FLUROMETRIC ESTIMATION OF QUININE SULPHATE

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ABSTRACT
The emitted radiation (fluorescence intensity) is directly proportional to the concentration of the substance present which can be measured by fluorimeter. Molecules such as quinine sulphate having conjugated double bonds especially pi bonds are particularly suitable for fluorescence. Quinine sulphate in 0.05 N sulphuric acid gives blue fluorescence and the fluorescence intensity can be measured by fluorimeter with the excitation wave length of 360 nm using primary filter and with the emission wave length of 475 nm using secondary filter.

Keywords - Emitted Radiation , Quinine sulphate, flurometric

INTRODUCTION

Fluorimetry
It is measurement of fluorescence intensity at a particular wavelength with the help of a filter fluorimeter or a spectrofluorometer.

Principle of fluorescence spectroscopy
Absorption of UV or visible radiation causes transition of electrons from singlet ground state to the singlet excited state. As this state is not stable, it emits energy in the form of UV or visible radiation and returns to singlet ground state.[1]

Advantages
1. It’s one of the newer methods and its potentialities are still largely unexplored.
2. It also affects precision. Up to 1% can be achieved easily in Flourimetric.
3. The method is very sensitive and also possesses specificity because there is a choice of wavelength not only for the radiation emitted, but also for the light which excites it.
Limitations

1. Ultraviolet light used for excitation may cause photochemical changes or destruction of the fluorescent molecule.
2. The presence of dissolved oxygen may cause increased photochemical destruction.
3. The method is not suited for determination of major constituents of a sample, because the accuracy is very less for large amounts.

INSTRUMENTATION

Fluorometer Components

INSTRUMENTATION

Sources:

1. Xenon arc Lamp:
   It consists of two tungsten electrodes form an arc at a specific distance and xenon gas is stored (under pressure) in quartz or fused silica tube. It emits radiation with a higher intensity (500 nm) than a hydrogen discharge lamp.
   Wavelength: 750-1000 nm.[2]
2. Tungsten Halogen Lamp:
It is also known as a halogen lamp. It is an incandescent light source. It consists of a filament made up of tungsten enclosed in a quartz vessel containing an inert gas and a small quantity of Iodine or bromine (Halogen).

3. Mercury Vapor Lamp:
These lamps are ideal light sources that provide high-intensity light in the deep UV to visible regions. It consists of 2 alloys (tungsten) electrodes which are placed together in a medium containing mercury vapor and 25-50 torr of pure argon gas. These electrodes are enclosed in an elliptically shaped in a silica glass tube. It provides clear white light, high intensity with 24000 Hrs. of life.[2]

![Mercury vapor lamp](image)

**Monochromators:** they convert polychromatic light into monochromatic light. They can isolate a specific range of wavelength or a particular wavelength of radiation from a source.
- Excitation monochromators: provides suitable radiation for excitation of molecule
- Emission monochromators: isolate only the radiation emitted by the fluorescent molecules.

**Sample cells:**
These are meant for holding liquid samples. These are made up of quartz and can have various shapes ex: cylindrical or rectangular.[2]

**DETECTOR:**

1. **Photomultiplier tubes:**
These are incorporated in expensive instruments like spectrofluorimeter. Its sensitivity is high due to measuring weak intensity of light.

The principle employed in this detector is that, multiplication of photoelectrons by secondary emission of electrons.

This is achieved by using a photo cathode and a series of anodes (Dyanodes). Up to 10 dyanodes are used.[3]
Validation of analytical methodology

Validation is defined as, it's proved substantiation which gives a high degree of assurance that a process, system, installation will co product meeting its destined specifications and quality attributes.

System confirmation is a process of proving that an Analytical system is respectable for its willed purpose. For pharmaceutical system/method, guidelines from United State Pharmacopoeia (USP), International Conference on Harmonization (ICH), World Health Organization (WHO), and the Food and Drug Administration (FDA) provides a framework for performing similar confirmation. ICH guidelines Q2A and Q2B (confirmation of Analytical procedure methodology) were the expert working group for enrollment of medicinal for mortal use. ICH Q2A also provides descriptions of typical confirmation parameters, measured, and which subset of its parameter is suitable for validation of analytical system. The discussion of confirmation of Analytical process has been divided into three common orders of Analytical procedures:[7]

- Identification test.
- Quantitative test for contaminant content, limit test for control of contaminations.
- Quantitative tests of active moiety in bulk substance or medicinal product or named ingredient in the product.

