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# Development and validation of stability indicating RP-HPLC method for the estimation of Cobicistat in bulk and tablet dosage form

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## ABSTRACT

A validated new stability indicating RP-HPLC method for the quantitative estimation of Cobicistat in bulk and Tablet dosage form was developed as per ICH guidelines. The analyte was injected into Symmetry ($C_{18}$) Column, 250 mm x 4.6 mm i.d. and 5µm particle size, maintained at ambient temperature and effluent was monitored at 247 nm. The mobile phase was consisted of HPLC Grade Methanol and HPLC Grade Water in the ratio of 35:35 v/v. The flow rate was maintained at 1.0 mL/min. The calibration curve for Cobicistat was linear from 0 to 70µg/mL (r2=0.999). The inter-day and intra-day precision was found to be within limits. The Limit of detection (LOQ) and Limit of quantification (LOQ) for Cobicistat was found to be 0.09 and 0.27µg/mL respectively. The average % recovery for Cobicistat was 98-102% and repeatability was found to be satisfactory. This RP-HPLC method is suitable for determining the concentration of Cobicistat in bulk and Tablet dosage form and it can applied for routine analysis for estimation of the Cobicistat.

**Keywords:** Cobicistat, RP-HPLC, Method Development, Validation.

## INTRODUCTION

Cobicistat, trade name Tybost (formerly GS-9350), is a licensed drug for use in the treatment of infection with human immunodeficiency virus (HIV). Although it does not have any anti-HIV activity, cobicistat acts as a pharmacokinetic enhancer by inhibiting cytochrome P450 3A isoforms (CYP3A) and therefore increases the systemic exposure of co administered agents [1, 2] that are metabolized by CYP3A enzymes. More specifically, Cobicistat is indicated to increase systemic exposure of atazanavir or darunavir (once daily dosing regimen) in combination with other antiretroviral agents in the treatment of HIV-1 infection. Increasing systemic exposure of anti-retrovirals (ARVs) without increasing dosage allows for better treatment [3] outcomes and a decreased side effect profile. Cobicistat is a CYP3A inhibitor indicated to increase systemic exposure of atazanavir or darunavir (once daily dosing regimen) in combination with other

antiretroviral agents in the treatment of HIV-1 infection. It is not interchangeable [4] with ritonavir to increase systemic [5, 6] exposure of darunavir 600 mg twice daily, fosamprenavir, saquinavir, or tipranavir due to lack of exposure data. The use of cobicistat is not recommended with darunavir 600 mg twice daily, fosamprenavir, saquinavir or tipranavir. Complex or unknown mechanisms of drug interactions preclude extrapolation of ritonavir drug interactions [7, 8] to certain cobicistat interactions. Cobicistat and ritonavir when administered with either atazanavir or darunavir may result in different drug interactions when used with concomitant medications. The IUPAC Name [9] of Cobicistat is (1,3-thiazol-5-yl)methyl N-[(2R,5R)-5-[(2S)-2-{[methyl ({[2-(propan-2-yl)-1,3-thiazol-4-yl] methyl}) carbamoyl] amino}-4-(morpholin-4-yl) butanamido]-1,6 diphenylhexan-2-yl] carbamate. The molecular formula [10, 11] for Rilpivirine is $C_{40}H_{53}N_7O_5S_2$. The Chemical Structure of Cobicistat is follows.

