Stability Indicating Method Development and Validation for the Estimation of Cobicistat by RP-HPLC in Bulk and Pharmaceutical Dosage Form

Kabilan T[1], Suchitra D[2], Paul Richards M[3], B A Vishwanath4

1. Department of Pharmaceutical Analysis, Aditya Bangalore Institute of Pharmacy Education, and Research, Bangalore, Karnataka, India.
2. Professor, Department of Pharmaceutical Analysis, Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka, India.
3. Professor& HOD, Department of Pharmaceutical Analysis, Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka, India.
4. Principal, Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, Karnataka, India.

Corresponding Author*
Paul Richards M
Professor & HOD
Department of Pharmaceutical Analysis
Aditya Bangalore Institute of Pharmacy Education and Research
Email: richie2626@gmail.com

Abstract

For the simultaneous measurement of Cobicistat in tablet dose form, a straightforward, accurate, and precise method was developed. Chromatogram was processed using Symmetry (C18) 250 x 4.6 mm, 5m. A 1ml/min flow of mobile phase, which was composed of Methanol and Water in the following proportions of 35:65. A constant buffer temperature of 30°C was used. For Cobicistat, a 247nm wavelength was optimal. Cobicistat's retention time was found to be 3.46 minutes, with RSD is 0.7% and 99.57% of the assay was achieved and 0.09 and 0.27 micrograms per ml are the values for LOD and LOQ were calculated via regression equations. The method created was straightforward and cost-effective, and retention durations were shortened as a result. The new approach was cost-effective and it could be used for routine quality control tests in industries because retention times and run time were decreased.

Keywords: *Cobicistat, RP-HPLC, Chromatogram, Assay and Retention time.*

1. Introduction

Cobicistat is approved for use in treating human immunodeficiency virus infection (HIV). When combined with other antiretroviral medications, cobicistat is used to increase the systemic exposure of atazanavir or darunavir for the treatment of HIV-1 infection.Cobicistat serves as a pharmacokinetic enhancer by inhibiting cytochrome P450 3A isoforms (CYP3A), despite the fact that it has no anti-HIV effect. As a result, it increases the systemic exposure of co-administered drugs that are processed by CYP3A enzymes. In order to treat HIV-1 infection more specifically, Cobicistat is advised to increase systemic exposure of atazanavir or darunavir (one daily dose schedule). Better treatment outcomes and a reduced side effect profile are possible when anti-retrovirals (ARVs) are exposed to the body more frequently without raising the dosage. Based up on the literature review it was found that little analytical methods such as (HPLC, HPTLC, GC and LC-MS, UV-Visible analysis) were reported for the determination of Cobicistat. The purpose of the developed method is to develop simple and accurate methods for the estimation of Cobicistat by RP-HPLC method in pharmaceutical dosage forms.

2. Materials and methods

2.1 Instruments: The present study was carried on HPLC (WATERS), which comes with an auto sampler injector with variable UV detector, High Precision Electronic Balance (SHIMADZU ATY224), Ultra Sonicator (Wensar wuc-2L) with Symmetry (C18) Column, 250 mm x 4.6 mm and 5µm particle size with pH Analyzer (ELICO) and Vaccum filtration Apparatus (BOROSIL).

2.2 Reagents & Chemicals: Cobicistat was obtained as gifted sample. Methanol, Acetonitrile is of HPLC grade and Triple distilled water was used. For the assay, Potassium dihydrogen orthophosphate, and Orthophosphoric acid were used.

2.3 Standard & sample preparation for UV-spectrophotometer analysis

A 10 ml volumetric flask was filled with 10 mg of the Cobicistat standard, which was then dissolved and made up to volume with mobile phase. Transferring 0.5 ml of the aforementioned solution into a 10 ml volumetric flask and bringing it up to volume with mobile phase served as a further dilution step. The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Cobicistat, so that the same wave number can be utilized in HPLC UV detector for estimating the Cobicistat. While scanning the Cobicistat solution we observed the maxima at 247 nm. The UV spectrum was recorded on ELICO SL-159 make UV-Visible spectrophotometer model UV-2450.