ICH Q2B is mandatory to ICH Q2, which presents a discussion of characteristics that should be considered during confirmation of logic practice, it's generally possible to design the experimental work similar that the applicable confirmation characteristics confirmation can contemporaneously to give a sound, overall knowledge capabilities of Analytical procedure.

1) Accuracy - The accuracy of the method was ascertained by recovery method. When the method was used for subsequent analysis of the drug in the dosage form after spiking with 50%, 100%, and 150% of the drug, the recovery was 100.05% (99.7% to 100.9%).

2) Precision - The repeatability of sample and measurement of peak area were expressed as %RSD. Repeatability and intermediate precision at three different concentrations (0.4, 0.6, and 0.8 μg/ml) for both within-day and day-to-day analysis were always

   a) Repeatability
b) Intermediate precision

c) Reproducibility

3) **Specificity** - A different set of condition for elution of the TAB was changed as discussed in previous sections, viz. formic acid content and temperature; then in spite of these changes, no additional peak was found, although a very small change was observed in retention times and peak shapes. The specificity of the method was ascertained by analyzing drug standard solution and samples of equivalent concentration (0.8 μg/ml). The identity of the peak in the sample was confirmed by comparison of the retention time and flurometry of the peak from the sample with those of the peak from the standard. Peak purity for the drug was assessed by comparing the flurometry spectra acquired at the peak start, peak apex, and peak end. The specificity of the method was also ascertained by analyzing the alkaline degraded samples; when degraded sample was chromatographed, the concentration of the TAB was reduced and other peaks were observed at different retention time.[5]

4) **Linearity** - Linearity was evaluated by analyzing different concentrations of the standard solutions of the TAB. Response was a linear function of concentration over the range of 0.2 to 2 μg/ml which was used as the working range of the method. Peak area and concentration were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient. The linearity of the calibration plots was confirmed by the high value of correlation coefficients \( r^2 = 0.9998 \pm 0.001 \), and %RSD for the correlation coefficients was less than 2.

5) **Robustness** - There was no significant change in the result of developed method, after the introduction of small deliberate changes in temperature (±5°C) and formic acid content in aqueous phase of the mobile phase (±5%). The standard deviation of peak areas was calculated for each set of conditions .[4]

**Drug Profile: Quinine sulphate (API)**

![Quinine sulphate molecule](image)
**Molecular Formula**: C20H26N2O6S

**Synonyms** - quinine sulfate, Quinidine sulfate, NSC5362, NSC10004, Quinicardine

**Molecular Weight**: 422.5 g/mol

Quinine Sulfate is the sulfate salt form of the quinidine alkaloid isolate quinine. Quinine has many mechanisms of action, including reduction of oxygen intake and carbohydrate metabolism; disruption of DNA replication and transcription via DNA intercalation; and reduction of the excitability of muscle fibers via alteration of calcium distribution. This agent also inhibits the drug efflux pump Pglycoprotein which is overexpressed in multi-drug resistant tumors and may improve the efficacy of some antineoplastic agents. (NCI04)

An alkaloid derived from the bark of the cinchona tree. It is used as an antimalarial drug, and is the active ingredient in extracts of the cinchona that have been used for that purpose since before 1633. Quinine is also a mild antipyretic and analgesic and has been used in common cold preparations for that purpose. It was used commonly and as a bitter and flavoring agent, and is still useful for the treatment of babesiosis. Quinine is also useful in some muscular disorders, especially nocturnal leg cramps and myotonia congenita, because of its direct effects on muscle membrane and sodium channels. The mechanisms of its antimalarial effects are not well understood.[7]

**Fig no.5 quinine sulphate API**

**Quinine sulphate tablet (300mg)**

Quinine 300 MG Tablet is an effective antimalarial medicine obtained from the bark of the cinchona tree. It is used alone or with other medicines to treat uncomplicated Plasmodium falciparum malaria. It may also be used to treat and prevent night leg cramps in adults and the elderly population when these cramps disrupt the sleep constantly.