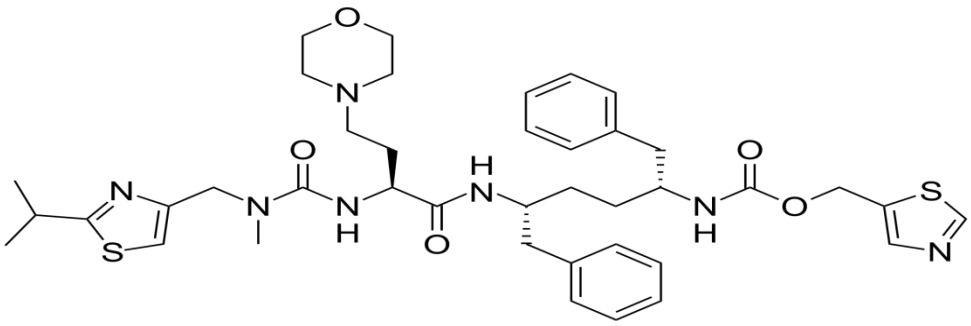

**Fig-1: Chemical Structure of Rilpivirine**

## METHODOLOGY

### Instruments used

**Table-1: Instruments used**

| S. No. | Instruments/Equipments/Apparatus |
|---|---|
| 1. | **HPLC WATERS** with Empower2 Software with Isocratic with UV-Visible Detector (L-2400). |
| 2 | **ELICO SL-159** UV – Vis spectrophotometer |
| 3. | High Precision Electronic Balance **(SHIMADZU ATY224)** |
| 4. | Ultra Sonicator **(Wensar wuc-2L)** |
| 5. | Thermal Oven |
| 6. | **Symmetry ($C_{18}$) Column, 250 mm x 4.6 mm i.d. and 5µm particle size** |
| 7. | $P^H$ Analyzer **(ELICO)** |
| 8. | Vaccum filtration Apparatus **(BOROSIL)** |

### Chemicals / reagents used

**Table-2: Chemicals used**

| S. No. | Name | Specifications | | Manufacturer/Supplier |
|---|---|---|---|---|
| | | Purity | Grade | |
| **1.** | Doubled distilled water | 99.9% | HPLC | Sd fine-Chem ltd; Mumbai |
| **2.** | Methanol | 99.9% | HPLC | Loba Chem; Mumbai. |
| **3.** | Acetonitrile | 99.9% | HPLC | Loba Chem; Mumbai. |
| **4.** | Potassium dihydrogen orthophosphate | 99.9 | L.R. | Sd fine-Chem ltd; Mumbai |
| **5.** | Orthophosphoric acid | 99.9 | L.R. | Sd fine-Chem ltd; Mumbai |

## METHOD DEVELOPMENT

### HPLC instrumentation & conditions

The HPLC system employed was HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector and automatic sampling system having Symmetry (C18) Column, 250 mm x 4.6 mm i.d. and 5µm particle size.

### Chromatographic conditions

The analysis was carried on HPLC Symmetry $C_{18}$, 5µm, 25cmx4.6mm i.d. column with detection wavelength of 247.0 nm. Injection volume of 20.0 µL and maintaining a flow rate at 1.0ml/min.

### Mobile phase

The mobile phase can be prepared by taking the HPLC Grade Methanol and HPLC Grade Water in the ratio of 35:65 v/v. The mobile phase [14] can be filtered through the 0.45 µm filter membrane and degassed by using ultra sonication process. The prepared mobile phase is pumped through the stationary phase maintained at a flow rate of 1.0 ml/min.

### Diluent

Mobile Phase preparation can be used as a diluent [15].

### Preparation of Standard Solution:

Weighed exactly and transferred 10mg of Cobicistat Standard working [16] into a 10ml clean dry volumetric flask, add 6ml of diluent, sonicated for 15 minutes and volume make up to the mark with the same diluent. From the above prepared stock [17] solution, 5ml was pipette out in to a 10ml volumetric flasks and then make up to the volume with the diluent preparation.

### Preparation of Test Solution

First take 10 tablets weighed and measure the average weight of each tablet. Then the weight is equivalent [18] to 1 tablet was weighed and transferred into a clean and 100ml volumetric flask, 50ml of diluent added and sonicated for 30 minutes, further the volume made up with the diluent preparation 9mobile phase) and filtered. From the resulted filtered [19] solution 1ml was pipette out into a 10ml volumetric flask and make up the volume up to mark with the 10ml of diluent.

