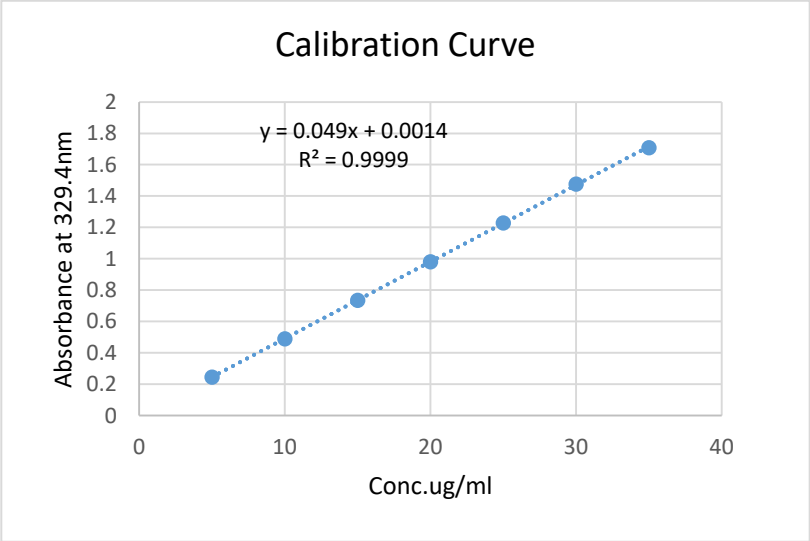

Fig. 3 (a)

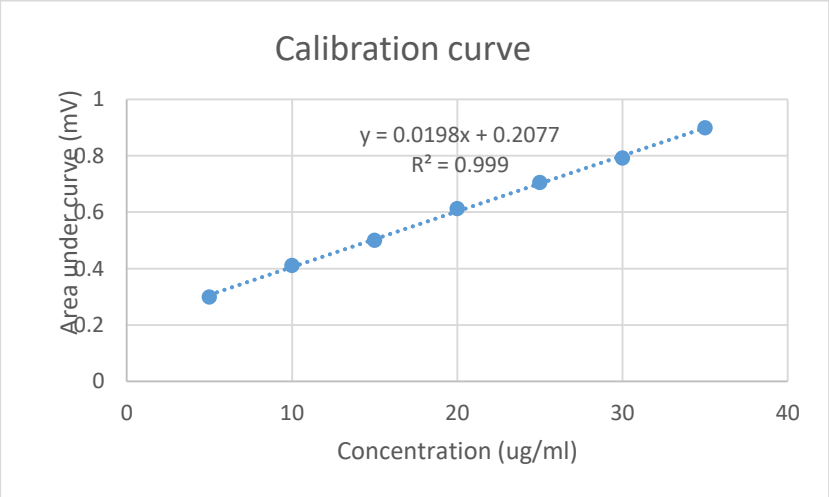

Fig. 3 (b)

Figure 3 (a & b) - (a) Calibration courve for absorbance & (b) Calibration curve for area under curve by Beer's Lamberts' plot of Hydroxychloroquine sulphate at 329.4nm.

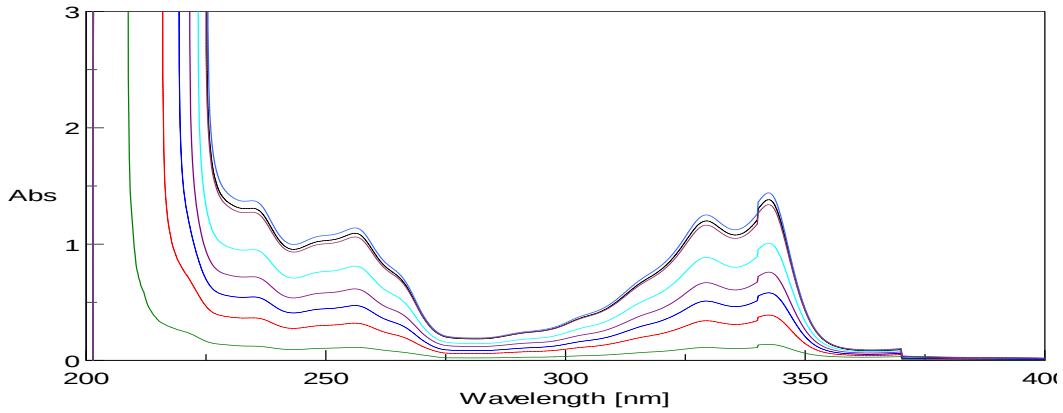

Figure 4- Overlay of API Sample of Hydroxychloroquine Sulphate.

