METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION HYDROXY CHLOROQUINE SULPHATE BY UV-SPECTROMETRY

P.Shravani *, D. Snigdha
Talla Padamavathi Pharmacy College, Urus, Warangal-506002, AP.

E.Mail:

ABSTRACT

A simple, reproducible and efficient spectroscopic method development and validation of hydroxy chloroquine sulfate (HCS) in tablet dosage form. The drug was determined by using methanol as a solvent for this study, which is determined by spectrophotometrically at 224-nm. The percentage recovery study of the drug for the proposed method range from 99-100%w/v indicating no interferences of the tablet excipients, by using methanol as a solvent. Linearity was obtained in the concentration range 10-50 μg/ml for the hydrochlorothiazide with correlation coefficient of 0.9916. The result analysis was validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.

Key Words: Hydroxy Chloroquine, UV, Anti-malarial

INTRODUCTION [1, 2, 3,]

Hydroxychloroquine sulphate is a colourless crystalline solid, soluble in water to at least 20 percent; chemically the drug is 2-[(4-[(7-Chloro-4-quinolyl) amino] pentyl] ethylamino] ethanol sulfate (1:1).

Hydroxychloroquine sulfate has the following structural formula:

![Fig.1: Hydroxychloroquine sulphate](image)

Each tablet for oral administration contains 200 mg hydroxychloroquine sulfate (equivalent to 155 mg hydroxychloroquine). Inactive ingredients: Dibasic calcium phosphate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, polyethylene glycol, povidone, sodium bicarbonate and titanium dioxide.
The drug possesses antimalarial actions and also exerts a beneficial effect in lupus erythematosus (chronic discoid or systemic) and acute or chronic rheumatoid arthritis. The precise mechanism of action is not known.

Hydroxychloroquine sulfate is indicated for the suppressive treatment and treatment of acute attacks of malaria due to Plasmodium vivax, P. malariae, P. ovale, and susceptible strains of P. falciparum. It is also indicated for the treatment of discoid and systemic lupus erythematosus, and rheumatoid arthritis. There are very few methods (Lastra, et al., 2003) (Dougles A Skoog 1996, 4-7) appearing in the literature for the method development and validation of hydroxychloroquine sulfate in tablet dosage for, since these methods were based on RP-HPLC, (P. D Sethi, 50-53) electrophoresis (Nafisur Rahman et al., 2006) only but there was no method has been developed by using methanol as a solvent in UV spectrophotometrically and also this procedure was convenient for determination of the hydroxychloroquine sulfate in tablet dosage form in 224 nm, thus an attempt was made to develop a simple, precise and economical method for development and validation of hydroxychloroquine sulfate in UV spectroscopy by tablet dosage form.

**DRUG PROFILE [4-10]**

**Mechanism of Actions:**

Antiprotozoal—Malaria: Unknown, but may be based on ability of hydroxychloroquine to bind to and alter the properties of DNA. Also has been found to be taken up into the acidic food vacuoles of the parasite in the erythrocyte. This increases the pH of the acid vesicles, interfering with vesicle functions and possibly inhibiting phospholipid metabolism. In suppressive treatment, hydroxychloroquine inhibits the erythrocytic stage of development of plasmodia. In acute attacks of malaria, it interrupts erythrocytic schizogony of the parasite. Its ability to concentrate in parasitized erythrocytes may account for their selective toxicity against the erythrocytic stages of plasmodial infection.

**Side Effects:**
Nausea, vomiting, stomach upset, cramps, and loss of appetite, diarrhea, tiredness, weakness or headache may occur the first several days as your body adjusts to the medication. If these effects continue or become bothersome, inform your doctor. Notify your doctor if you develop: vision changes (such as blurred vision, trouble seeing at night or problems focusing clearly), ringing in the ears, difficulty hearing. In the unlikely event you have an allergic reaction to this drug, seek immediate medical attention. Symptoms of an allergic reaction include: rash, itching, swelling, dizziness, breathing trouble. If you notice other effects not listed above, contact your doctor or pharmacist.