## RESULTS AND DISCUSSION

### Accuracy

To establish the accuracy of the developed method and the recovery studies [12, 13] was performed by adding different quantities (80%, 100%, and 120%) of pure drug of Cobicistat was taken and added to the pre-analyzed formulation of concentration about 50µg/ml. From that % recovery values was determined. The attained results were shown in following Table-3.

**Table-3: Accuracy Readings**

| Sample ID | Concentration (µg/ml) | | Peak Area | % Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Amount Injected | Amount Recovered | | | |
| $S_1$ : 80 % | 40 | 40.634 | 98329 | 101.585 | Mean= 100.605% |
| $S_2$ : 80 % | 40 | 40.204 | 97322 | 100.51 | S.D. = 1. 0.936122 |
| $S_3$ : 80 % | 40 | 39.888 | 96582 | 99.72 | % R.S.D.= 0.930493 |
| $S_4$ : 100 % | 50 | 50.982 | 122563 | 101.964 | Mean= 99.90667% |
| $S_5$ : 100 % | 50 | 49.440 | 108952 | 98.88 | S.D. = 1.781704 |
| $S_6$ : 100 % | 50 | 49.438 | 118948 | 98.876 | % R.S.D.= 1.783369 |
| $S_7$ : 120 % | 60 | 59.94 | 143561 | 99.911 | Mean= 99.868% |
| $S_8$ : 120 % | 60 | 59.202 | 141816 | 98.67 | S.D. = 1.177089 |
| $S_9$ : 120 % | 60 | 60.614 | 145123 | 101.023 | %R.S.D. = 1.178645 |

## PRECISION

### Repeatability

The precision of every method was determined separately from the peak areas and retention times obtained by real estimation [14] of 6 replicates of a fixed amount of drug. Cobicistat (API). The percent relative standard deviations[15] were calculated for Cobicistat are presented in the Table-4.

**Table-4: Repeatability Readings**

| HPLC Injection Replicates of Cobicistat | Retention Time | Peak Area |
|---|---|---|
| Replicate – 1 | 3.464 | 1036653 |
| Replicate – 2 | 3.463 | 1034698 |
| Replicate – 3 | 3.464 | 1036524 |
| Replicate – 4 | 3.463 | 1036524 |
| Replicate – 5 | 3.462 | 1036254 |
| Replicate – 6 | 3.461 | 1036542 |
| **Average** | **3.462833** | **1036199** |
| **Standard Deviation** | **0.001169** | **747.1333** |
| **% RSD** | **0.03376** | **0.072103** |

### Intermediate precision

### Intra-assay & inter-assay

The intra & inter day variation [16] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD< 2%) within a day & day to day variations for Cobicistat revealed that the proposed method is precise [17, 18].

**Table-5: Results of intra-assay & inter-assay**

| Conc. Of Cobicistat (API) (µg/ml) | Observed Conc. Of Cobicistat (µg/ml) by the proposed method | | | |
|---|---|---|---|---|
| | Intra-Day | | Inter-Day | |
| | Mean (n=6) | % RSD | Mean (n=6) | % RSD |
| 40 | 39.96 | 0.97 | 40.02 | 0.89 |
| 50 | 50.03 | 0.85 | 49.86 | 0.09 |
| 60 | 59.89 | 0.56 | 60.02 | 0.63 |

### Linearity & range

The calibration curve showed good linearity [18] in the range of 0 – 70 µg/ml, for Cobicistat (API) with correlation coefficient ($r^2$) of 0.999 (Fig-2). A typical calibration curve has the regression [19] equation [20] of y = 26249x + 15500 for Cobicistat.

