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A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form

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ABSTRACT

The literature review reveals that there are some analytical methods reported for Cobicistat by RP-HPLC method and most of the work was done on biological fluids. Present study aims to develop a specific, precise, accurate, rapid, sensitive and faster elution RP-HPLC method for the estimation of Cobicistat in bulk and pharmaceutical dosage form, using laboratory chemicals. Chromatography was carried on reverse phase C_{18} (4.6 x 100 mm, 5µm, Make: Phenomenex) column, in an isocratic mode using a combination of methanol and water in the ratio of 20:80 v/v as mobile phase at a flow rate of 0.8 ml/min with UV detection at 249 nm. The selected chromatographic conditions were found to analyze Cobicistat (retention time = 4.7 min). The different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection and limit of quantification were determined. The linearity of the calibration curve for each analyte in the desired concentration range is good (r^2>0.9) The recovery of the method was between 98-102%. Hence, the proposed method is highly sensitive, precise and accurate and it may be successfully applied for the reliable quantification of API content in the commercial formulation of Cobicistat.

Key words: Cobicistat, Estimation, RP-HPLC, Validation

INTRODUCTION

Cobicistat is a potent inhibitor of $CYP_{450\ 3A}$ enzymes, including the important CYP_{3A4} subtype. It is chemically described as Thiazol-5-ylmethyl *N*-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl)methyl-methyl-carbamoyl]amino]-4-morpholino-butanoyl]amino]-5-phenyl-pentyl]carbamate. Its molecular formula is $C_{40}H_{53}N_7O_5S_2$. Its structural formula is shown in figure 1 [1].

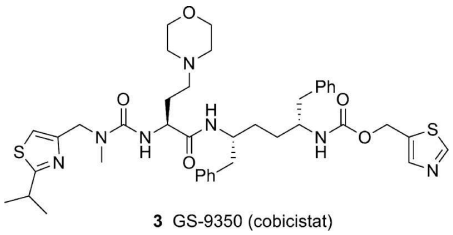


Fig 1: Chemical Structure of Cobicistat

When administered as a fixed dose combination tablet (Elvitegravir 150 mg, Emtricitabine 200 mg, Tenofovir 300 mg, Cobicistat 150 mg) in healthy volunteers, Cobicistat's AUC_{inf} and C_{max} each increases 3% with a light meal, and decreases 17% and 24% respectively with a high-fat meal. The estimated oral bioavailability by means of non-compartmental and compartmental approaches resulted in 74% and 76.4%, respectively. It is extensively

metabolized via CYP_{3A4} and 2D6 (minor) and primarily eliminated in the feces (86%). Renal elimination is a minor pathway (<10% of a dose). Cobicistat has a serum half-life of 3.5 hours.

Cobicistat is a licensed drug for use in the treatment of infection with the human immunodeficiency virus (HIV). By combining Cobicistat with Elvitegravir, higher concentrations of the latter are achieved in the body with lower dosing, theoretically enhancing Elvitegravir's viral suppression while diminishing its adverse side-effects. Cobicistat has no anti- HIV effect of its own.[2]

MATERIALS AND METHODS

Chemicals and materials: Cobicistat was obtained as a gift sample from Mylan Laboratories. HPLC grade water, Acetonitrile and Methanol were obtained from Merck.

Instrumentation: Quantitative HPLC was performed on Waters liquid chromatograph, with UV detector equipped with automatic injector with injection volume 20 µl, and 515 pump. A symmetry C_{18} column (4.6 x 100 mm, 3.5 µm, Make: Phenomenex) was used.

Method development and optimization

To optimize the chromatographic conditions, the effect of a chromatographic variable such as mobile phase was studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of Cobicistat in bulk drug and tablet dosage form. Mobile phases tried for this purpose were Acetonitrile : Methanol : Water (10 : 20 : 70), Methanol : Water (40 : 60), Methanol : Water (20 : 80). The condition that gave best resolution and symmetry was selected. HPLC conditions are given in Table-1.

TABLE 1: Optimized Chromatographic Conditions

PARAMETERS	CONDITIONS
Column (Stationary phase)	Symmetry C_{18} (4.6 x 100 mm, 3.5 µm, Make: Phenomenex) or equivalent
Mobile phase	Methanol : Water (20:80)
Flow rate	0.8 ml/min
Run time	8 min
Column temperature	Ambient
Volume of injection loop	20 µl
Detection wavelength	249 nm
Retention time	4.7 min

Preparation of mobile phase:

200 ml of Methanol was mixed with 800 ml of water. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Diluent Preparation:

Mobile phase is used as diluent.