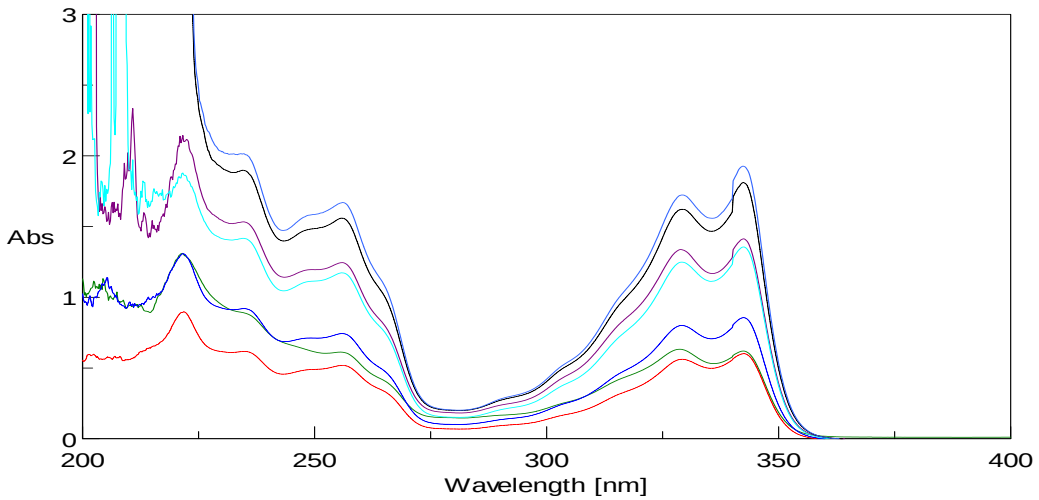

Figure 5- Overlay of Marketed Sample of Hydroxychloroquine Sulphate.

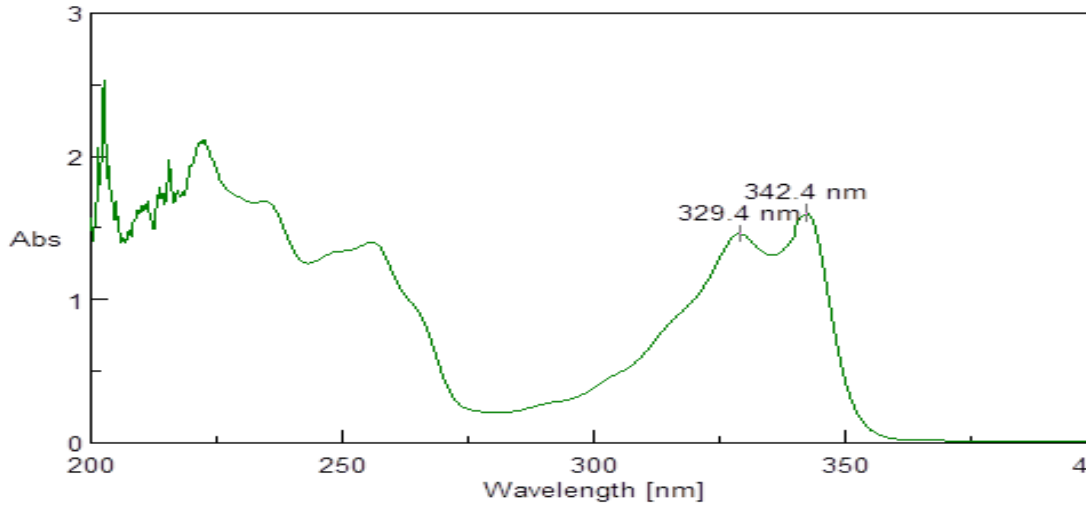

Figure 6a- By Instrument 1 spectrum of marketed hydroxychloroquine sulphate solution.

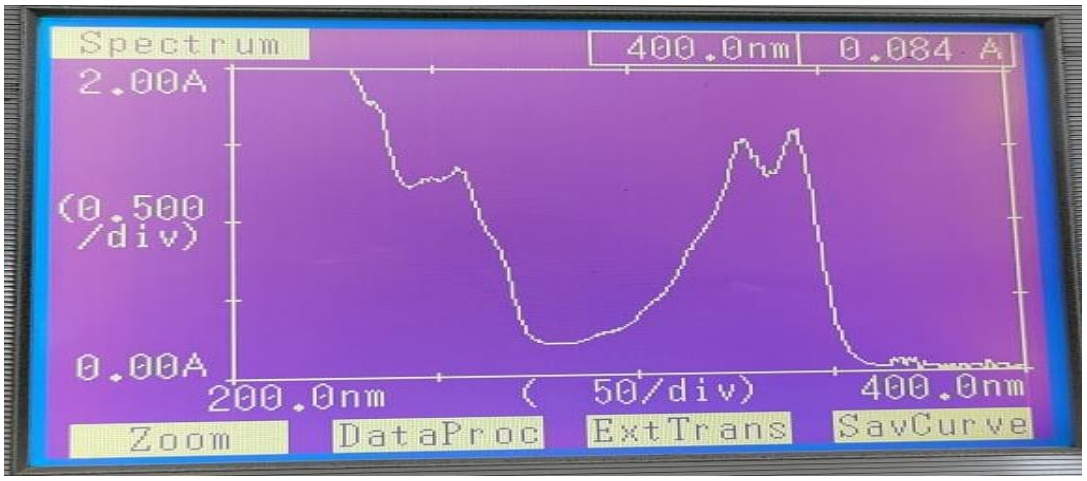

Figure 6b- By Instrument 2 spectrum of marketed hydroxychloroquine sulphate solution.

