**Investigation of Spectrophotometric for Determination of Cloxacillin Sodium in Different Brands of Pharmaceutical Preparations**

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**ABSTRACT**

This article comprises a very effective, sensitive economical and precise spectrophotometric method for the fortitude of cloxacillin sodium in different brands of pharmaceutical preparations. Studied method is based on complexation of cloxacillin sodium with Cu ions by heating in water bath. The method has investigated the parameters of wavelength 450nm concentration of Cu ions (1000 ppm) and volume of the Cu ions 20 mL with incubation time of 10 minutes, for 40 ppm of cloxacillin sodium. The limit of detection was found 0.3099 with quantification limit of 1.033. The standard deviation was found 0.1033, and relative standard deviation of 1.033. The method was applied and found very promising for the determination of cloxacillin sodium in various pharma brands with labeled claims of the assay. The investigated method is recommended for the quality control analysis of the cloxacillin sodium in pharma industry.

**Keywords:** Spectrophotometric method, Cloxacillin sodium, Pharmaceutical Preparations.

**INTRODUCTION**

Cloxacillin is a semi-synthetic antibiotic related to penicillin,¹ and is also isoxazolyl penicillin.² Cloxacillin is used in treatment of infections due to staphylococci resistance to benzyl penicillin. It is administered by orally or by injection as sodium salt.² Cloxacillin is a bactericidal with mode of action similar to benzyl penicillin. It’s active against penicillinase- producing and non-penicillinase-producing staphylococci. It’s activity against streptococci such as Streptococcus pneumonia and str. Pyogenes is less than that of benzylpenicillin, but sufficient to be useful when these organisms are present with penicillin resistant staphylococci. Cloxacillin is virtually ineffective against Enterococcus faecalis Resistance.²

Cloxacillin is incompletely absorbed from the gastrointestinal tract, after an oral dose of 500mg, peak plasma concentration of 7-15 µg/mL in fasting subjects in 1-2 hours. Absorption is more complete when given intramuscular injection and peak plasma concentration of about 15 µg/mL is observed in 30 minutes after a dose of 500mg. drug has been reported to have plasma half life of 0.5-1 Hour.²

The chemical structure of Cloxacillin Sodium is shown in Figure 1.³⁵

**Figure 1:** The structural formula of cloxacillin sodium

**Chemical formula:** C₁₉H₁₇ClN₂NaO₅S, H₂O.⁴⁵

**Molecular weight:** 475.⁹⁴⁵

**Characters**

A white, hygroscopic, crystalline powder, soluble in methanol, alcohol and freely soluble in water. In the present study an attempt was made to develop an innovative, less expensive, simple and more authentic method for the determination of Cloxacillin Sodium in pure state and its pharmaceutical preparations.³

**Spectrophotometric determination method development strategy for Cloxacillin sodium**

The presence of carboxylic acid functional group in the formula of drug suggests the possibility of complexation with transition metal offering a possibility for its spectrophotometric determination. Therefore in the proposed method Cloxacillin sodium was tried for its complex formation with various transition metals such as Cr, Ni, V, Cu to give a colored product. The colored product thus formed was used for the determination of Cloxacillin sodium.

**MATERIALS AND METHODS**

Preliminary Study of the chelation potential for spectrophotometric determination of Cloxacillin sodium

Preliminary determinations were conducted to explore the possibility of the formation of expected colored complex between metal ion and Cloxacillin sodium. Initially higher concentration of Cloxacillin sodium (1000ppm) was treated with different metal ion solution along with heating to check the formation of expected
colored product. The colored product indicates the chances of reaction and subsequent formation of Cloxacillin sodium complex by this method. Further studies were focused on the optimization of various parameters for maximum complexation and are discussed below.

**Investigation of appropriate wavelength for the estimation of Cloxacillin metal complex**

**Instruments**

Digital analytical balance, thermostatic water bath and UV/VIS spectrophotometer were used during this investigation.

**Reagents**

Analytical reagent grade copper nitrate trihydrate and Cloxacillin sodium were used during this work.

**Solution Preparation**

**Metal Ion Solution**

Metal Ion solution (1000 ppm) was prepared by dissolving 0.380 g of Cu(NO$_3$)$_2$.3H$_2$O in distilled water and diluted to 100 mL with distilled water.

**Standard Cloxacillin Sodium solution**

Cloxacillin sodium (1000 ppm) stock solution was prepared by dissolving 0.1 g of authentic standard Cloxacillin sodium in distilled water and diluted upto 100 mL with distilled water.

