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Development and Validation of a UV Spectrophotometric Method for the Determination of Cobicistat in Pharmaceutical Formulations

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Abstract: A novel UV spectrophotometric method was devised and tested to appropriately quantify Cobicistat (COBI) in pharmaceutical formulations. It was discovered that the COBICISTAT wavelength of maximum absorbance was 243.8 nm. For COBI, it was discovered that the system and technique accuracy, given as the % RSD of six duplicate measurements, was 0.4712. It was shown that the COBI mean per cent of recovery ranged from 100.84. For COBI, the limits of detection and quantitation were 0.79 µg/ml and 2.154 µg/ml, respectively. The relationship between absorbance and COBI concentration was linear between 7.5–37.5 and 15–75 µg/ml. The Evotaz tablet dosage form's COBI test resulted in a result of 99.52%. So, it was found that the new way was easy, quick, and good for checking quality control. It is suggested for testing COBICISTAT in pure and tablet forms in any lab for quality control.

Keywords: Cobicistat, UV spectrophotometric, Method development, Validation, Stability

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Introduction

Cobicistat, (Fig. 1) an antiviral medicine classed as a protease inhibitor, is used to treat HIV/AIDS. It is widely used in conjunction with powerful antiretroviral medication for its capacity to suppress host enzymes that activate other protease inhibitors. This inhibition increases the plasma concentration of the final medication, allowing doctors to reduce dosage and dosing frequency while improving clinical effectiveness. Cobicistat has been determined using a variety of analytical methods, including UV spectrophotometric and HPLC procedures (Ganji and Satyavati, 2015; Purnima *et al.*, 2016; Desai *et al.*, 2019).

Materials and Methods

Standards and reagents:

Cobicistat medicine with a strength of 99.8% were generously donated as gift sample from Mylan Labs and Hetero Drugs Ltd., Hyderabad, India. Good quality chemicals like methanol, hydrochloric acid, sodium hydroxide, hydrogen peroxide, and water, were bought from Merck India for the tests.

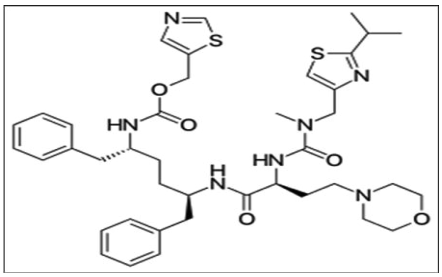

Fig. 1: Structure of Cobicistat

PREPARATION OF SOLUTIONS:

Preparation of individual stock solutions:

15 mg of cobicistat working standards were precisely weighed and put to 100 ml volumetric flasks. Next, 70 ml of methanol was added to each flask, followed by 5 min of sonication to ensure thorough dissolution. The solutions were vacuum-filtered using a 4.5 µ filter before being diluted with water. COBI concentrations were measured to be 150 µg/ml.

Preparation of sample stock solution:

The mean weight of ten tablets was determined and crushed into a fine powder. An amount of this powder equivalent to 150 mg of Cobicistat was precisely weighed and transferred into a clean, dry 100 ml volumetric flask. The powder was dissolved in 70 ml of methanol and subjected to sonication for 5 min to ensure complete dissolution. The resultant solution was then filtered through a 4.5 µ filter using vacuum filtration and adjusted to the mark with water. The concentration of COBI in this solution was confirmed to be 150 µg/ml (Saha and Ahmed, 2014; Kalyani and Anuradha, 2015; Rao and Rao, 2019).

DEVELOPMENT METHOD:

Choice of solvent:

The selection of a solvent for spectrophotometric analysis is a critical decision, as it must be a pure solvent that does not interact with the substance being analyzed and shows minimal absorption at the chosen wavelength. Typically, polar solvents such as water, methanol, and ethanol are commonly used for organic compounds and drugs. It is noteworthy to consider the cut-off wavelengths for these solvents: water's cut-off wavelength is at 192 nm, ethanol at 209 nm, and methanol at 220 nm. This knowledge is indispensable for choosing an appropriate solvent that ensures accurate measurement of the substance's absorption without interference.