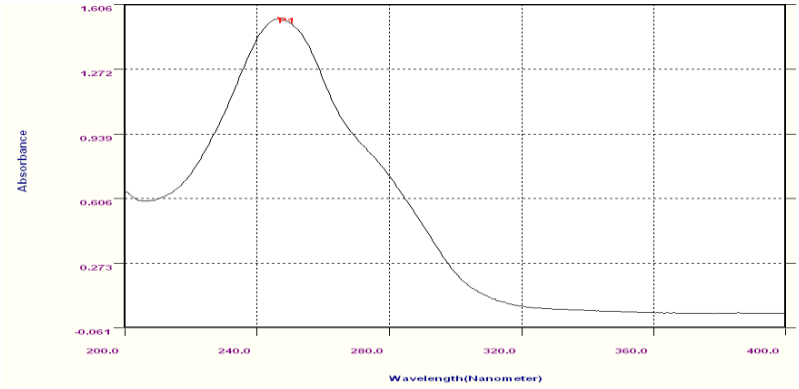

Figure. 1. Cobicistat UV spectrum

2.4 Mobile phase preparation: The methanol and HPLC water used in this estimation are combined in a 35:65 ratio as the mobile phase. A homogeneous solution was achieved after 350 ml of this methanol solution was added and well mixed with 650 ml of HPLC grade. Before being used in the experiment, this prepared mobile phase was filled and sonicated for 15 minutes.

2.5 Sample and Standard Preparation 10 mg of Cobicistat standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was complete by transferring 0.5ml of the above prepared solution into a 10 ml volumetric flask and make up to volume up to mark.

2.6 Optimization of Chromatographic Conditions: The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength and different diluents for sample preparation etc. The Optimum conditions obtained from experiments are Methanol (HPLC Grade) Water (Triple distilled) are used as mobile phase in the ratio of 35:65, wavelength as adjusted to 247, flow rate was 1.0 ml/min with a run time of 6 minutes using C18 column for the study. The retention time for Cobicistat was found to be 3.4 min.

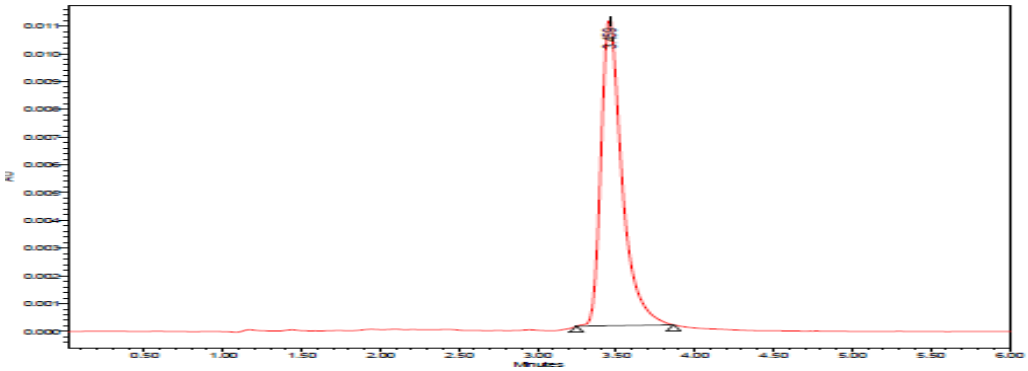

Figure. 2. Chromatogram of Cobicistat

3. Results and Discussion

3.2.1 Accuracy: To establish the accuracy of the developed method and the recovery studies 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.