**Side effects**:

- Severe diarrhea
- Nausea and Vomiting
- Severe abdominal pain
- Black or tarry stools
- Bloody stool
- Blurred vision
- Anxiety and nervousness
● Difficulty in breathing
● Severe back pain
● Ringing or buzzing in the ears
● Swelling of the eyes, ears, and inside of nose
● Slurred speech
● Excessive hunger
● Malaria

This medicine is used to treat uncomplicated malaria characterized by fever, chills, nausea and vomiting, headache, muscle pain, etc.

This medicine may be used to treat and prevent night leg cramps in adults and the elderly population when these cramps disrupt the sleep constantly.

![Fig no.6- quinine sulphate tablet](image)

**AIM AND OBJECTIVE**

**AIM:**
Literature review reveals that only limited analytical methods have been reported for determination of Quinine sulphate. In present work an attempt was made to provide newer, simple, accurate flurometric method for determination of Quinine sulphate. Optimization and validation of the flurometric conditions were completed according to the standard ICH guidelines.

**OBJECTIVES:**

To fluorometric method for determination of Quinine sulphate.

To validate the developed methods as per the ICH guidelines.

**Material and Method –**

**REQUIREMENTS:**

**Reagents:** Distilled water, Quinine sulphate, con.sulphuric acid

**Glassware:** Volumetric flask 100 ml (5), 10 ml pipette, tissue paper, Whatman filter paper, glass funnel.
Instrument/Apparatus: fluorimeter apparatus

Preparation of 0.05M sulphuric acid

Dissolve 1.35 ml sulphuric acid in 500 ml distilled water.

Preparation of standard Quinine (API) solution-

a) Weigh accurately 10 mg of quinine sulphate and dissolve in 1000 ml of 0.05 M sulphuric acid.

b) Take 4, 8, 12, 16, 20 and 24 ml of the above solutions and dilute to 10 ml with 0.05M sulphuric acid to get the resulting solutions of 0.4, 0.8, 1.2, 2.0 and 2.4 ug/ml

Preparation of sample and formulation solution:

Marketed Formulation:

Table no- 1 Marketed formulation of QUNINE SULPHATE

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Brand Name</th>
<th>Strength</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QUININ</td>
<td>300 mg/tablet</td>
<td>HAB pharmaceuticals &amp; research Ltd</td>
</tr>
</tbody>
</table>

a) Take a tablet of quinine sulphate.
b) Weigh the tablet.
c) Weight of tablet is 0.356g
d) Weigh accurately 12 mg of quinine sulphate tablet and dissolve in 100 ml of 0.05 M sulphuric acid.
e) Pipette out 0.8, 1.0, 1.2, 1.6 ml of the above solution and dilute to 10 ml with 0.05M sulphuric acid to get the resulting solutions of 8, 10, 12 and 16 ug/mL

Operation of fluorimeter:

a) Switch on the instrument and stabilize for 10 minutes.
b) Keep the primary and secondary filters at the wave lengths of 360 nm and 485 nm. respectively in the instrument.
c) Set the fluorescence intensity to 0 using 0.05M sulphuric acid as blank.
d) Set the fluorescence intensity to 100% by using highest concentration of the standard solution (24ug/mL).
e) Measure the percentage fluorescence intensity of different standard solutions (4, 8, 12, 16 and 20 μg/mL).
f) Measure the percentage fluorescence intensity of the sample solution.
g) Plot a graph between concentration versus fluorescence intensity and determine the concentration of sample by using linearity equation.

Validation Parameter:

1. Linearity
2. Accuracy
3. Precision
4. Robustness
5. Recovery formulation

Experimental work;

1) Fluorometric Method:

- Selection of solvent-

In order to select suitable solvent for the estimation of Quinine sulphate, the solubility and stability of various solvents was checked.