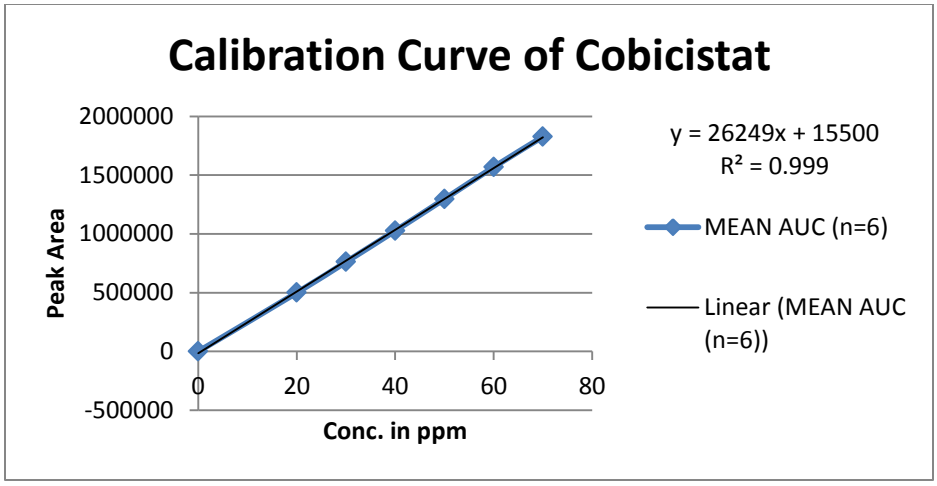


**Fig-2: Calibration curve of Cobicistat (API)**

**Table-6: Linearity-Results**

| CONC.(µg/ml) | MEAN AUC (n=6) |
|---|---|
| 0 | 0 |
| 20 | 499584 |
| 30 | 761241 |
| 40 | 1026412 |
| 50 | 1296542 |
| 60 | 1568542 |
| 70 | 1826541 |

## METHOD ROBUSTNESS

The effect of minute changes in the optimized chromatographic conditions [21] such as Temperature (±2$^0$C), Acetonitrile content in mobile phase (±2%), Wavelength of detection (±2nm) and change in flow rate (± 0.1ml/min) calculated to establish the robustness [22] of the proposed method. (Table-7, % RSD < 2%).

**Table-7: Result of Method Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.35 |
| Flow (0.9 ml/min) | 0.39 |
| Wavelength of Detection (249 nm) | 0.27 |
| Wavelength of detection (245 nm) | 0.37 |

## LOD & LOQ

The Minimum concentration level [23] at which the analyte can be reliable detected (LOD) & quantified (LOQ ) [24] were found to be 0.09 & 0.27 µg/ml respectively.

### System suitability parameter

System suitability parameters are vital part of a lot of analytical procedures. These parameters are based on the concept that the electronics, analytical operations, equipment and samples to be analyzed comprise an important system that can be assessing as such. Following system suitability test parameters [25, 26] were established. The data are shown in Table-8.

**Table-8: Data of System Suitability Parameter**

| S. No. | Parameter | Limit | Result |
|---|---|---|---|
| 1 | Resolution | Rs > 2 | 8.29 |
| 2 | Asymmetry | T ≤ 2 | Cobicistat = 0.13 |
| 3 | Theoretical plate | N > 2000 | Cobicistat = 3426 |

## Evaluation of COBICISTAT in Pharmaceutical Dosage Form

### COBICISTAT- 150 mg

20 tablets were taken and the I.P. method was followed to estimate the average weight. Above weighed tablets were finally powdered and triturated well. An amount of powder equivalent to the 100mg of drug was transferred to 100ml volumetric flask and 70 ml of methanol was added and solution was sonicated for 15 minutes. Then make up the volume up to the mark with the mobile phase. Then add 10 ml of the prepared solution and diluted to 100 ml with methanol. The resulted solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From stock solution (0.5 ml) was transferred to 5 different 10ml volumetric flasks and volume was made up to the 10 ml with mobile phase [27]. The prepared solution was injected into the HPLC system and the observations were recorded.