**How to use:**
Take Hydroxychloroquine Sulfate with food to prevent stomach upset. Take Hydroxychloroquine Sulfate exactly as prescribed. Do not stop taking it without consulting your doctor. It is important to continue taking this for the length of time prescribed. Stopping therapy too soon may not treat the infection and can lead to a reinfection. While taking Hydroxychloroquine Sulfate, your doctor may schedule lab tests to check your eyesight, hearing and blood.
Uses:
Hydroxychloroquine Sulfate is used to treat rheumatoid arthritis and lupus; and to treat or prevent malaria.

Precautions:
Tell your doctor if you have: liver disease, blood disorders, psoriasis, allergies (especially drug allergies). Hydroxychloroquine Sulfate should be used only if clearly needed during pregnancy. Since small amounts of Hydroxychloroquine Sulfate are found in breast milk; consult your doctor before breast-feeding.

Interactions:
Tell your doctor of any over-the-counter or prescription medication you may take. Do not start or stop any medicine without doctor or pharmacist approval.

Overdose
If overdose is suspected, contact your local poison control center or emergency room immediately. Symptoms of overdose may include blurred vision; severe drowsiness or dizziness; fainting; irregular heartbeat; headache; excessive excitability; slow, shallow breathing; seizures; and loss of consciousness.

Notes:
Children are very sensitive to the effects of this medication. It is important to keep this and all medications out of the reach of children.

Missed dose:
If you miss a dose, use it as soon as you remember. If it is near the time of the nextdose, skip the missed dose and resume your usual dosing schedule. Do not double the dose to catch up.

Storage:
Store at room temperature between 59 and 86 degrees F (between 15 and 30 degrees C) away from moisture and heat.

OBJECTIVE OF WORK
➢ Method development and validation of Hydroxy chloroquine sulfate in tablet dosage form using U.V spectroscopy.
➢ Analyzing the prepared hydroxy chloroquine sulfate tablet by performing reliable procedures.
➢ Performing Assay of Hydroxy chloroquine tablets using different validation techniques.

EXPERIMENTAL WORK [11-14]

Instruments
A Shimadzu UV-Visible Spectrophotometer (UV-1700) with a matched pair of 10 mm quartz cells were used for experimental purpose

Materials
Hydroxy chloroquine sulfate was procured as gift sample from Torrent Research Center, Ahmedabad. The obtained hydroxy chloroquine sulfate was recrystallized in methanol for experimental purpose. Freshly prepared 0.1N HCl, methanol and all other chemicals and reagents were of analytical
grade. The commercially available two marketed tablet brands containing Hydroxy chloroquine sulfate, 200 mg in each tablet have been used for estimation.

**Preparation of Standard Stock Solution**

The standard stock solution was prepared by dissolving Hydroxy chloroquine sulfate in 0.1N HCl to make final concentration of 100 μg/ml. Different aliquots were taken from stock solution and diluted with 0.1N HCl separately to prepare series of concentrations from 2-24 μg/ml. The $\lambda_{\text{max}}$ was found by UV spectrum of Hydroxy chloroquine sulfate in 0.1NHCl, in the range of 200-400 nm and it was found to be 233 nm. Absorbance was measured at 233 nm against 0.1 N HCl as blank. The calibration curve was prepared by plotting absorbance versus concentration of hydroxy chloroquine sulfate.

**Application of the Proposed Procedure for the Determination in Tablets**

The proposed procedure was adopted for determination of hydroxy chloroquine sulfate in tablets in following manner. The marketed tablet formulations of Hydroxy chloroquine sulfate were used for the purpose of analysis. Twenty tablets were weighed and average weight was calculated, crushed to fine powder. The powder equivalent to 25 mg of hydroxy chloroquine sulfate was transferred in 100 ml volumetric flask and dissolved in 0.1NHCl by intermittent shaking. The volume was made up to mark to get final concentration of 600 μg/ml. The solution was then filtered through Whatmann filter paper (no.41). This solution was used as stock solution.

