USE OF BROMOTHYMOL BLUE AS CHROMOGENIC REAGENT FOR THE COLORIMETRIC DETERMINATION & VALIDATION OF COBICISTAT IN ITS BULK FORM

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ABSTRACT
Cobicistat a CYP3A inhibitor, indicated to increase systemic exposure of Atazanavir, Darunavir or Elvitegravir in combination with other antiretroviral agents in the treatment of HIV-1 infection. It is only a pharmacokinetic enhancer, but has no antiviral activity. The review of literature reveals not much spectrophotometric work being carried out for its estimation & hence with the need to explore, in the present work a colorimetric method was developed for its estimation in bulk form based on the principle of ion-pair complex using Bromothymol blue as chromogenic reagent. The method developed was observed to have absorption maxima at 411nm, linear (r=0.9999) over the range 10 – 50µg/ml, precise and robust (%RSD). Thus this validated method can be used for the determination of Cobicistat.

KEYWORDS: Cobicistat; Colorimetric method development; Bromothymol blue.

INTRODUCTION
Cobicistat is a potent inhibitor of CYP450 3A enzymes, including the important CYP3A4 subtype. By combining with Atazanavir, Darunavir or Elvitegravir (once daily dosing regimen) their increased systemic exposure are achieved in the body with lower dosing, theoretically enhancing their viral suppression while diminishing adverse side-effects.1 Marketed formulations of Cobicistat include STRIBILD and TYBOST, by Gilead Sciences approved by FDA in August 2012 for use in USA. Review of literature shows lack of simple spectrophotometric methods for the estimation of Cobicistat and hence leaves us with a scope to explore novel spectrophotometric methods. In analytical methods usually conversion of functional groups of a molecule to other form which is readily adaptable to the technique at hand is known as derivatisation or derivative formations. This principle is utilized in formation of ion pairs between drugs and dye molecules at pH values where the dyes can serve as charge donors. Subsequently, the ion pairs are extracted into organic solvents and spectra overlay is carried to determine the wavelength of maximum absorption. Chloroform, being highly nonpolar and immiscible with water has found the greatest relevance in the extraction of the ion pair complexes. Bromoresol blue (BCB), Bromoresol purple (BCP), Bromothymol blue (BTB) and Bromoresol green (BCG) have found great relevance as ion pair donors in most reported methods. In the present work Bromothymol blue (BTB) is used as chromogenic reagent for the formation of ion-pair complex with Cobicistat. When a solution of BTB in suitable solvent, is mixed with the drug solution, an intense yellow colour ion pair complex is produced immediately. This is due to the conversion of the dye into an open quinoidal anionic derivative, which subsequently forms an ion pair with drug, which could be directly measured from extraction with chloroform.

Fig 1: Structure of Cobicistat

2. EXPERIMENTAL
2.2 Chemicals & Reagents: The drug sample of Cobicistat as an active pharmaceutical ingredient was obtained from IICT, Hyderabad. All chemicals & reagents used were of analytical grade.

2.2 Instrument: Shimadzu 1800, UV/Visible spectrophotometer was used for absorption
measurements with matched glass cuvettes. All weights were taken on electronic balance.

3. EXPERIMENTAL
3.1 Preparation of Buffer pH 4: 8.2 g sodium acetate dissolved in water and volume was made up to 1000ml. 5.9ml of glacial acetic acid made up to 1000ml with water. 153ml of sodium acetate solution and 847ml of glacial acetic acid solution were mixed.

3.2 Preparation of 0.1N HCL: 85ml of HCL was taken in a 1000ml volumetric flask and volume was made up to 1000 ml with distilled water.

3.3 Standard Preparation: Standard Stock solution of Cobicistat was prepared by dissolving 100 mg of Cobicistat in 100ml of 0.1N HCL to obtain a concentration of 1000ug/ml.

3.4 Method Development
Ion pair formation & determination of Absorption maxima- Different aliquots of concentrations ranging from 10ug/ml to 50ug/ml were prepared by pipetting out 0.1 ml of stock solution in a 10ml volumetric flask. To this through method optimization 2.5 ml of Bromothymol blue, 2ml acidic buffer of pH 4 was added. The volume was made up to 10 ml with 0.1N HCL and mixed well. A yellow colour complex was formed and extracted with chloroform using a separating funnel.