Determination of absorption spectra:

The UV-spectrophotometric technique development procedure included determining the wavelength of maximum absorption for the chosen medicines in various solvents. Because the chosen medicines were completely soluble in water, methanol, diethyl ether, and ethanol, the wavelength of maximum absorbance and the stability of absorbance were measured in these solvents. Methanol was chosen as the most suitable solution for method development. To identify the wavelength of maximum absorbance, precisely 2 ml of the standard stock solution was added to two separate 10 ml volumetric flasks and diluted up to the mark with water, resulting in individual final concentrations of COBI of 30 µg/ml. A reagent blank was made in a similar fashion but without COBI. The absorbance of the solutions was then measured as a function of wavelength in comparison to the reagent blank.

The wavelength of maximum absorbance was found to be 243.8 nm based on the representation of the COBI absorption spectra in Figure 2. This specific wavelength was identified as optimal for accurately determining the absorbance of COBI in methanol solution (Stenlake, 1976; Skoog *et al.*, 2007; Fatima *et al.*, 2014; Kumar *et al.*, 2016).

VALIDATION METHOD:

Method validation:

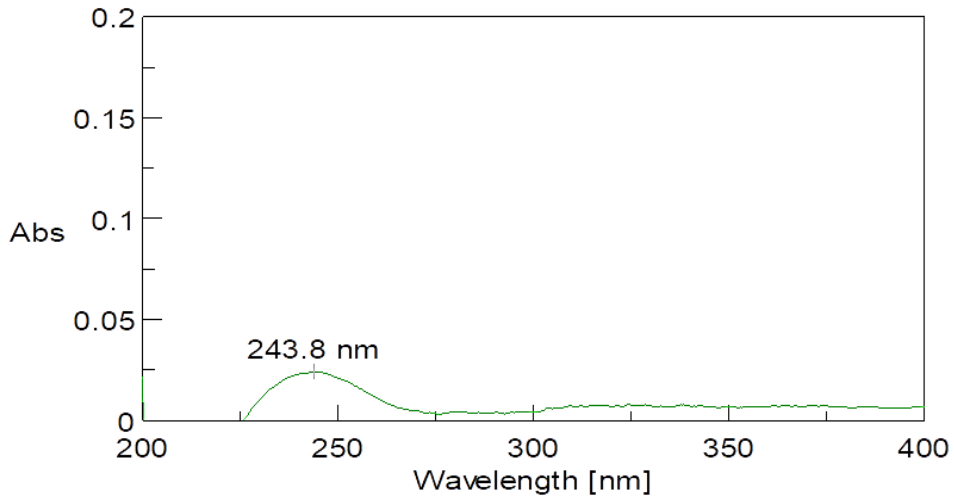

Fig. 2: Absorption spectrum of cobicistat (30 µg/ml) in methanol solvent.

Analytical procedure validation serves to demonstrate whether a new analytical method is appropriate for the intended use or not. By essentially giving a quantitative evaluation of the active pharmaceutical components in a sample, the purpose of assay techniques is to ascertain the content of the analyte present in that sample.

Precision:

The degree of agreement or scatter between a set

of measurements made from numerous samplings of the same homogenous sample under particular conditions is characterized as the precision of an analytical method. The mean and standard deviation of replicate measurements can be used to calculate the per cent relative standard deviation (%RSD), which is the statistical expression for precision. Essentially, it assesses how consistently the analytical procedure gives results when applied to the same material many times.

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Absorption measurements for the standard solution were carried out similarly, but on separate days, with possibly different analysts and equipment, in order to assess intermediate precision. Both intra-day and inter-day precision were found in this experiment. On two distinct days, absorption measurements were conducted using working standard solutions of COBI in comparison to the reagent blank at 243.8 nm. For measurements taken both within and between days, the mean, standard deviation, and percentage RSD were found (Table 1).

Accuracy:

The degree of agreement between the found value and the true or most likely value indicates how accurate an analytical technique is. Either an absolute method or a comparative method can be used to assess accuracy in analytical methods.