The working solution of drug (6 μg/ml) was prepared from standard stock solution in 0.1NHCl. The absorbance of working solution was measured and amount of hydroxy chloroquine sulfate was calculated from the calibration curve. The readings were taken in triplicate and same procedure was repeated with another marketed tablet formulation.

All the marketed tablet formulations contain excipients which are added along with active pharmaceutical ingredient. These substances may cause some interference during estimation of active pharmaceutical ingredient. Recovery study was carried out on marketed tablet formulations and the results obtained showed that, there was no interference from excipients. From the results of recovery study it can be claimed that, the method can be used for estimation of hydroxy chloroquine sulfate in tablet forms. A typical UV spectrum obtained from a standard and sample is shown in figure 2 and 3.
RESULTS & DISCUSSION [15]

Fig. 2: UV curve for standard hydroxy chloroquine sulfate at 224nm

Fig. 3: UV curve for sample hydroxy chloroquine sulfate at 224nm
Table 1: Ultra violet spectroscopy determination

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount Labeled (gm)</th>
<th>Amount taken for Assay (gm)</th>
<th>Ultraviolet spectrometry determination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amount found</td>
</tr>
<tr>
<td>Hydroxy chloroquine sulfate</td>
<td>25</td>
<td>0.1510</td>
<td>24.17mg</td>
</tr>
</tbody>
</table>

METHODS OF VALIDATION [16-19]

The method was validated in accordance with ICH guidelines for linearity, accuracy, precision, specificity, ruggedness and robustness (ICH, 1996).

METHOD DEVELOPMENT BY UV-VISIBLE SPECTROMETRY

Principle of the method: Because there is no specific monograph in Brazilian official compendia, the method was based on the identification of the test drug by UV absorption which is recommended by United States Pharmacopeia (USP) 9. Thus, it was used as initial parameters the reading concentration of the sample solution and the diluent solution specified: 10 μg.mL⁻¹ and 0.01 M HCl, respectively. For the preparation of the sample, it was considered also the USP assay method of HCQ sulfate by HPLC-UV. To achieve the reading concentration (10 μg.mL⁻¹) two successive dilutions should be made, which may be the source of random errors. The absorbance analysis was realized in samples solutions with spectrophotometric scans performed in the range of 200 to 700 cm⁻¹, in triplicate.

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Preparation of sample solution:

The samples of HCQ sulfate were analytically weighed and solubilized in 0.01 M HCl to yield a concentration of 50 μg.mL⁻¹ (stock solution). Then, it was realized a first dilution (1:20) and a second (1:5), in order to obtain a final concentration of 10 μg.mL⁻¹, in triplicate.

Preparation of the calibration curve:

Similar to the procedure performed to prepare the sample solution, the analytical standard was used to obtain the stock solution of 50μg.mL⁻¹ and to dilute with 0.01 M HCl to obtain the following concentrations: 8, 9, 10, 11 and 12 mg.ml⁻¹. The control curve was prepared each day of analysis and used to calculate concentration.
Optimization of the analytical method developed:

Sample preparation was demonstrated as the first critical point to guarantee security analytics: the realization of two dilutions requires a longer running time of the experiment and can result in greater number of analytical errors. In order to overcome this problem a statistical comparison was performed (by Student's t-test) between the two successive dilutions (1:20 followed by 1:5) and only one direct dilution (1:100) to reach the concentration reading (10 μg.mL⁻¹).

The remaining optimization process was proposed to evaluate the actual need of agitation by ultrasonic bath and the use of 0.01 M HCl as a diluent solution, these conditions recommended by the specific drug monograph in the American pharmacopoeia 9.

From the identification of the U.S. Pharmacopoeia test, a comparative analysis of the assay was performed under the following conditions: with (for 15 min) and without stirring in an ultrasonic bath in order to reduce the experimental time of the analytical method and reduce operational costs due to the use of equipment. Next, it was tested the variation of the diluent solution used (0.01 M HCl and distilled water) whereas the use of water would enable to reduce time, reagents and analytical waste.

