EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 6
372-378
Year: 2021

# NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANIOUS ESTIMATION AND FORCED DEGRADATION STUDIES OF COBICISTAT AND ATAZANAVIR IN TABLET DOSAGE FORM

**K. Renuka*, P. Sunitha and Dr. Vijaya Kuchana**

M.Pharmacy, Dept. of Pharmaceutical Analysis, T.K.RCollege of pharmacy, Hyderabad India.

***Corresponding Author: K. Renuka**
M.Pharmacy, Dept. of Pharmaceutical Analysis, T.K.RCollege of pharmacy, Hyderabad India.

**Article Received on 24/03/2021** **Article Revised on 14/04/2021** **Article Accepted on 04/05/2021**

## ABSTRACT

**Objective**: A New method was established for simultaneous estimation of Cobicistat and Atazanavir by RP-HPLC method and Its force degradation study. **Methods**: Chromatographic separations were carried using inertsil (ODS 250 x 4.6 mm, 5.), column with a mobile phase composition of 0.1 M $Na_2HPO_4$ (45) and ACN (55%).have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 HPLC system with PDA detector at wavelength 270 nm. **Results**: The retention time for Cobicistat and Atazanavir were 1.678 and 3.895 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 50-150 µg/ml for Cobicistat and 50-150 µg/ml for Atazanavir respectively. For accuracy The total recovery was found to be 100.63 % and 101.79 % for Cobicistat and Atazanavir. LOD and LOQ for Cobicistat 1.32 and 4.01. LOD and LOQ for Atazanavir 0.40 and 1.22. Cobicistat and Atazanavir were subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Cobicistat and Atazanavir are more sensitive towards acidic and Thermal degradation. **Conclusion**: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of of Cobicistat and Atazanavir in tablet dosage form.

**KEYWORDS:** Cobicistat, Atazanavir, RP-HPLC, Simultaneous estimation.

## INTRODUCTION

Cobicistat, marketed under the name Tybost (formerly GS-9350), indicated for treating 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 coadministered agents 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 outcomes and a decreased side effect profile.[1] IUPAC name is (1,3-thiazol-5-yl)methylN-[(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.Molecular weight 776.03. Molecular formula $C_{40}H_{53}N_7O_5S_2$. The solubility of cobicistat is approximately 10 mg/ml in ethanol and 20 mg/ml in DMSO and DMF. Cobicistat is sparingly soluble in aqueous buffers.

Atazanavir (formerly known as BMS-232632) is an antiretroviral drug of the protease inhibitor (PI) class. Like other antiretrovirals, it is used to treat infection of human immunodeficiency virus (HIV). Atazanavir is distinguished from other PIs in that it can be given once-daily (rather than requiring multiple doses per day) and has lesser effects on the patient's lipid profile (the amounts of cholesterol and other fatty substances in the blood). Like other protease inhibitors, it is used only in combination with other HIV medications. Used in combination with other antiretroviral agents for the treatment of HIV-1 infection, as well as postexposure prophylaxis of HIV infection in individuals who have had occupational or nonoccupational exposure to potentially infectious body fluids of a person known to be infected with HIV when that exposure represents a substantial risk for HIV transmission[2-4]. IUPAC name is methyl N-[(1S)-1-{N'-[(2S,3S)-2-hydroxy-3-[(2S)-2-[(methoxycarbonyl)amino] -3,3-dimethylbutanamido] -4-phenylbutyl] -N'- { [4- (pyridin-2-yl) phenyl]methyl}

hydrazinecarbonyl} -2, 2-dimethylpropyl]carbamate. Molecular weight 704.8. Molecular formula $C_{38}H_{52}N_6O_7$.