Preparation of standard solution:

10 mg equivalent of Cobicistat tablet powder (working standard) was accurately weighed and transferred into a 10 ml dry volumetric flask. To it, 10 ml of diluent was added to obtain a solution with concentration 1000 µg/ml. 0.3 ml of Cobicistat was pipetted out of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with the diluent, to obtain a final solution with concentration 30 µg/ml.

Preparation of sample solution:

10 mg equivalent of Cobicistat tablet powder was accurately weighed and transferred into a 10 ml dry volumetric flask. To it, 7 ml of diluent was added and sonicated for 3-5 minutes to dissolve the drug completely. The volume was made up to the mark with the same solvent to obtain a solution with concentration 1000 µg/ml. 0.3 ml of Cobicistat was pipetted out of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with the diluent, to obtain a final solution with concentration 30 µg/ml.

Assay:

20 µl of the standard and sample solutions were injected into the chromatographic system and the areas of peaks for Cobicistat were measured and the % assay was calculated by using the formula

Formula:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Average\ Weight}{Label\ claim} \times 100 = Assay\ \%$$

Where:
AT = average area count of sample preparation
AS = average area count of standard preparation
WS = weight of working standard taken in mg
WT = weight of working sample taken in mg
DS = dilution of standard
DT = dilution of test sample
P = percentage purity of working standard
Label claim in mg/ml.

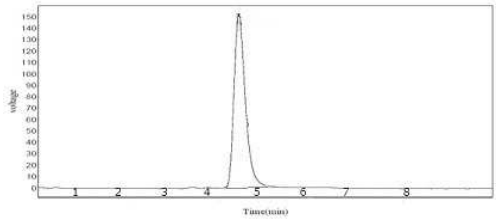


Fig 2: Chromatogram of standard solution

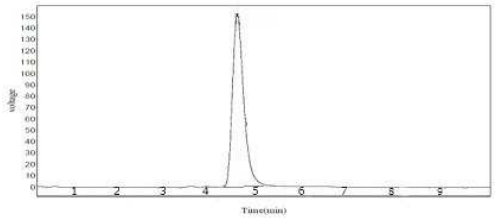


Fig 3: Chromatogram of sample solution

Method validation:
The method was validated for the following parameters such as linearity, precision, accuracy, limits of detection and quantification, ruggedness and robustness.

System Suitability:
System suitability was performed daily during the entire validation period of this method. The results of system suitability were presented in Table 2.

TABLE 2: System Suitability Parameters

S. No.	Parameter	Drug
1	Retention Time	4.717
2	Theoretical Plates	2159.320
3	Tailing Factor	1.342
4	Area	3715063

Accuracy:
The accuracy of an analytical method expresses the closeness of agreement between the value (which is accepted reference value) and the value found. Accuracy studies were done by the standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet. It was measured in drug products by spiking known amounts (50%, 100%, 150%) of the analyte into the analyzed tablet powder and calculating the percent recovered. QC samples were taken, intermediate to standard concentrations, and area was calculated using the standard graph. Percentage deviation from the theoretical concentration was calculated. The recovery data for accuracy studies was shown in Table 3. The accuracy chromatograms for the respective concentrations were shown in fig 4, 5 and 6.