**Procedure**

Cloxacillin sodium 2 mL from (1000 ppm) stock solution was slowly transferred to 50 mL volumetric flask. To this 10 mL of metal ion solution from (1000 ppm) stock solution of metal ion was added with little dilution with water followed by incubation in boiling water bath for 20 minute. The contents were allowed to cool to room temperature in tape water tub, and diluted to 50 mL with distilled water. Blank solution was prepared by the same procedure without addition of Cloxacillin sodium. The absorbance of yellow colored complex was measured at 450 nm using Genesys 5 spectrophotometer. The results are given in Table 1 and are shown in Figure 2.

**Investigation of the effect of metal ion solution and incubation time on the formation and absorbance behavior of complex.**

**Instrument:** The same as mention before  
**Reagent:** The same as mention before  
**Solution:** The same as mention before  
**Procedure**

Standard Cloxacillin sodium solutions 2 mL from (1000 ppm) stock solutions were transferred to six separate 50 mL volumetric flasks and to each of these flasks, varied volumes of metal ion solution from (1000 ppm) stock solution was added along with little dilution with distilled water and was incubated in water bath for 20 minute. The resulting colored complex was allowed to cool and diluted to 50 mL with distilled water. Blank was prepared in the same manner without the addition of Cloxacillin sodium. The absorbance of the resulting complex was measured at 450 nm using Genesys 5 spectrophotometer. The results are given in Table 2 and are shown in Figure 3.

**For studying the effect of incubation time** Standard Cloxacillin sodium solution 2 mL (1000 ppm) stock solution was transferred to six separate 50 mL volumetric flasks. To each of these flask 20 mL of metal ion solution form stock solution and little volume of water was added followed by incubation in boiling water bath for varied times, in a range of 0-25 minutes. The resulting yellow colored complex was cooled and diluted to 50 mL with distilled water. Blank was prepared in the same manner without the addition of Cloxacillin sodium. The absorbance of the resulting complex was measured at 450 nm using Genesys 5 spectrophotometer. The results are given in Table 3 and are shown in Figure-4.
The effect of concentration on the absorbance behavior of Cloxacillin Cu complex

**Instruments:** The same as mentioned before

**Reagents:** The same as mentioned before

**Solutions:** The same as mentioned before

**Procedure**

Varied amount of standard Cloxacillin sodium solution with final concentration after dilution ranging from 2-120 ppm taken in fifteen separate 50 mL volumetric flasks. To each of these flask 20mL of metal ion solution from (1000 ppm) stock solution and little volume of distilled water was added followed by the incubation in boiling water bath for 10 minutes. The resulting colored complex was cooled and diluted to 50 mL with distilled water. Blank solution was prepared in same manner without the addition of Cloxacillin sodium. The absorbance of the resulting colored complex was measured at 450 nm using Genesys 5 spectrophotometer to find out absorbance behavior. The results are given in Table 4 and are shown in Figure 5.

![Figure 5](image)

Figure 5: The effect of concentration on the absorbance behavior for cloxacillin sodium at lower concentration using spectrophotometric method

**Analysis of Cloxacillin sodium in various pharmaceutical preparations using investigated method and its comparison with official method**

**Instruments:** The same as mentioned before

**Reagents:** The same as mentioned before

**Solutions:** The same as mentioned before

**Procedure**

Accurately weigh 100 mg of reference standard cloxacillin sodium in 100 mL volumetric flask. Dissolved it in distilled water by continues shaking and made up to mark. Pipette out 3 mL of the solution into 50 mL volumetric flask, to this 20 mL of (1000 ppm) Cu\(^{++}\) ion solution was added after little dilution and incubation was carried out in water bath for 10 minutes. The solution was allowed cooling in tape water tub and diluted upto mark with distilled water. Blank solution was prepared in the same manner without the addition of Cloxacillin sodium. The absorbance of the resulting colored complex was measured at 450 nm using Genesys 5 spectrophotometer.

**Sample preparation**

**Procedure**

Weigh 20 capsules and take contents of powder equivalent to 100 mg cloxacillin in 100 mL volumetric flask. Dissolved and made the volume with distilled water, shaked well sonicated for 5 minutes. Filtered the solution using filter paper # 1, Pipette out 3 mL of the filtrate into 50 mL volumetric flask, to this 20 mL of (1000 ppm) Cu\(^{++}\) ion solution was added along with little volume of water followed by incubation in water bath for 10 minutes, allow it to cool and diluted upto mark by distilled water and measure the absorbance at 450 nm. In cause of injection proceed same as for standard preparation.