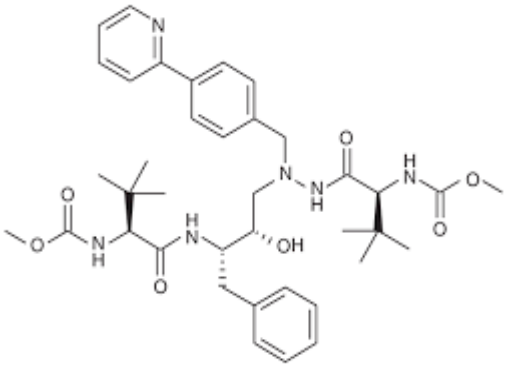

**Figure 1: Structure of Cobicistat.**

Atazanavir is soluble in organic solvents such as ethanol, DMSO.

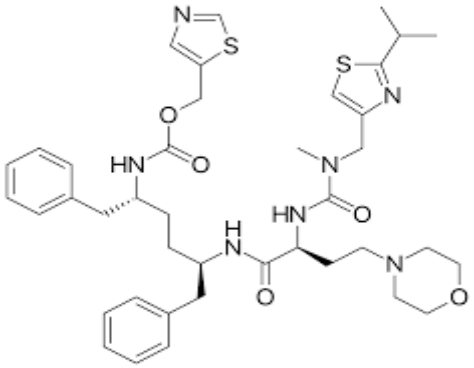

**Figure 2: Structure of Atazanavir.**

The review of literature revealed that very few methods are available for the determination of Atazanavir and Cobicistat individually and simultaneously. Reported methods are [5-14] reveals that different methods RP-HPLC, UV, LCMS for its analysis in formulations. The present study is to establish an apt stability indicating RP-HPLC method for evaluation of Cobicistat and Atazanavir in tablets and bulk form simultaneously and validate developed method as per frames and rules of ICHQ2 (R1).

## MATERIALS AND METHODS

**Chemicals and Reagents:** Cobicistat and Atazanavir were obtained from Sun Pharma India Limited, Hyderabad. $NaH_2PO_4$ was analytical grade supplied by Sd Fine-chem limited, Orthophosphoric acid (Sd Fine-chem limited), and Water for HPLC (Sd Fine-chem limited), Methanol for HPLC (Sd Fine-chem limited).

**Equipment and Chromatographic Conditions:** The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector and Empower 2 software. Analysis was carried out at 270 nm with column inertsil (ODS 250 x 4.6 mm, 5**)**, dimensions at $30^0$C temperature. The optimized mobile phase consists of 0.1 M $Na_2HPO_4$ (45%) and ACN (55%). Flow rate was maintained at 1 ml/min and run time for 6 min.

### Preparation of solutions

### Preparation of buffer

Pipette out 1ml of Ortho Phosphoric Acid was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.0 with NaOH.

### Preparation of mobile phase

Sodium dihydrogen phosphate (0.1M) and methanol blended in 60:40 volume/volume proportion. Orthophosphoric acid was utilized for pH (4.0) alteration. This blend was utilized as diluent, moreover, to make standard solutions.

### The diluents

The Mobile phase was used as the diluent.

### Preparation of standard stock solution

Accurately weigh 150 mg of Cobicistat and 300 mg of Atazanavir working standards and transfer into a 100 ml volumetric flask containing 60 mL of Diluent and sonicate to dissolve it completely. Make up the final volume to 100mL with diluent (Stock solution). Pipette out 1.5 ml of the stock solution into a 10ml volumetric flask and make up to the mark with diluent.

.

### Preparation of Sample stock solution

Accurately weigh 150 mg of Cobicistat and 300 mg Atazanavir equivalent tablet powder and transfer into a 100mL volumetric flask containing 60 mL of diluent and sonicate it for 30 mins to dissolve the drugs completely. Make up the final volume to 100mL with diluent (Stock solution). After filtering the solution pipette out 1.5 ml of the stock solution into a 10ml volumetric flask and make up to the mark with diluent

**Procedure:** 10 µL of standard and sample solutions were injected into the LC-system and measure the peak areas for Cobicistat and Atazanavir.

## METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

**System suitability parameters:** To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into inertsil (ODS 250 x 4.6 mm, 5.), the mobile phase of composition Buffer and Acetonitrile taken in the ratio (45:55)was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor

and USP theoretical plate count of the developed method are shown in table 1.