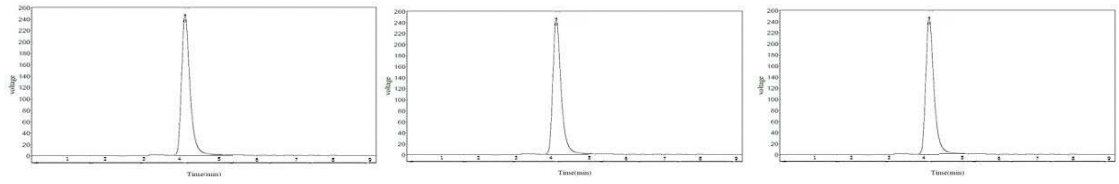


Fig 4: Accuracy chromatograms for 50% solution

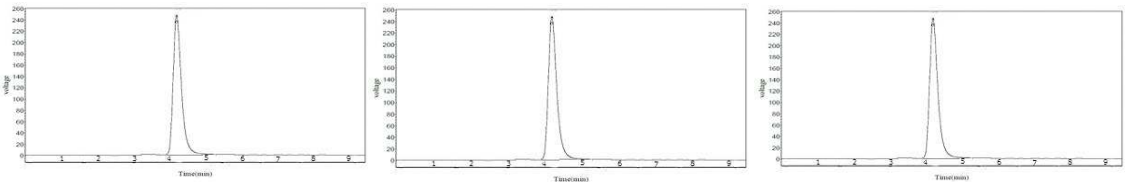


Fig 5: Accuracy chromatograms for 100% solution

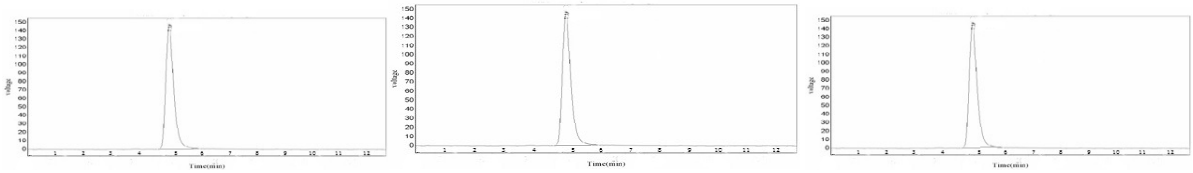


Fig 6: Accuracy chromatograms for 150% solution

TABLE 3: Accuracy Observations of Cobicistat

% concentration (at specification level)	Area	Amount added (µg)	Amount found (µg)	% recovery
50%	3593373.33	22.5	22.481833	99.74667
100%	3436102.33	30	29.81348	98.73333
150%	3473680.33	37.5	37.82457	98.64

Precision:

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements.

$$\%CV = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

The standard solution was injected five times and the area of peak was measured for all five injections in HPLC. The % RSD for the areas of peaks of five replicate injections was found to be within the specified limits. Results were reported in Table 4. Chromatograms were reported in fig 7.

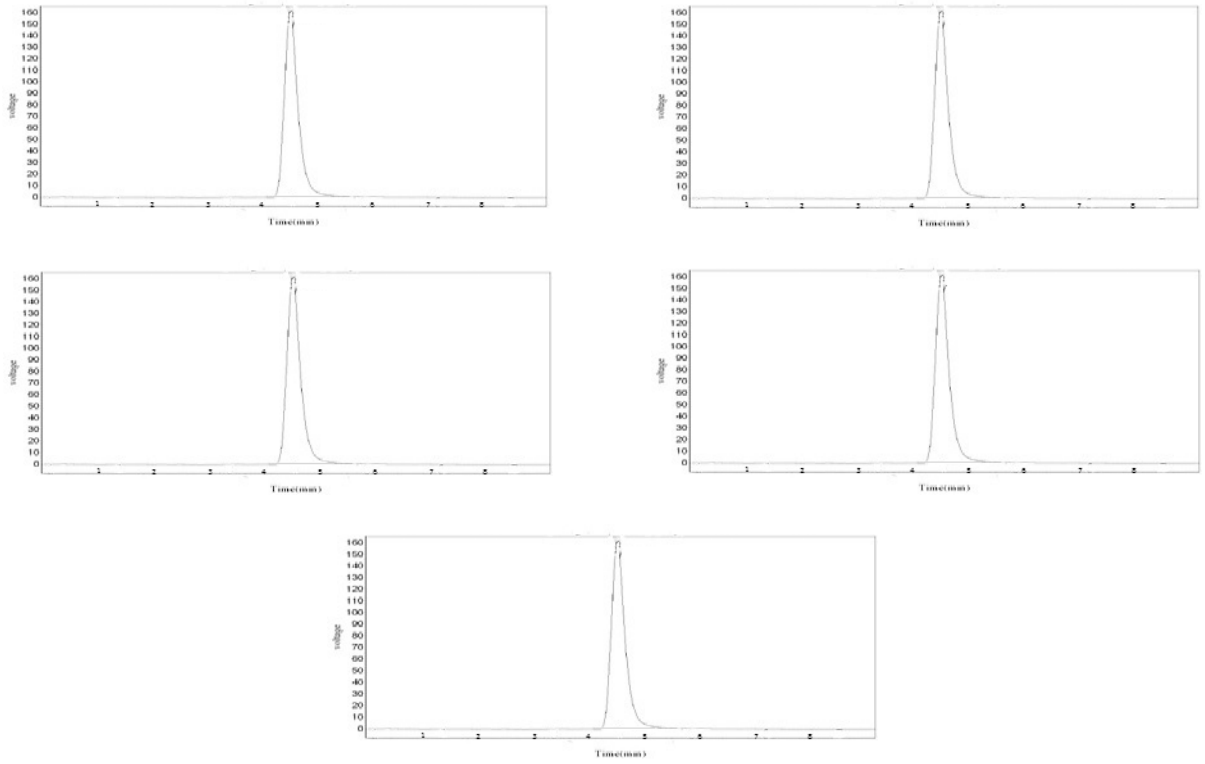


Fig 7: Precision chromatograms of Cobicistat

TABLE 4: System Precision Results of Cobicistat

INJECTION	AREA
Injection 1	358382
Injection 2	358482
Injection 3	358389
Injection 4	358396
Injection 5	358329
Average	358400.167
Standard Deviation	50.5071
%RSD	0.01

Intermediate Precision/Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was performed on different days. For intermediate precision studies, 3 replicate formulation injections were injected. %RSD was determined for peak areas of Cobicistat. The acceptance limit should not be more than 2% and the results obtained were found to be within acceptance limits i.e. 1.84. Results were reported in Table 5.

