DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF HYDROXYCHLOROQUINE IN BULK AND TABLET FORMULATION

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ABSTRACT
Two simple, precise and economical UV spectrophotometric methods have been developed for the estimation of Hydroxychloroquine in pharmaceutical dosage form. Method A applied was area under curve (AUC) in which area under curve was integrated in the wavelength range of 251-261 nm using water as a solvent. Method B involves getting first order derivative spectrum of drug solution and measurement of derivative amplitude at 347.80 nm. Calibration curves were plotted for both methods by using instrumental response at selected wavelength and concentrations of analyte in the solution. Linearity for the detector response was observed in the concentration range of 2-12 μg/ml for both the methods. The correlation coefficient for first and second method was 0.9981 and 0.9975 respectively. %Recovery from pharmaceutical formulation was 99.38-100.9 and 99.75-100.33 for first and second method respectively. The proposed methods were validated as per ICH analytical method development guidelines. Limit of detection and limit of quantitation were determined for both methods.

Keywords: Hydroxychloroquine, Area under curve, first order derivative method, Validation

INTRODUCTION

Hydroxychloroquine, chemically (RS)-2-{[4-[(7-chloroquinolin-4-yl)amino]pentyl](ethyl)amino}ethanol. It is official in BP and USP. It is used in malaria and having antirheumatoid action. It is a class of 4-aminoquinolines. It is a very potent blood schizonticidal drug effective against the erythrocytic forms of all four plasmodial species but not have any effect on sprozoites, hypnozoites or gametocytes. A survey of literature has revealed Enantioselective analysis of the metabolites\(^2\), HPLC and UV detection in plasma\(^3\-\^8\), differential pulse voltammetry\(^9\), Impurity identification by HPLC, LC/IT/MS, LC/TOF/MS and NMR\(^10\), photochemical stability by preparative TLC\(^11\).
method for estimation of Hydroxychloroquine in bulk drug and formulation. The objective of the present study is to develop simple, precise, accurate and economic spectrometric methods for estimation of Hydroxychloroquine.

![Structure of Hydroxychloroquine](image)

**Figure 1**
Structure of Hydroxychloroquine

**MATERIALS AND METHODS**

**Instrument**
A double-beam Shimadzu UV/Vis spectrophotometer, 1800 with spectral bandwidth of 2 nm, wavelength accuracy of ±0.5 nm and a pair of 1-cm matched quartz cells, was used to measure absorbance of the resulting solution. All weighing were done on electronic balance (Model Shimadzu AUW-220D), Ultrasonicator model 5.5L150H were used.

**Chemicals and Reagents**
Pharmaceutical grade of Hydroxychloroquine was kindly gifted from Cadila Pharmaceutical Ltd., Ahmedabad. The commercially available marketed tablet, HCQS tab (IPCA) containing 200 mg of Hydroxychloroquine was used and it was procured from the local market. Distilled water was obtained by in house distillation.

**PROCEDURE**

**Preparation of standard stock solution**
Standard stock solution of Hydroxychloroquine was prepared by dissolving 10 mg in 10 ml (1000 µg/ml) volumetric flask using water as solvent. From resulting stock solution prepare 100 µg/ml with water.

**Preparation of working standard solution**
The prepared stock solution was further diluted with water in 10 ml volumetric flask to get working standard solution of 2-12 µg/ml to construct Beer’s law plot. The absorbance of each solution was measured at the area between 251-261 nm for method A. For method B spectras were converted into first order derivative and absorbance was measured at 347.80 nm.

Analysis of commercial dosage form

Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 200 mg of Hydroxychloroquine was accurately weighed and transferred to 100 ml volumetric flask containing water and sonicated for 5 min. That gives 500 µg/ml. The solution was filtered through whatman filter paper. The solution was further diluted with water to get the concentration of 6 µg/ml. The amount of drug present in the sample solution was determined using the calibration curve of standard drug.

![UV spectra of 6 µg/ml Hydroxychloroquine in 251-261 nm wavelength region](image)

Figure 2

UV spectra of 6 µg/ml Hydroxychloroquine in 251-261 nm wavelength region
**Figure 3**
Overlay UV spectra of Hydroxychloroquine (2-12 µg/ml)

**Figure 4**
Calibration curve of Hydroxychloroquine in AUC method (2-12 µg/ml)

\[ y = 0.0254x - 0.0025 \]
\[ R^2 = 0.9981 \]
Figure 5
Overlay spectra of Hydroxychloroquine in First order derivative method

Figure 6
UV spectra of 6 µg/ml Hydroxychloroquine in First order derivative method
Recovery studies
The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for drug. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 4 µg/ml of Hydroxychloroquine.