**Calculations**

\[
\text{Mg/caps/injection of cloxacillin} = \frac{\text{Au} \times \text{wt of std} \times 100 \times 5 \times 5 \times \text{potency of std} \times \text{average weight}}{\text{As} \times 100 \times 5 \times \text{Wt of sample} \times 3 \times 100}
\]

Where \(\text{Au}\) = Absorbance of Sample

\(\text{As}\) = Absorbance of Standard

Percentage Label Claim = \((\text{mg per caps/inj} \times 100) / (\text{LC mg per caps/inj})\)

**Determination of cloxacillin sodium by official method (HPLC method)**

**Buffer**

Prepared a 0.2 M solution of monobasic potassium phosphate in water, and adjusted with 2N NaOH to pH of 6.8.

**Mobile phase**

Prepared a mixture of buffer and acetonitrile (80:20).

**Standard preparation**

Prepared a solution of USP cloxacillin sodium in buffer having a concentration of 0.55 \(\mu\)g/mL.

**Sample Preparation**

Transferred 110 mg of cloxacillin sodium in 200 mL volumetric flask diluted with buffer to volume, and mixed using magnetic stirrer for five minutes to dissolve.

**Chromatograph parameters**

- **Wavelength:** 225 nm
- **Flow rate:** 1 mL/min
- **Column:** 4.6 mm \times 25 cm (Contains packing L1)

**Procedure**

Separately injected equal volume (20 microlites) of standard and sample into the 200(CE / W) (ru / rs)

\[C = \text{concentration in mg/ mL}\]

\[E = \text{cloxacillin equivalent, in microgram per mL}\]
W = weight in mg
ru and rs = cloxacin peak response obtained from assay and standard preparation respectively.

RESULTS AND DISCUSSION

The proposed method involves the complexation of the cloxacin sodium with Cu^{2+} ions leading to a yellow colored complex.

Various parameters like wavelength, volume of Cu^{2+} ion solution, incubation time were optimized for the formation of utmost colored complex.

After the preliminary experiment the complex formed, was investigated for the optimal wavelength. The results are given in Table 1 and are shown in Figure 2 as can be seen from Table 1 that resulting complex has a maximum absorbance at 450 nm, and was used as optimum wavelength for further investigation of Cloxacin sodium determination.

Cu^{2+} solution (1000 ppm) was used for the complexation. Various volumes of (1000 ppm) Cu^{2+} ion solution in the range of 5 to 30 mL were tried with 40 ppm of cloxacin sodium. The results are given in Table 2 and are shown in Figure 3. As can be seen from the Table 2 that 20 mL of Cu^{2+} ion (1000 ppm) solution was found to be optimum volume for the formation of maximum complex with 40 ppm of cloxacin sodium.

It was observed that incomplete complexation could be achieved at room temperature and it was necessary to heat the solutions in hot water bath. Therefore incubation time in range of 0-25 minute was investigated for maximum complexation in boiling water bath and the results are given in Table 3 and are shown in Figure 4. It was found that 10 minute incubation time in boiling water bath was the optimum incubation time for maximum complexation.

Effect of concentration at lower level on the absorbance behavior of cloxacin Cu^{2+} was investigated to calculate the limit of detection (LOD) and limit of quantification (LOQ) at optimum conditions. Cloxacin sodium 2 ppm was selected for investigation of detection limit as this was the minimum concentration for which the absorbance could be noted. Six replicate readings were taken for this concentration. The results are given in Table 4. The following formulas were used for calculation of LOD, LOQ, S.D and R.S.D.

Limits of detection (for concentration) = 3 x S

Limits of quantifications (for concentration) = 10 x S

Standard deviation, $S \sqrt{n/(n − 1)}$

Relative standard deviation, R.S.D = S/X x 100

Where as

X = Concentration in (ppm) found.
X = Average founded concentration (ppm) of six samples.

Table 1: Wavelength optimization for spectrophotometric determination of cloxacin sodium

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Absorbance</th>
<th>Wavelength (nm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>0.111</td>
<td>490</td>
<td>0.061</td>
</tr>
<tr>
<td>410</td>
<td>0.122</td>
<td>500</td>
<td>0.042</td>
</tr>
<tr>
<td>420</td>
<td>0.133</td>
<td>510</td>
<td>0.030</td>
</tr>
<tr>
<td>430</td>
<td>0.140</td>
<td>520</td>
<td>0.020</td>
</tr>
<tr>
<td>440</td>
<td>0.146</td>
<td>530</td>
<td>0.016</td>
</tr>
<tr>
<td>450</td>
<td>0.147</td>
<td>540</td>
<td>0.014</td>
</tr>
<tr>
<td>460</td>
<td>0.136</td>
<td>550</td>
<td>0.013</td>
</tr>
<tr>
<td>470</td>
<td>0.118</td>
<td>560</td>
<td>0.012</td>
</tr>
<tr>
<td>480</td>
<td>0.085</td>
<td>570</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Table 2: Optimization of the incubation time for the formation of complex