**Assay of pharmaceutical formulation:** The proposed validated method was successfully applied to determine Cobicistat and Atazanavir in their tablet dosage form. The result obtained for Cobicistat and Atazanavir was comparable with the corresponding labeled amounts and they were shown in Table-2.

**Validation of Analytical method**

**Linearity and Range:** Stock solution was prepared by dissolving the appropriate amount of Cobicistat and Atazanavir in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 75 µg/ml to 225 µg/ml of Cobicistat and 50 µg/ml to 150 µg/ml of Atazanavir. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

**Accuracy studies:** The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Cobicistat and Atazanavir and calculate the individual recovery and mean recovery values. The resulte are shown in table 4,5.

**Precision Studies:** precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

**Ruggedness:** To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 7.

**Robustness:** As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The Organic composition in the Mobile phase was varied from 35% to 45%. The resulte are shown in table 8.

**LOD and LOQ:** The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 9.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

**Forced degradation studies**

**Acid degradation condition**

Acid hydrolysis was performed by treating 10 ml of Cobicistat and Atazanavir tablet stock solution (1500 µg/ml Cobicistat and 1000 µg/ml Atazanavir) with 10 ml of 0.1 N HCI for 30 min with sonication at room temperature. 0.1 N NaOH was sufficiently added to neutralize the sample. Degradation sample was diluted in 100 ml volumetric flask using mobile phase to procure a final concentration of 150 µg/ml Cobicistat and 100 µg/ml Atazanavir. The stressed sample was quantified beside the Cobicistat and Atazanavir reference standard

**Alkali degradation condition**

Alkali hydrolysis was performed by treating 10 ml of Cobicistat and Atazanavir tablet stock solution (1500 µg/ml Cobicistat and 1000 µg/ml Atazanavir) with 10 ml of 0.1 N NaOH for 30 min with sonication at room temperature. 0.1 N HCl was sufficiently added to neutralize the sample. Degradation sample was diluted in 100 ml volumetric flask using mobile phase to procure a final concentration of 150 µg/ml Cobicistat and 100 µg/ml Atazanavir. The stressed sample was quantified beside the Cobicistat and Atazanavir reference standard

**Thermal-induced degradation condition**

Thermal degradation was performed by exposing 10 ml of Cobicistat and Atazanavir tablet stock solution (1500 µg/ml Cobicistat and 1000 µg/ml Atazanavir) to 105ºC in hot oven. Cooled the sample. Degradation sample was diluted in 100 ml volumetric flask using mobile phase to procure a final concentration of 150 µg/ml Cobicistat and 100 µg/ml Atazanavir. The stressed sample was quantified beside the Cobicistat and Atazanavir reference standard.

**Photolytic degradation condition**

Accurately 10 ml of stock sample was exposed to sunlight for about 6 h and then the sample diluted with 5 ml of mobile phase and percentage of degradation

**Oxidative degradation condition**

Peroxide mediated hydrolysis was performed by treating 10 ml of Cobicistat and Atazanavir tablet stock solution (1500 µg/ml Cobicistat and 1000 µg/ml Atazanavir) with 10 ml of 30% hydrogen peroxide for 30 min with sonication at room temperature. Degradation sample was diluted in 100 ml volumetric flask using mobile phase to procure a final concentration of 150 µg/ml Cobicistat and 100 µg/ml Atazanavir. The stressed sample was quantified beside the Cobicistat and Atazanavir reference standard.