Validation of the analytical method developed:

Once developed, the following parameters were evaluated: selectivity, robustness, linearity, limit of detection, limit of quantification, precision and accuracy. These parameters were evaluated according to the standards set by ICH 26, 27 and the Resolution No. 899 of 2003 (ANVISA) 21, since this work has been classified as an analytical method for the quantitative determination. The confidence level of the results was observed for the following treatments: coefficient of variation (CV %) less than 5% and statistical analysis using Student's t-test, One-Way and Two-Way ANOVA test when applicable.

Specificity:

The selectivity and/or specificity of a method ensures that the measurement of the sample of interest is not affected by the presence of metabolites, degradation products, co-administered drugs or adjuvants used in formulations 28. To evaluate the selectivity, a preliminary study of forced degradation of the drug was performed. For this, the samples were subjected to the following stress conditions: acid and basic hydrolysis (1M HCl and 1M NaOH, respectively), oxidation (hydrogen peroxide 5%) - in the ratio 3:1 degradative solution: sample - and photolysis (45 ± 5ºC/15 min). The solutions were maintained at room temperature and analyzed after 72 hours. By the method developed and compared with analysis by HPLC-DAD according to the U.S. Pharmacopoeia monograph 9. The specificity of the method was evaluated regarding the contamination of the sample with excipient ingredients of the reference medicine, a solid dosage form (tablet) Plaquenil®, which are: starch, talc and dibasic calcium phosphate. All physical mixtures were prepared in 2:1 drug: excipient and the samples were weighed with respect to the concentration of HCQ sulfate.
Robustness:

To evaluate the performance of the method compared to small and deliberate modifications, the following parameters were alternated and applied to sample preparation and/or analytical method: influence of light during sample preparation through the presence and absence of cold white light (for no more than 25 minutes); stability of the sample at times 0, 2 and 4 hours and reading wavelength: 341, 342 and 343 nm, mimicking the possible lack of calibration of the equipment.

Linearity:

Linearity was assessed with the aid of serially diluted calibration solution as mentioned above. The standard and sample were taken separately. Calibration curves were plotted on the basis of triplicate analysis of each calibration solutions. Linear concentrations were obtained over the range studied with correlation coefficients ≥ 0.99 for the drug. The regression equation was y = 0.02172x. Its shown in tables no 2 & 3.

**Table 2**: Linearity study for hydroxy chloroquine

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of Hydroxy chloroquine sulfate (µg/mL)</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0.156</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.215</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.364</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0.489</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.640</td>
</tr>
</tbody>
</table>

**Fig.4**: Linearity study for hydroxy chloroquine sulfate
Table 3: Linearity parameters for hydroxy chloroquine sulfate

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Slope</td>
<td>0.01242</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0002</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.991607</td>
</tr>
<tr>
<td>RSD</td>
<td>99.16</td>
</tr>
</tbody>
</table>

**Precision:**
The precision of the method was done by replicate (n=5) analysis of tablet preparations. The precision was also studied in terms of intraday changes in absorbance of drug solution on the same day and on three different days over a period of one week. The intra-day and inter day variations was calculated in terms of percentage relative standard deviation and the results are given in Table 4.

Table 4: Precision study for hydroxy chloroquine sulfate

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Amount Labeled mg per dose</th>
<th>Amount found</th>
<th>Recovery (% n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroxy chloroquine sulfate</td>
<td>25</td>
<td>24.57</td>
<td>99.71% w/v</td>
</tr>
</tbody>
</table>

**CONCLUSION:**
The proposed method of estimation of Hydroxy chloroquine sulfate by UV spectroscopy in tablet dosage forms is simple, precision, specific and highly accurate and less λ max for analysis would be recorded, so it can definitely be employed for the routine analysis. Hence this method development and validation of hydroxy chloroquine sulfate tablet dosage form in UV method is suitable for quality control; of raw materials and formulations and also for dissolution studies. It can be used for bio-equivalence studies in Pharma Industry.

**REFERENCES:**