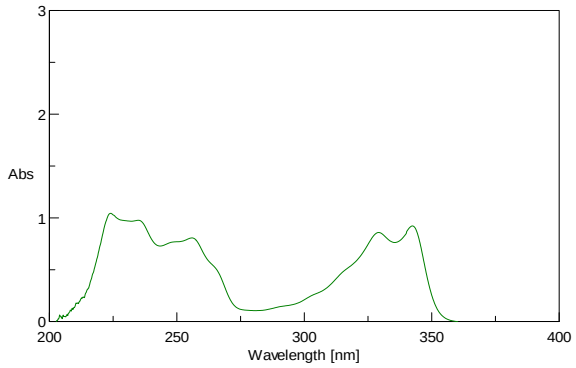

Figure 7a- Degradation at Neurtal Condition.

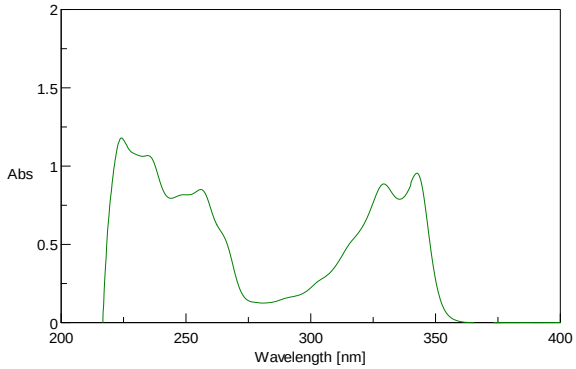

Figure 7b- Degradation at Acidic Condition.

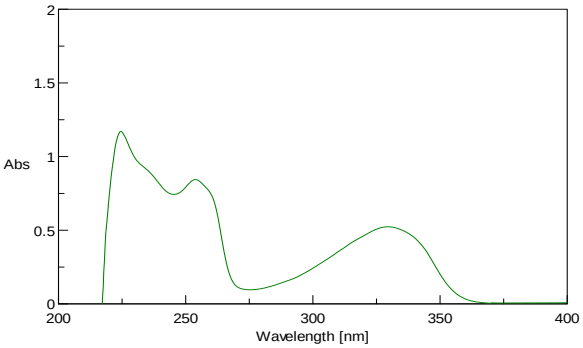

Figure 7c- Degradation at Basic Condition.

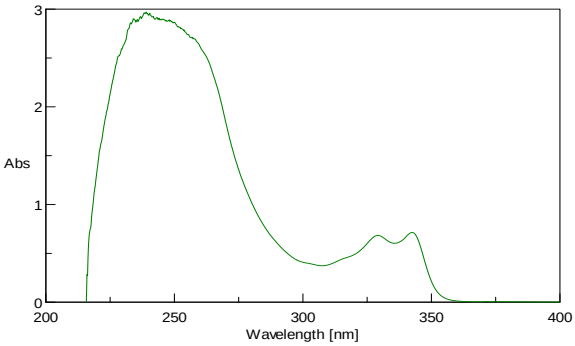

Figure 7d- Degradation at Oxidative Condition.

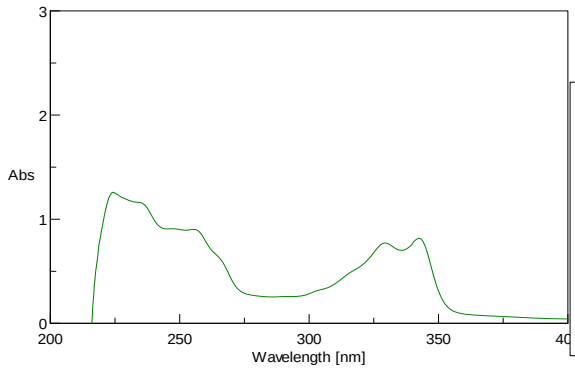

Figure 7e- Degradation at Photosensitive Condition.

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.

Table-1- A1% values at different concentrations.

Sr.No.	Conc.ug/ml	Absorbance (329.4nm)	A 1% Value	AUC(mV)
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

Table 2 -Results of % Estimation from tablets.

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 value is the mean of three reading.

Table 3- Recovery Study Results of % estimation from tablets.

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 value is the mean of three reading.

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Table 4- Results of 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 value is the mean of three reading.

Table 5- Results Obtained for the Analysis of the Robustness.

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

Table 6 - Results Obtained for the Analysis of the Ruggedness.

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 value is the mean of three reading.

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Table 7- Results of LOD and LOQ.

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

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