Force degradation results are shown in table 10,11

## RESULTS AND DISCUSSION

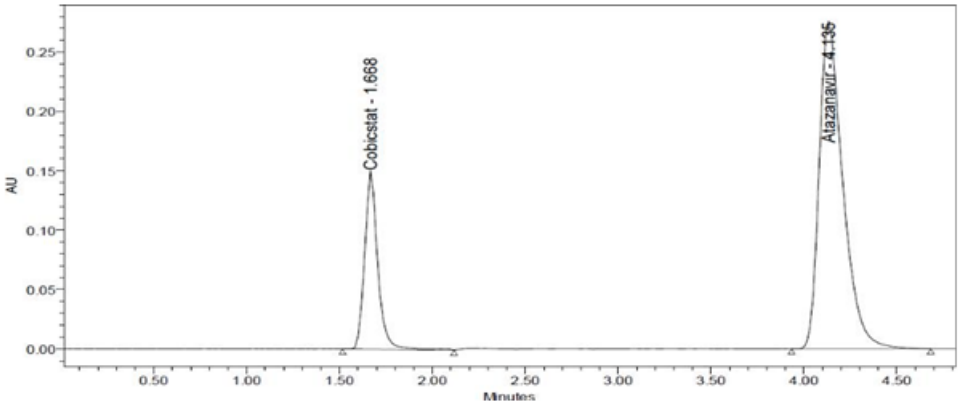


**Figure 3: Standard chromatogram.**

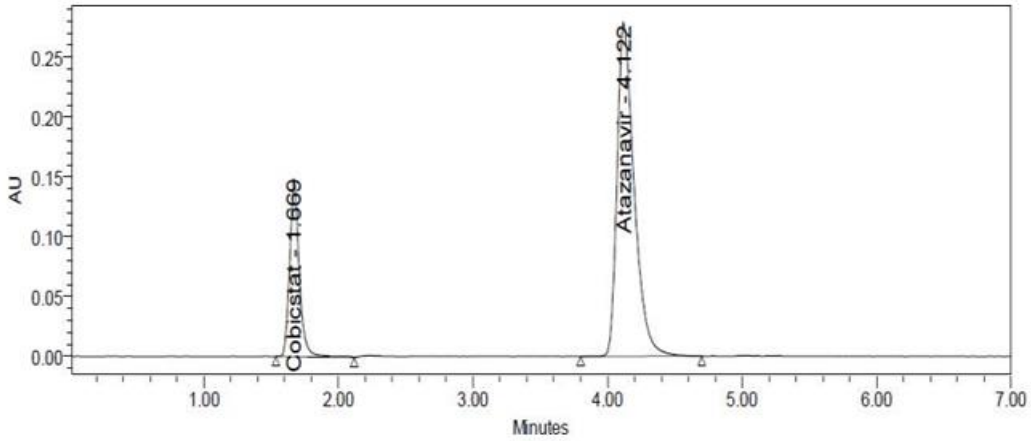


**Figure 4: Sample chromatogram**

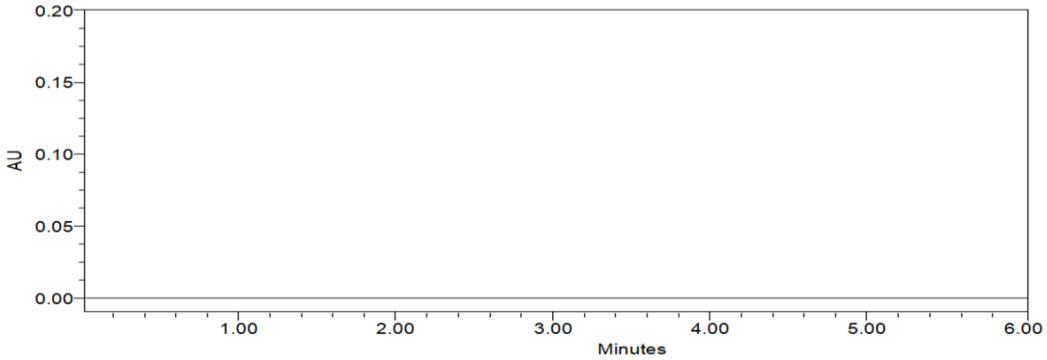


**Figure 5: Blank chromatogram.**

**Table 1: System suitability parameters.**

| Parameters | Cobicistat | Atazanavir |
|---|---|---|
| Retention time | 2.202 | 3.301 |
| USP Plate count | 5986 | 7522 |
| USP Tailing | 1.39 | 1.30 |

**Table 2: Assay results for Cobicistat and Atazanavir.**

| | Label Claim (mg) | % Assay |
|---|---|---|
| **Cobicistat** | 150 | 101.03 |
| **Atazanavir** | 300 | 99.95 |