A duplicate standard solution was also injected into the HPLC system and the chromatogram [28] was recorded. The obtained data are shown in following Table-48.

### Assay

% Assay=AT/AS×WS/DS×DT/WT×P/100×AW/LC×100

Where:

AT = Peak Area of Cobicistat obtained with test preparation

AS = Peak Area of Cobicistat obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Assay was performed as described in previous chapter. Results obtained are tabulated below:

**Table-9: Recovery Data for estimation Cobicistat in FADINE Tablets**

| Brand name of Capsules | Labelled amount of Drug (mg) | Mean (±SD) amount (mg) found by the proposed method (n=6) | Assay + % RSD |
|---|---|---|---|
| Tybost (Gilead Medical Information (UK & Eire) | 150 | 149.89 (±0.06) | 99.65% (±0.48) |

## RESULT & DISCUSSION

The assay of Tybost tablets containing COBICISTAT was found to be 99.65 %.

### Stability studies

The protocol was severely adhering to for forced degradation [29] of Cobicistat Active Pharmaceutical Ingredient.

The API was subjected to strain conditions in different ways to observe the rate and level of degradation that is probable to take place in the course of storage and/or after administration to body.

This is one category of accelerated stability studies [30] that helps us estimating the fate of the drug that is likely to happen after long time storage within a very small time as compare to the actual time or long term stability testing.

The different degradation pathways analysed are acid hydrolysis [31], basic hydrolysis, thermal degradation [32] and the oxidative degradation

### Acid hydrolysis

Accurately weigh 25 mg of pure drug was transferred to a clean and dry 25 ml volumetric flask. To which 0.1N Hydrochloric acid was added & make up to the mark & kept for 24 hrs. from that 0.1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions).

### Basic hydrolysis

An accurately weighed 10 mg. of pure drug was transferred to a clean & dry 10 ml volumetric flask. To which 0.1N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. From that 0.1 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of NaOH (after all optimized conditions).

### Thermal degradation

An accurately weighed 1 mg. of pure drug was transferred to a clean & dry 100 ml volumetric flask, make up to the mark with mobile phase & was maintained at 50 $^0$C. For 24 hrs. Then injected into the HPLC system against a blank of mobile phase (after all optimized conditions).

### Photolytic degradation

Approximately 10 mg. of pure drug was taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg. of the UV exposed drug was transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in methanol & make up to the mark. Then resulted solution injected into the system against a blank of mobile phase.

### Oxidation with (3%) $h_2o_2$

Accurately weighed about 1 mg of drug was transferred to a clean and dry 100ml volumetric flask. Then add 30 ml of 3% $H_2O_2$ and a little methanol to it in order to make soluble. Then keep it aside in dark for 24 hrs. The volume was made up to mark. The resulted sample solution was injected into the HPLC system.

### Results of degradation studies

The results of the stress studies [33] indicated the **specificity** of the method that has been developed. Cobicistat was stable in thermal & photolytic stress conditions. The results of forced degradation studies are given in the following Table-10.

**Table-10: Results of forced degradation studies of Cobicistat API**.

| Stress condition | Time | Assay of active substance | Assay of degraded products | Mass Balance (%) |
|---|---|---|---|---|
| Acid Hydrolysis (0.1 M HCl) | 24Hrs. | 87.85 | 12.15 | 100.0 |
| Basic Hydrolysis (0.I M NaOH) | 24Hrs. | 75.64 | 24.36 | 100.0 |
| Thermal Degradation (50 $^0$C) | 24Hrs. | 96.55 | 3.45 | 100.0 |
| UV (254nm) | 24Hrs. | 96.03 | 3.97 | 100.0 |
| 3 % Hydrogen peroxide | 24Hrs. | 25.37 | 74.63 | 100.0 |

## CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Cobicistat API. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. This proposed method can be used for the further analysis of Cobicistat in bulk and marketed formulations.

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