The absorbance was read immediately after extraction using matched glass cuvettes of path length 1 cm. Method Validation- The above developed method was validated on parameters of linearity precision, robustness, LOQ & LOD. For linearity studies concentration of 10-50ug/ml were prepared and read for absorbance at the absorption maxima. Intra Day Precision (Repeatability) - 6 solutions of concentration 30ug/ml were prepared and read for absorbance. Robustness studies were made by small amount (2%) deliberate changes in amount of BTB and amount (ml) of acetate buffer. LOD and LOQ were calculated by using the formula 3.3 S.D/S and 10 S.D/S where S.D is the standard deviation of Y-intercept and S is the slope of the calibration curve.

4. RESULTS AND DISCUSSION
4.1 Optimizing the reaction conditions: After observing the spectra with different amounts of Bromothymol blue, 3ml of the reagent showed maximum absorption. Similarly, pH ranging from 2 to 6 gave a parabolic curve with a maximum absorption at pH 4. The reaction product gave a maximum absorption with 2ml of buffer of pH 4 and the highest absorption was noted with zero time intervals. (Fig 2-8)
4.2 Method Validation

a) Absorption maxima: The proposed procedures are based on the reaction between Cobicistat and BTB, resulting in the formation of yellow ion-pair complex which could be measured directly in chloroform. Cobicistat features a benzene ring. This structure suggests the possibility of utilizing anionic dyes as chromogenic agents. In chloroform, Cobicistat does not absorb the visible region. Also, the dyes used have almost negligible absorbance. But when a solution of BTB is mixed with the drug solution, in the presence of acetate buffer of pH 4 and 0.1N HCL an intense yellow colour is immediately produced with the absorption maximum at 411nm (Fig: 9).

![Absorption Maxima of Cobicistat-BTB ion pair](image)

**Fig 9: Absorption Maxima of Cobicistat-BTB ion pair**

b) Linearity: The absorbance of the reaction product followed Beer-Lambert’s law over the concentration range of 10ug/ml to 50 ug/ml. The linearity is determined to be 0.9997(Table 1; Fig 10, 11).

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentrations (ug/ml)</th>
<th>Absorbance at 411nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10ug/ml</td>
<td>0.160</td>
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<tr>
<td>2.</td>
<td>20ug/ml</td>
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<td>3.</td>
<td>30ug/ml</td>
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<tr>
<td>4.</td>
<td>40ug/ml</td>
<td>0.631</td>
</tr>
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<td>5.</td>
<td>50ug/ml</td>
<td>0.805</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient(r²)</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

![Absorption spectrum for linearity](image)

**Fig 10: Absorption spectrum for linearity**
Fig 11: Calibration curve for Cobicistat-BTB ion pair

Table 2: Results for precision (Repeatability)

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (ug/ml)</th>
<th>Absorbance at 411nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>0.391</td>
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<tr>
<td>2</td>
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<tr>
<td>8</td>
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<tr>
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<td>11</td>
<td>%RSD</td>
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</tr>
</tbody>
</table>

5. CONCLUSION

Cobicistat, a pharmacokinetic enhancer on combining with Atazanavir, Darunavir or Elvitegravir, higher concentrations of them are achieved in the body with lower dosing. The potential of Cobicistat with other drugs can be explored and hence studies on various methods for the determination of Cobicistat are important. In present work colorimetric method using Bromothymol blue as chromogenic reagent to form ion pair complex with Cobicistat has been developed and validated. The method developed showed absorbance maxima at 411nm with linearity in the range of 10-50ug/ml and correlation coefficient 0.9997. The method was found to be precise and robust with %RSD less than 2. Therefore this simple colorimetric method can be extended for the determination of Cobicistat in industries, laboratories, academics and to any other relevant field.

CONFICT OF INTEREST

We are glad to express no conflict of interest exists between any authors or with any person involved in this research work.

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REFERENCES

2. Aidsinfo Drug Database, Elvitegravir/Cobicistat/Emtricitabine /Tenofovir Disoproxil Fumarate. [Online], Available at: https://aidsinfo.nih.gov/drugs/507/stribild/0/patient
4. I started my personal revolution, Stribild. [Online], Available at: https://www.stribild.com/