**Table 3: Linearity results for Cobicistat and Atazanavir**

| S.no | Concentration Cobicistat (µg/ml) | Response | Concentration Atazanavir (µg/ml) | Response |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 2 | 37.5 | 191498 | 6.25 | 225114 |
| 3 | 75 | 390010 | 12.5 | 414984 |
| 4 | 112.5 | 546140 | 18.75 | 622694 |
| 5 | 150 | 738926 | 25 | 837022 |
| 6 | 187.5 | 946014 | 31.25 | 1049477 |
| 7 | 225 | 1125330 | 37.5 | 1273064 |

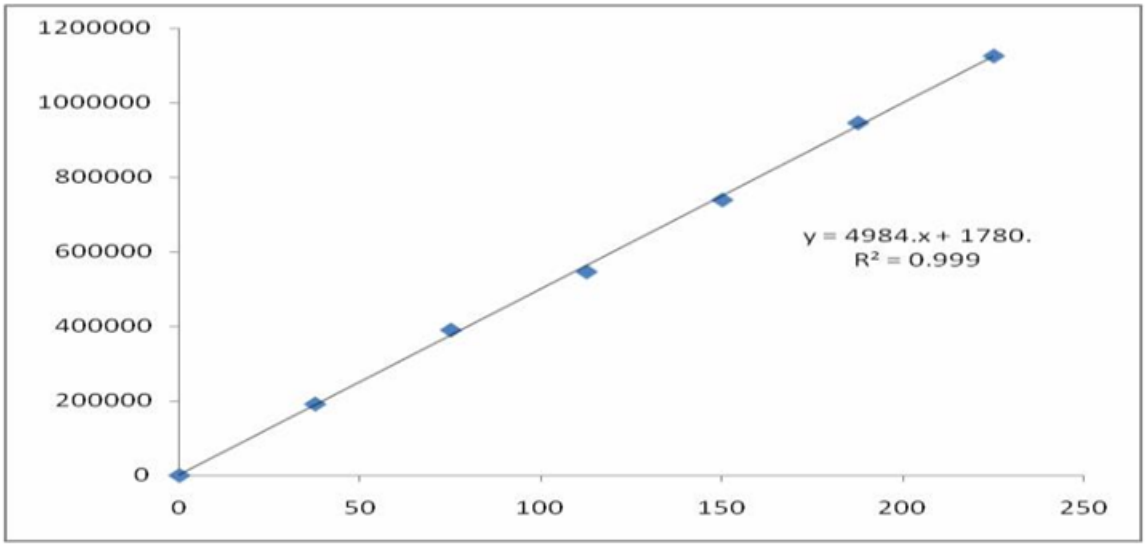


**Figure 4: Linearity graph for Cobicistat.**

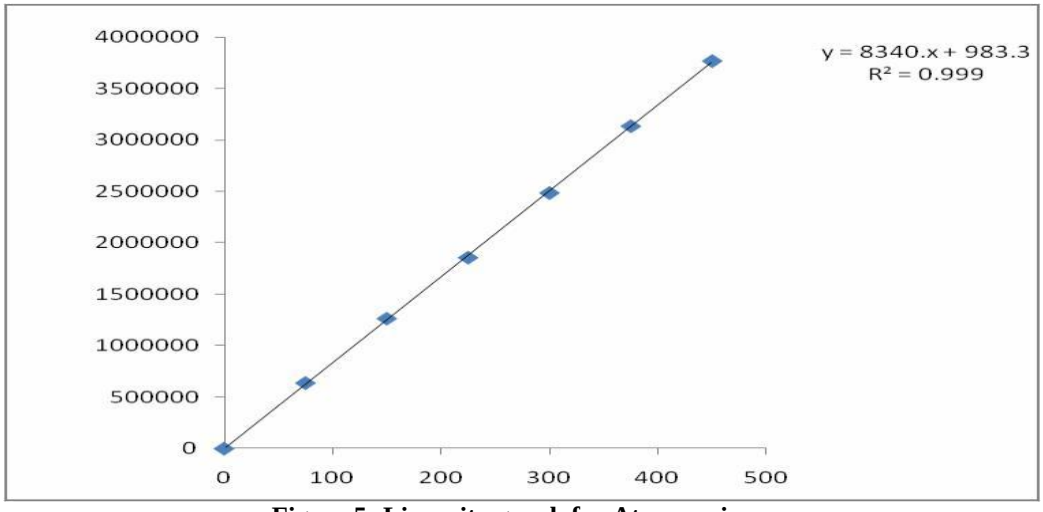


**Figure 5: Linearity graph for Atazanavir.**

**Table 4: Showing accuracy results for Cobicistat**

| %Concentration (at specification Level) | Area | Amount Added (µg/ml) | Amount Found (µg/ml) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 47085 | 75 | 74 | 99.12 | 99.18 |
| 100% | 94332 | 150 | 149 | 99.22 | |
| 150% | 140531 | 225 | 224 | 199.24 | |

**Table 5: Showing accuracy results for Atazanavir.**

| %Concentration (at specification Level) | Area | Amount Added (µg/ml) | Amount Found (µg/ml) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 4224 | 150 | 151 | 100.16 | 100.18 |
| 100% | 8503 | 300 | 303 | 100.08 | |
| 150% | 12734 | 450 | 455 | 100.32 | |

**Table 6: Precision results for Cobicistat and Atazanavir.**

| Sr. No. | Cobicistat | Atazanavir |
|---|---|---|
| 1 | 718678 | 2466350 |
| 2 | 715658 | 2466490 |
| 3 | 738088 | 2456197 |
| 4 | 740141 | 2453992 |
| 5 | 739309 | 2453388 |