Determining the quantity of the intended element (reported as per cent recovery) in pharmaceutical formulations or solid laboratory-prepared samples of desired composition requires the use of two or more supposedly "accurate" procedures that are fundamentally different from

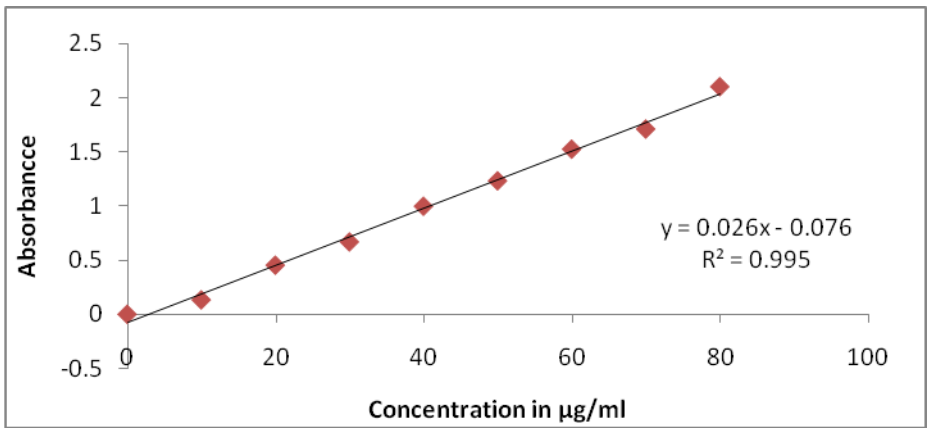

Fig. 3: Linearity plot between peak area and concentration of COBI.

Table 1: Method precision of the developed method

S. No.	Absorbance of COBI at 243.8 nm
1	0.472
2	0.474
3	0.472
4	0.473
5	0.472
6	0.476
Mean	0.473
Standard deviation	0.002
%RSD	0.4712

Table 2: Result of LOD and LOQ of COBI

Constituent	Property	Concentration
COBI	LOD	0.79 µg/ml
	LOQ	2.154 µg/ml

one another. There is not much determinate error if the findings from various approaches are comparable.

It is recommended to perform at least nine determinations across at least three concentration levels that extend the specified range in order to accurately assess accuracy. To establish a rigorous assessment, for example, the entire analytical method should be conducted with three concentrations and three replicates each. The per cent recovery of the known additional analyte amount in the sample or the difference between the mean and the acknowledged real value, together with confidence intervals, should be used to indicate accuracy. This methodology ensures the dependability of the analytical data by enabling a thorough comprehension of the correctness of the method at different concentration levels.

Table 3: Accuracy studies (%recovery) of COBI at three spiked levels

Spiked level	Amount added	Amount found	% Recovery	Mean % recovery
50%	15.0	15.76	102.07	100.22
	15.0	15.12	99.80	
	15.0	14.97	98.80	
100%	30.0	29.47	98.23	98.56
	30.0	29.86	99.53	
	30.0	30.28	97.93	
150%	45.0	45.98	100.18	99.78
	45.0	44.97	100.00	
	45.0	45.52	99.16	
Mean				99.52

Table 4: Assay of COBI formulation

Brand Name	Name of the drug	Label claimed	Amount found ± SD	% of assay ± RSD
Evotaz	COBI	150	149.5±0.429	99.61±1.024

Preparation of 50%/100% and 150% sample solution:

The cobicistat working standard was precisely weighed and divided into three distinct 100 ml volumetric flasks for the analysis: 7.5 mg, 15 mg, and 22.5 mg. About 70 ml of diluent were put in each flask, and to ensure complete dissolution, the contents were sonicated for 5 min. Next, using the same solvent, the volume was adjusted to the correct amount. Then, exactly 2 ml of each of the above solutions was added to distinct 10 ml volumetric flasks and diluted with water to the appropriate level. The final individual standard COBI concentrations were 15, 30, and 45 µg/ml.