RESULT AND DISCUSSION
Under experimental conditions described, calibration curve, assay of tablets, recovery studies and precision studies were performed. Using appropriate dilutions of standard stock solution, the solutions were scanned. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient is shown in Table I. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision. Beer’s law obeyed in the concentration range 2-12µg/ml and with correlation coefficient of 0.9981 and 0.9975 for method A and method B respectively. The proposed method was also evaluated by the assay of commercially available tablets containing Hydroxychloroquine. The % assay was found to be 99.84 %
for method A and 99.67% for method B. Results of recovery studies are shown in Table II and table III. For Hydroxychloroquine, the recovery study results ranged from 99.38% - 100.9% and 99.66-100.5% respectively. The LOD and LOQ were found to be 0.3700 g/ml and 1.1212 g/ml respectively for method A and 0.3145 and 0.9530 respectively for method B.

**TABLE 1: OPTICAL CHARACTERISTIC AND VALIDATION PARAMETER**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC method</th>
<th>Derivative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer's range (mcg/ml)</td>
<td>2-12 g/ml</td>
<td>2-12 g/ml</td>
</tr>
<tr>
<td>Wavelength</td>
<td>251-261 nm</td>
<td>348 nm</td>
</tr>
<tr>
<td>Regression Equation</td>
<td>y = 0.0254x – 0.0025</td>
<td>y = -0.0042x + 0.0001</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9981</td>
<td>0.9975</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0254</td>
<td>-0.0042</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0025</td>
<td>0.0001</td>
</tr>
<tr>
<td>Limit of Detection ( g/ml)</td>
<td>0.3700</td>
<td>0.3145</td>
</tr>
<tr>
<td>Limit of Quantification ( g/ml)</td>
<td>1.1212</td>
<td>0.9530</td>
</tr>
<tr>
<td>Intraday precision (n=3) (%RSD)</td>
<td>0.9326</td>
<td>0.6723</td>
</tr>
<tr>
<td>Interday precision (n=3) (%RSD)</td>
<td>1.2608</td>
<td>0.9652</td>
</tr>
</tbody>
</table>

**TABLE 2: RECOVERY STUDY OF HYDROXYCHLOROQUINE IN AUC METHOD**

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Amount taken ( g/ml)</th>
<th>Amount spiked ( g/ml)</th>
<th>Total amount ( g/ml)</th>
<th>Amount recovered ( g/ml)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>6.04</td>
<td>100.67</td>
</tr>
<tr>
<td>100%</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>7.95</td>
<td>99.38</td>
</tr>
<tr>
<td>150%</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>10.09</td>
<td>100.9</td>
</tr>
</tbody>
</table>

**TABLE 3: RECOVERY STUDY OF HYDROXYCHLOROQUINE IN FIRST ORDER DERIVATIVE METHOD**

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Amount taken ( g/ml)</th>
<th>Amount spiked ( g/ml)</th>
<th>Total amount ( g/ml)</th>
<th>Amount recovered ( g/ml)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>5.98</td>
<td>99.66</td>
</tr>
<tr>
<td>100%</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>8.04</td>
<td>100.5</td>
</tr>
<tr>
<td>150%</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>10.02</td>
<td>100.2</td>
</tr>
</tbody>
</table>
TABLE 4: RESULT OF ANALYSIS OF HYDROXYCHLOROQUINE IN TABLET DOSAGE FORM

<table>
<thead>
<tr>
<th>Method</th>
<th>Label amount</th>
<th>Amount found</th>
<th>% Label claim (Mean ± SD)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>200mg</td>
<td>24.87 mg</td>
<td>99.48 ± 0.44</td>
<td>0.4422</td>
</tr>
<tr>
<td>B</td>
<td>200 mg</td>
<td>24.74 mg</td>
<td>98.97 ± 0.2203</td>
<td>0.2225</td>
</tr>
</tbody>
</table>

CONCLUSION
The validated Spectrophotometric method employed here proved to be simple, fast, accurate, and precise and sensitive thus can be used for routine analysis of Hydroxychloroquine in combined tablet dosage form without prior separation.

ACKNOWLEDGEMENT
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REFERENCES


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