TABLE 5: Intermediate Precision Results of Cobicistat

INJECTION	AREA
Injection 1	3486640
Injection 2	3376630
Injection 3	3378630
Mean	3413966.6666666665
Standard Deviation	62944.8968
%RSD	1.84

This indicates that the method has good reproducibility (inter-day variation is within specified limits).

Linearity:

Aliquots of standard Cobicistat stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Cobicistat were in the range of 5-30 µg/ml. Each of these drug solutions (20 µL) was injected into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 249 nm and a Calibration graph was obtained by plotting peak area versus concentration of Cobicistat (Fig 8). The linearity chromatograms were presented in Fig 9. Results were reported in table 6.

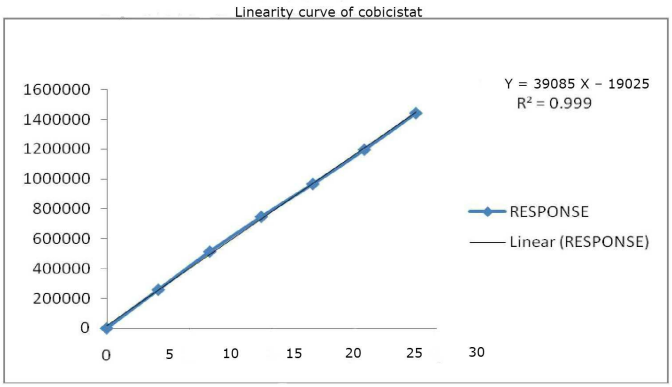
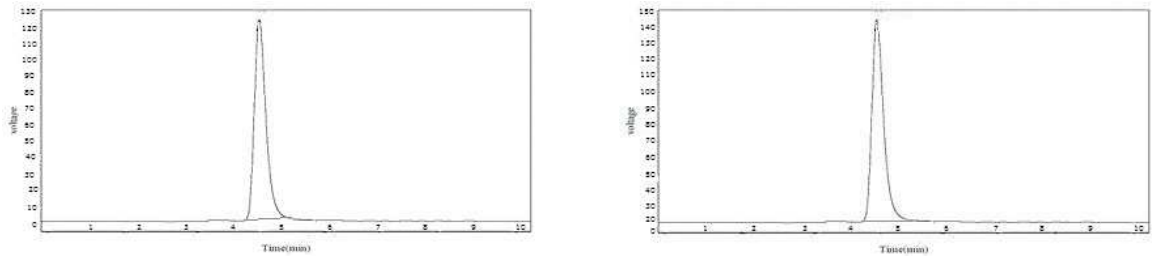