A similar procedure was also used to generate reagent blanks, although COBI was not included. At 243.8 nm, the absorbance of each solution was measured in triplicate in comparison to the reagent blank. Tables 3 and 4 give the recovery experiment findings. By comparing the discovered concentrations with the known added quantities of the analyte, these tables probably demonstrate the analytical procedure's correctness.

Linearity studies:

As mentioned in the experimental section, a stock solution of cobicistat was made in order to evaluate the linearity of the suggested method. Subsequent dilutions in the linearity investigation were conducted using this stock solution.

Six volumetric flasks with a capacity of 10 ml were made for the linearity investigation. Each flask was precisely filled with various aliquots of the stock solution, ranging from 0.5 ml to 3.0 ml, and the volume was adjusted with the proper solvent. At 243 nm, the absorbance of these solutions was measured in duplicate against the reagent blank.

The mean peak area was then plotted against the COBI concentration to create a linearity plot. For every example, the linearity, slope, intercept, and correlation coefficient were calculated; the

results are shown in Table 1. This investigation provides insight on the method's capacity to yield test findings that, within the given range, are exactly proportionate to the analyte concentration in the sample.

Limit of Detection and Limit of Quantification:

In analytical terms, sensitivity describes how an instrument reacts to low analyte concentrations. The limit of detection (LOD) and the limit of quantification (LOQ) are frequently used to describe this sensitivity. The signal-to-noise ratio or the calibration curve's slope (s) and response standard deviation (σ) are used to calculate these values.

The formulas for calculating LOD and LOQ are as follow:

$$LOD = 3\sigma/s$$

$$LOQ = 10\sigma/s$$

The results of LOD and LOQ are shown in Table 4, which offers crucial details regarding the sensitivity of the procedure and its capacity to identify and measure analyte concentrations at low levels (Table 2).

ASSAY STUDIES:

Standard Solution Preparation:

15 mg of cobicistat was taken into a dry, clean 100 ml volumetric flask. To ensure that the cobicistat was completely dissolved, added methanol to the flask and sonicated the contents. Next, ensured proper mixing by using the same solvent to make up the remaining volume to the mark.

Sample Solution Preparation:

2.0 ml of standard stock or sample stock was carefully pipetted into a 10 ml volumetric flask and diluted to the mark with the diluent. At 243.8 nm, the absorbance of the final solution was measured in triplicate in comparison to the reagent blank.

Ultimately, the absorbance values of the standard and sample solutions were used to calculate the assay and percentage of COBI assay. The findings are shown in Table 4.

Results and Discussion

The UV spectrophotometric approach was created with the goal of simultaneously studying the stability of the drug sample under multiple degradation circumstances, including acid-base, peroxide hydrolysis, photo, and light decomposition, and cobicistat (COBI) in both its pure and dose forms. Individual drug absorption spectra were collected, and the wavelength of maximum absorbance for COBI was found to be 243.8 nm.Six measurement replicates were used to evaluate the system's accuracy, and the COBI yielded a percentage RSD of 0.3668. By making the functioning standard solution six times, measuring absorbance, and calculating a %RSD of 0.8290 for COBI, the method's accuracy was assessed.

By estimating the per cent recovery from the quantity added and the amount recovered, the proposed method's accuracy was assessed at three different concentration levels. The mean per cent recovery for COBI was found to be 99.52%. The absorbance in the range of 7.5-37.5 and 15-75 µg/ml was found to be linearly related to the COBI concentration.

Conclusion

The created approach was judged to be quick, easy to use, and appropriate for drug sample analysis. As such, it is advised that COBI be analyzed in quality control laboratories using both pure and tablet dose forms. The UV spectrophotometric method developed was identified as straightforward, discerning, and highly sensitive. It was subsequently utilized to determine the assay of pharmaceutical formulations and further expanded to investigate degradation under various forced degradation conditions. Consequently, the method proposed is recommended for use in any quality control laboratory for assessing the quality of both pure cobicistat (COBI) and pharmaceutical formulations, adhering to standard values.

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