Table. 1. Accuracy results of Cobicistat

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.D. = 1. 0.936122 % R.S.D.= 0.930493
S_2: 80 %	40	40.204	97322	100.51	
S_3: 80 %	40	39.888	96582	99.72	
S_4: 100 %	50	50.982	122563	101.964	Mean= 99.90667% S.D. = 1.781704 % R.S.D.= 1.783369
S_5: 100 %	50	49.440	108952	98.88	
S_6: 100 %	50	49.438	118948	98.876	
S_7: 120 %	60	59.94	143561	99.911	Mean= 99.868% S.D. = 1.177089 %R.S.D. = 1.178645
S_8: 120 %	60	59.202	141816	98.67	
S_9: 120 %	60	60.614	145123	101.023	

3.2.2 Precision:

Repeatability: The peak areas and retention periods obtained by real estimation of six replicates of a fixed dose of the medication Cobicistat were used to determine the precision of each approach individually (API). The following is a presentation of the percentage relative standard deviations for Cobicistat.

Table. 2. Repeatability results of Cobicistat

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 – 6	3.461	1036542
Average	3.462833	1036199
Standard Deviation	0.001169	747.1333
% RSD	0.03376	0.072103

3.2.2.1 Intermediate precision:

Intra-assay & inter-assay:

The intra & inter day variation 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.

Table. 3. Results of intra-assay & inter-assay of Cobicistat

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

3.3 Linearity & Range: The calibration curve showed good linearity in the range of 0-70 µg/ml, for Cobicistat (API) with correlation coefficient (R^2) of 0.999. A typical calibration curve has the regression equation of y = 26249x + 15500 for Cobicistat.

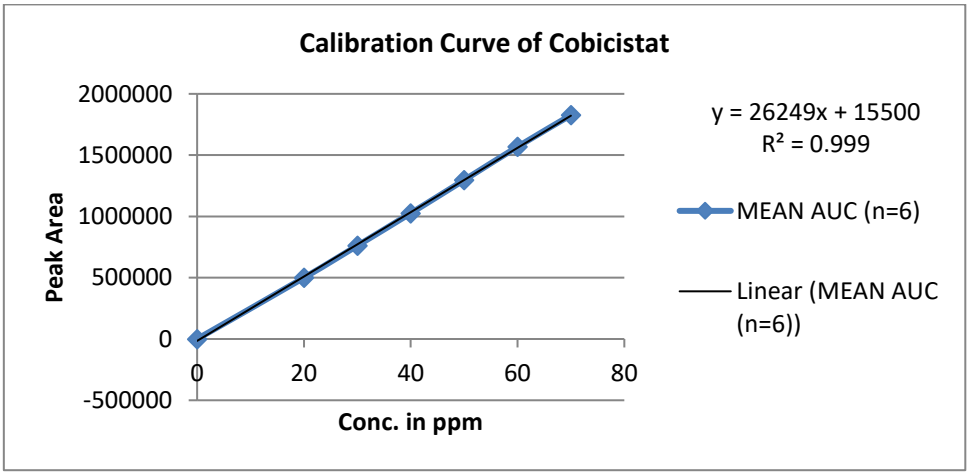

Figure. 3. Calibration curve of Cobicistat

Table. 4. Linearity Results Figure. 6. Calibration curve of Cobicistat (API)

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

3.4 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 were established. The data are shown below

Table. 5. System Suitability Parameter of Cobicistat

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

3.5 Robustness: The effect of minute changes in the optimized chromatographic conditions such as Temperature (±20C), Acetonitrile content in mobile phase (±2%), Wavelength of detection (±2nm) and change in flow rate (± 0.1ml/min) calculated to establish the robustness of the proposed method.

Table. 6. Robustness results of Cobicistat

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

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

3.7 Assay - Evaluation of Cobicistat (150 mg) in Pharmaceutical Dosage Form

20 tablets were taken and the I.P. method was followed to estimate the average weight. Finally, the above-weighed pills were pulverized and thoroughly triturated.. 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. 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 was recorded.