Fig 8: Linearity Calibration Curve of Cobicistat



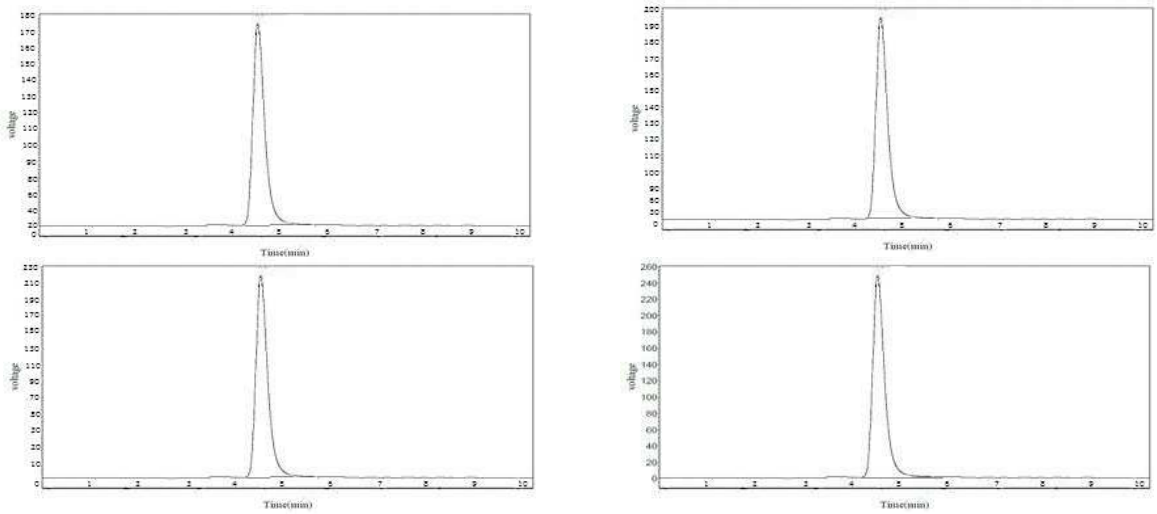


Fig 9: Linearity Chromatograms of Cobicistat

TABLE 6: Results of Linearity of Cobicistat

S.No.	LINEARITY LEVEL	CONCENTRATION (µg/ml)	AREA
1.	1	5	358382
2.	2	10	554246
3.	3	15	787701
4.	4	20	957616
5.	5	25	1297640
6.	6	30	1542910
Correlation Coefficient			0.999183

Limit of Detection [LOD] and Limit of Quantification [LOQ]:

The LOD and LOQ were determined for Cobicistat, based on the standard deviation of (SD) of the response and slope (S) of the regression line as per ICH guideline according to the formulae given below.

$$LOD = \frac{3.3 \times SD}{S}$$

$$LOQ = \frac{10 \times SD}{S}$$

The Cobicistat LOD was found to be 0.4 µg/ml and the LOQ was found to be 1.2 µg/ml.

Method Robustness:

Robustness of the method was determined by small deliberate changes in flow rate and column oven temperature. The content of the drug was not adversely affected by these changes as is evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 7. The chromatograms for flow rate variation were shown in fig 10 and 11 respectively.

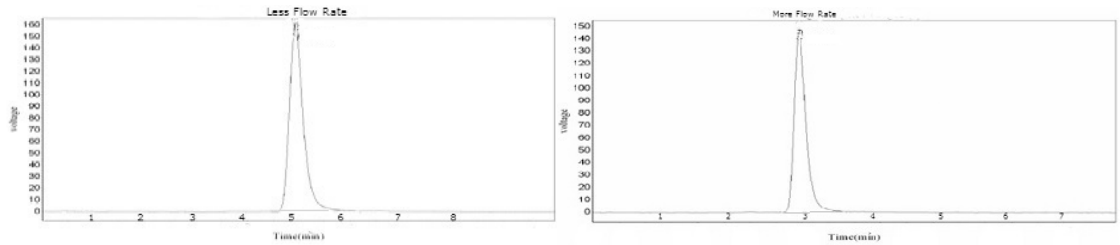


Fig 10: Chromatogram for less flow rate **Fig 11: Chromatogram for more flow rate**

TABLE 7: Results for changes in flow rate

S. No.	Flow Rate	System Suitability Results	
		USP Plate Count	USP Tailing
1.	0.6 ml/min	2155.130	1.351
2.	*0.8ml/min	2155.420	1.349
3.	1.2ml/min	2156.740	1.326

Results for actual flow have been considered from assay standard.

RESULTS AND DISCUSSION

To optimize the mobile phase, various combination ratios of solvents were analyzed. Mobile phase composition was determined and the method development was started by symmetry C_{18} (4.6 x 100 mm, 3.5 µm, Make: Phenomenex) column and with a flow rate of 0.8 ml/min, and detection wavelength at 249 nm. Injection volume was 20 µL, and run time was for 8 min. The mobile phase consists of water and methanol in the ratio of 80:20 v/v. The retention time of Cobicistat was found to be 4.7 minutes. The assay result was found to be 99.775%. Quantitative linearity was observed over the concentration range of 5-30 µg/ml. The correlation coefficient was found to be 0.99. The number of theoretical plates obtained was 2159.32, which indicates the efficiency of the column. The limit of detection and limit of quantification were found to be 0.4 and 1.2 µg/ml respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate and sensitive.

CONCLUSION

The proposed HPLC method was found to be specific, precise, accurate, rapid and economical for estimation of Cobicistat in bulk and pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness, ruggedness and results were validated statistically according to ICH guidelines. A simple, rapid and reproducible HPLC method was developed and validated for the estimation of Cobicistat and separation was achieved on C_{18} column using a combination of methanol and water in the ratio of 20:80 v/v as mobile phase. The flow rate was set at 0.8 ml/min. The analyte was monitored at 249 nm wavelength. The retention time for Cobicistat was found to be 4.717 minutes. The sample recoveries in all formulations were in good agreement with their respective label claims and this method can be used for routine analysis.

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