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>0.00</th>
<th>5.0</th>
<th>10.0</th>
<th>15.0</th>
<th>20.0</th>
<th>25.0</th>
<th>25.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.004</td>
<td>0.101</td>
<td>0.179</td>
<td>0.172</td>
<td>0.148</td>
<td>0.139</td>
<td>0.139</td>
</tr>
</tbody>
</table>

Table 3: Optimization of volume of metal ion solution for formation of complex

<table>
<thead>
<tr>
<th>Volume used in mL (1000 ppm)</th>
<th>05</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.178</td>
<td>0.172</td>
<td>0.197</td>
<td>0.218</td>
<td>0.211</td>
<td>0.200</td>
</tr>
</tbody>
</table>

Table 4: The effect of concentration on the absorbance behavior for cloxacin sodium at lower concentration using spectrophotometric method

<table>
<thead>
<tr>
<th>Cloxacin sodium Conc. (ppm)</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.012</td>
<td>0.017</td>
<td>0.034</td>
<td>0.045</td>
<td>0.070</td>
<td>0.089</td>
<td>0.098</td>
<td>0.115</td>
</tr>
<tr>
<td>Cloxacin sodium Conc. (ppm)</td>
<td>32</td>
<td>36</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Absorbance</td>
<td>0.140</td>
<td>0.164</td>
<td>0.206</td>
<td>0.270</td>
<td>0.365</td>
<td>0.438</td>
<td>0.532</td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Replicate readings for 2 ppm concentration of cloxacillin sodium

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>Concentration (ppm) found (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>2.2</td>
</tr>
<tr>
<td>0.011</td>
<td>2.0</td>
</tr>
<tr>
<td>0.012</td>
<td>2.2</td>
</tr>
<tr>
<td>0.012</td>
<td>2.2</td>
</tr>
<tr>
<td>0.011</td>
<td>2.0</td>
</tr>
<tr>
<td>0.012</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 6: Results of investigated method

<table>
<thead>
<tr>
<th>Linear range</th>
<th>1-120μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max</td>
<td>450nm</td>
</tr>
<tr>
<td>standard deviation</td>
<td>0.1033</td>
</tr>
<tr>
<td>R.S.D</td>
<td>4.849</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.996</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>5.04*10^{-5}</td>
</tr>
<tr>
<td>Σ</td>
<td>1.08*10^{2}</td>
</tr>
<tr>
<td>L.O.D</td>
<td>0.3099</td>
</tr>
<tr>
<td>L.O.Q</td>
<td>1.033</td>
</tr>
</tbody>
</table>

Table 7: Application of investigated method for the analysis of cloxacillin sodium in various pharmaceutical preparations and comparison with official method

<table>
<thead>
<tr>
<th>Name of Drug</th>
<th>Label Claim</th>
<th>Develop method</th>
<th>Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auropen capsules</td>
<td>250 mg/capsule</td>
<td>251.31±1.13 mg/capsule</td>
<td>251.83±1.41 mg/capsule</td>
</tr>
<tr>
<td>Auropen inj</td>
<td>250 mg/vial</td>
<td>2.49±0.59 mg/vial</td>
<td>250.4±0.87 mg/vial</td>
</tr>
<tr>
<td>Cloxazan Capsules</td>
<td>250 mg/capsule</td>
<td>253.2±1.64 mg/capsule</td>
<td>252.9±0.92 mg/capsule</td>
</tr>
</tbody>
</table>

CONCLUSION

Spectrophotometric method for the determination of cloxacillin sodium involves complexation of cloxacillin sodium with Cu^{2+} ions followed by heating in water bath. Various analytical parameters like wavelength, concentration and volume of metal ion solution and incubation time in water bath were optimized for spectrophotometric determination of cloxacillin sodium and were found to be 459nm, 20 ml (1000 ppm), and 10 minutes respectively for 40 ppm of cloxacillin sodium. The limit of quantification and limit of detection for the investigated method were calculated using authentic reference standard and were found to be 1.033 and 0.3099 respectively. The relative standard deviation and standard deviation were established to be 1.033 and 0.1033 respectively.

The method was found linear in range of 1-120 ppm. The developed method was effectively useful for determination of cloxacillin sodium in various pharmaceutical formulations and method was found in good conformity with labeled claim in pharmaceutical preparations.

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REFERENCES


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