Table. 7. Assay results of 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)

3.9 Stability Studies

3.9.1 Acid Hydrolysis

Accurately weigh 25 mg of pure drug was transferred to a clean and dry 25 ml volumetric flask. To which 0.1 ml was put in to a 10 ml volumetric flask and made up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl. To which 0.1N Hydrochloric acid was added and made up to the mark and stored for 24 hours.

3.9.2 Base Hydrolysis

A 10 mg. dose of pure medication, precisely weighed, was put to a dry, spotless 10 ml volumetric flask. Which was then given 0.1N sodium hydroxide, adjusted to the proper strength, and maintained for 24 hours? From there, 0.1 ml was transferred to a 10 ml volumetric flask and diluted to the proper volume with mobile phase before being injected into the HPLC apparatus in comparison to a NaOH blank.

3.9.3 Thermal Degradation

1 milligrams of pure medication, precisely weighed, was put to a clean, dry 100 ml volumetric flask, made up to the appropriate level with mobile phase, and kept at 50°C for 24 hours then against a mobile phase blank, injected into the HPLC apparatus.**3.9.4 Photolytic Degradation**

In a tidy, dry Petri dish, 10 mg of pure medication were introduced. It was continuously stored in a UV cabinet operating at a 254 nm wavelength for a full day. One milligram of the UV-exposed medication, accurately weighed, was transferred to a clean, dry 10 ml volumetric flask. The UV-exposed medication was first dissolved in methanol and adjusted as needed. Then, against a blank of mobile phase, the resultant solution was introduced into the system.

3.9.5 Oxidation with (3%) H_2O_2

One milligram of the medication, accurately weighed, was put to a dry, clean, 100-milliliter volumetric flask. Then, to make it soluble, add 30 ml of 3% H2O2 and a small amount of methanol to it. Next, leave it alone in the dark for 24 hours. The volume was calibrated. The HPLC system was then filled with the resulting sample solution.

3.9.6 Results of studies on degradation: The results of the stress studies showed how specific the approach that was created was. Cobicistat remained stable under conditions of thermal and photolytic stress. The results of experiments on forced degradation are provided in the table below.

Table. 8. Results of forced degradation studies of Cobicistat

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1M HCl)	24Hrs.	87.85	12.15	100.0
Basic Hydrolysis	24Hrs.	75.64	24.36	100.0

(0.1M NaOH)				
Thermal Degradation (50^{o}C)	24Hrs.	96.55	3.45	100.0
UV (254nm)	24Hrs.	96.03	3.97	100.0
3 % Hydrogen peroxide	24Hrs.	25.39	74.68	100.0

4. Conclusion

For the examination of Cobicistat, a precise, linear, specific, and stability indicating RP-HPLC method was used. Various chromatographic conditions were applied, and the results observed are presented. Isocratic elution is straightforward, only needs one pump, and flat baseline separation for simple and repeatable results. Therefore, it was used for the current investigation instead of gradient elution. In the case of RP-HPLC, a variety of columns are available, but Symmetry (C18) Column, 250 mm x 4.6 mm column was recommended since using this column's peak shape, resolution, and absorbance were good. The best analysis was determined to be at a flow rate of 1 ml/min. The outcome demonstrates that the developed method is ideal for impurity studies and assays related to stability that can aid in the investigation of Cobicistat in various formulations.