- Preparation of standard stock solution-

Four tablets of Quinine sulphate were finely powdered and well mixed. An amount equivalent was weighed, and transferred into the flask and dissolved in solvent (methanol: distilled water). Ultra-sonication of the solution was carried out for one and half hour, filtered, cooled and completed the volume using methanol: distilled water. This solution was used as a standard stock solution for analysis.

- Selection of concentration range and preparation of calibration curve

For the preparation of calibration standards, aliquots portion 1.1,1.4,1.8,2.2,2.6,3.0 ml was pipetted out from standard stock solution and transferred to series of 10 ml volumetric flasks and volume was made up with solvent to get a concentration range from 10-30µg/ml. the absorbance was measured three times for each concentration. Absorbance of each solution was measured against as a blank at 23 quinine sulphate 9nm.

- Analysis of marketed formulation-

For the estimation of in marketed formulation, 1 tablet of QUININE SULPHATE were finely powdered and well mixed. And 10 mg amount equivalent was weighed, and transferred into the flask and dissolved in 50 ml solvent (methanol: Distilled water). ultra-sonication of the solution was carried out for one and half hour, The solution was filtered through whatman filter paper no.41, cooled and completed the volume up to 100 mL using methanol : distilled water gives 100
µg/ml. This solution was used as a standard stock solution for analysis.

2. Method validation:
   - According to ICH Q2 (R1) guidelines for developed method was validated to assure the reliability of the analysis for different parameters like linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness.
   - The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. The linearity was accessed by plotting calibration curve for quinine sulphate. For these, 6 different concentrations of quinine sulphate ranging from 10-30 µg/ml were prepared and analyzed respectively.

- Linearity-

- Precision-

  - The precision study of analytical method expresses the closeness of agreement obtained from the multiple sampling of the same homogenous sample under the prescribed conditions. Intraday precision (repeatability) was performed by taking three different concentration (18, 22, 26 µg/ml of quinine sulphate) covering specified range in the triplicates and were analyzed three times within a day with same operator and with same equipment. Interday precision was determined by analyzing three different concentrations (18, 22, 26 µg/ml of quinine sulphate) in triplicates on three days within same laboratory conditions.

- Accuracy-

  - Accuracy of an analytical procedure is closeness of test results to the true value. Accuracy was determined by standard addition method. The study was determined by spiking known amount of standard stock to the test solution prepared from tablet formulation at three different spiking level 80%, 100%, 120% of target concentration.

- Limit of Detection (LOD)-

  The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD was calculated using the following formula, LOD = 3.3 σ/S
Where,

\( \sigma \) is the standard deviation of the response, \( S \) is the slope of the calibration curve.

- **Limit of Quantification**-

The limit of Quantification is ability of analytical method that the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

\[
\text{LOQ} = 10 \sigma / S
\]

Where,

\( \sigma \) is the standard deviation of the response, \( S \) is the slope of the calibration curve.

**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. The robustness was performed by change in temperature of surrounding, changing strength of solvent.

**RESULT AND DISCUSSION:**

- **Fluorometric Method**

  Fluorometric method was developed for estimation of Quinine Sulphate in bulk and tablet dosage form. The wavelength was found to be 239nm for quinine sulphate.

  **Linearity studies of Quinine sulphate**

  The method was found to be linear in the concentration range of 4-24\( \mu \)g/ml (Table No 2) with regression equation as \( y = 3.55x + 16.40 \) and coefficient of regression (\( R = 0.9997 \)) as shown in figure No.2.

  mg of standard quinine sulphate was transferred to 100 ml volumetric flask, dissolved and make up volume with 100ml to obtain 100\( \mu \)g/ml concentration. Further dilution was done by transferring 10 ml of the prepared solution into a 10ml volumetric flask and subsequent dilutions were made with 10 ml to obtain 10 \( \mu \)g/ml concentration.