5. References

1. Sigamala S. Kumar, Donthireddy Sai Priyanka and Paul Richards M., RP-HPLC method development and validation for simultaneous estimation of Cobicistat and darunavir in tablet dosage form, World Journal of Pharmacy and Pharmaceutical Sciences, Volume 5, Issue 6, 490-499.
2. Urooj Fatima, T. Mamatha1 and Rajesh Goud Gajula, A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form, Pelagia Research Library der Pharmacia Sinica, 2014, 5(5):99-105.
3. J. Sathish Kumar Reddy, K. R. S. Prasad1 and K. Suresh Babu, A stability indicating RP-HPLC method for simultaneous estimation of darunavir and Cobicistat in bulk and tablet dosage form, Scholars Research Library der Pharmacia Lettre, 2016, 8 (12):89-96.
4. S.H.Rizwan, V. Girija Sastry , Shaik Gazi , Q.Imad, Khatija Mohammed Bhameshan5, A New and Validated Stability Indicating RP-HPLC Analysis of Darunavir and Cobicistat in Bulk Drug and Tablet Dosage Form, International Journal of Pharmaceutical Sciences Review and Research, Int. J. Pharm. Sci. Rev. Res., 36(1), January – February 2016; Article No. 31, Pages: 180-185.
5. Sridhar Siddiraju, Stability indicating RP-HPLC method development and validation for the simultaneous estimation of darunavir and Cobicistat in pharmaceutical dosage form, International Conference and Exhibition on Advances in HPLC & Chromatography Techniques, March 14-15, 2016, Volume 7, Issue 2 (Suppl), page-69.
6. K.R. Gupta, A.D. Mahapatra, A.R. Wadodkar S.G. Wadodkar, Simultaneous UV Spectrophotometric Determination of Valsartan and Amlodipine in tablet International Journal of Chem Tech Research, Jan-Mar 2010; 2(1): 551-556.

7. Charushila HB, Shivanand NH. Stability indicating RP-HPLC method for the determination of Darunavir in bulk and dosage form. Drug Invention Today, 2013; 5: 81–86.
8. Reddy BV, Jyothi G, Reddy BS, Raman NV, Reddy KS, Rambabu C. Stability-indicating HPLC method for the determination of darunavir ethanolate. J Chromatogr Sci., 2013; 51: 471-476.
9. Tulsidas Mishra, Pranav SS. Validation of Simultaneous Quantitative Method of HIV Protease Inhibitors Darunavir, Darunavir and Ritonavir in Human Plasma by UPLC- MS/MS. The Scientific World Journal, 2014; 1-12.
10. Urooj Fatima, Mamatha T, and Rajesh Goud Gajula. A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form. Der Pharmacia Sinica, 2014; 5: 99-105.
11. D. Sindu Priya and D. Gowri Sankar Stability indicating RP-HPLC Method Development and Validation for Simultaneous Determination of Darunavir and Cobicistat in Bulk and Pharmaceutical Formulation, Am. J. PharmTech Res., 2015; 6(1).
12. Rowthula Prasanna Surya Bhavani, R. P. S. B., and M. M. M.Sandhya Maduri. "Stability indicating method development and validation for the simultaneous estimation of ledipasvir and sofosbuvir in bulk drug by using rp-hplc". World Journal of Current Medical and Pharmaceutical Research, Nov. 2020, pp. 307-18, doi:10.37022/wjcmpr.vi.159.
13. K.V.G., T. K, and S. P. P. "A New Stability Indicating Analytical Method Development and Validation for the Quantitative Determination of Emitricitabine and Lamivudine by RP-HPLC". World Journal of Current Medical and Pharmaceutical Research, Vol. 2, no. 2, May 2020, pp. 184-90, doi:10.37022/WJCMPR.2020.2219.
14. G.A. Shabir, "Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities between Validation Requirements of FDA, the US Pharmacopeia and the ICH," J. Chromatogr. A. 987(1-2), 57-66 (2003).
15. GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (PubMed-index for MEDLINE) validation of Cobicistat of bulk and pharmaceutical dosage form
16. Watnabe, M.et al. Synthesis and biological activity of methane sulfonamide pyramiding and N-methane sulfonyl pyrrole-substituted 3, 5-dihydroxy-heptonates, a novel series of HMG-CoA reductase inhibitors. Bioorg.Med.Chem. 5, 437-444, (1997).
17. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," Federal Register 62(96), 27463–27467 (1997).
18. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability," Federal Register (Notices) 65(169), 52776–52777 (2000).