  The calibration curves were linear in the concentration range of 10-30\( \mu \)g/ml for quinine sulphate.
Table no- 2  Linearity of Quinine sulphate

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Intensity of fluorescence</th>
<th>MEAN</th>
<th>SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>33.3</td>
<td>33.4</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>8</td>
<td>41.6</td>
<td>41.5</td>
<td>0.09</td>
<td>0.21</td>
</tr>
<tr>
<td>12</td>
<td>57.9</td>
<td>58.0</td>
<td>0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>16</td>
<td>72.8</td>
<td>72.7</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>20</td>
<td>91.4</td>
<td>91.3</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>24</td>
<td>100.1</td>
<td>100.1</td>
<td>0.14</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Calibration curve for Quinine sulphate

![Calibration curve](chart.png)
Optical characteristics of Quinine Sulphate:

Table no: 3

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\lambda_{\text{max}}$</td>
<td>475 nm</td>
</tr>
<tr>
<td>2</td>
<td>Beer’s Law</td>
<td>$Y=3.55x+16.40$</td>
</tr>
<tr>
<td>3</td>
<td>Regression equation</td>
<td>$Y=3.55x+16.40$</td>
</tr>
<tr>
<td>4</td>
<td>Slope</td>
<td>3.55</td>
</tr>
<tr>
<td>5</td>
<td>Intercept</td>
<td>16.40</td>
</tr>
<tr>
<td>6</td>
<td>Coefficient of Correlation</td>
<td>$r^2 = 0.9947$</td>
</tr>
<tr>
<td>7</td>
<td>Limit of Detection</td>
<td>1.09</td>
</tr>
<tr>
<td>8</td>
<td>Limit of quantitation</td>
<td>3.32</td>
</tr>
</tbody>
</table>

Accuracy

Accuracy of the proposed method was determined by performing recovery study at 80, 100 and 120% level for Quinine sulphate. The recovery study was done by adding pure drug solution to pre analysed tablet sample solution and concentrations ppm were determined by using calibration graph showing satisfactory accuracy. Results of recovery study are shown in Table no.

Table no: 4 Accuracy observations of Quinine sulphate

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Recovery added in %</th>
<th>Total Concentration</th>
<th>Concentration observed in ppm</th>
<th>% amount found</th>
<th>Mean</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>18</td>
<td>17.21</td>
<td>95.6</td>
<td>95.7</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.25</td>
<td>95.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.25</td>
<td>95.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>20</td>
<td>18.19</td>
<td>90.9</td>
<td>91.13</td>
<td>0.16</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.25</td>
<td>91.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.25</td>
<td>91.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Precision

The precision of an analytical method was expressed as the SD and % RSD of the series of measurements. It was ascertained by replicate estimation of standard drugs. It involves intraday and Interday precision. For intraday precision triplicates of the solutions containing 8, 12, 16 ppm were carried out three times on the same day and for inter-day precision triplicates of the solutions were carried out for the three consecutive days at the same concentration level. Results are summarized in Table. The percent RSD value for 8, 12, 16 ppm was found to be less than 2% for repeatability, intraday and interday showing good precision, respectively.

Intraday precision

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. µg/ml (2hrs interval)</th>
<th>Mean % found</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 pm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>92.46</td>
<td>0.85</td>
<td>0.91</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>96.6</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>97.9</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 pm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>94.5</td>
<td>0.76</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>96.7</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>98.8</td>
<td>0.27</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Interday precision

Table no- 6 Interday precision observations of Quinine sulphate

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. µg /ml (2hrs interval)</th>
<th>Mean %amount found</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>91.45</td>
<td>0.97</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>98.37</td>
<td>0.44</td>
<td>0.44</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>99.7</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>90.60</td>
<td>0.87</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>96.30</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>98.57</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Robustness

Robustness study was carried out by changing the λ max (± 2 nm) The % RSD was found to be less than 2 %. It shows the method was robust.

Table no-7 Robustness observations of Quinine sulphate

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Conc. µg /ml (2hrs interval)</th>
<th>Mean %amount found</th>
<th>SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>91.5</td>
<td>0.286</td>
<td>0.31</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>99.3</td>
<td>0.285</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Conclusion

The fluorimetry method was developed and validated for estimation of Quinine sulphate in tablet dosage form. The selected method was found to be sensitive, reproducible and accurate for analysis of Quinine sulphate in tablet formulation.

REFERENCE-

13. Lloyd JB. Synchronized excitation of fluorescence emission spectra. Naj