| 6 | 740816 | 2447690 |
| Mean | 732115 | 2457351 |
| Std. Dev. | 11652.8 | 7565 |
| %RSD | 1.592 | 0.3 |

**Table 7: Intermediate precision resultes for Cobicistat and Atazanavir.**

| Sr. No. | Cobicistat | Atazanavir |
|---|---|---|
| 1 | 736679 | 2470266 |
| 2 | 738088 | 2463991 |
| 3 | 747936 | 2460592 |
| 4 | 739309 | 2454003 |
| 5 | 721714 | 2455955 |
| Mean | 737722 | 2461678.5 |
| Std. Dev. | 8811.1 | 6079 |
| %RSD | 1.19 | 0.2 |

**Table 8: Robustness results for Cobicistat and Atazanavir.**

| S.NO | Robustness condition | Cobicistat %RSD | Atazanavir %RSD |
|---|---|---|---|
| 1 | Flow minus | 0.8 | 1.3 |
| 2 | Flow Plus | 0.2 | 0.4 |
| 3 | Mobile phase minus | 0.2 | 2.5 |
| 4 | Mobile phase Plus | 0.1 | 0.7 |
| 5 | Temperature minus | 0.3 | 2.5 |
| 6 | Temperature Plus | 0.6 | 0.4 |

**Table 9: LOD, LOQ of Cobicistat and Atazanavir.**

| Drug | LOD | LOQ |
|---|---|---|
| **Cobicistat** | 1.32 | 4.01 |
| **Atazanavir** | 0.40 | 1.22 |

**Table 10: Degradation results for Cobicistat.**

| Sample Name | Cobicistat | | |
|---|---|---|---|
| | %Assay | % Degraded | Peak purity |
| **Acid** | 96.88 | 3.12 | Passes |
| **Base** | 97.40 | 2.60 | Passes |
| **Peroxide** | 94.35 | 5.65 | Passes |
| **Thermal** | 99.60 | 0.40 | Passes |
| **Photo** | 99.13 | 0.87 | Passes |

**Table 11: Degradation results for Atazanavir.**

| Sample Name | Atazanavir | | |
|---|---|---|---|
| | %Assay | % Degraded | Peak purity |
| **Acid** | 96.89 | 3.11 | Passes |
| **Base** | 97.92 | 2.08 | Passes |
| **Peroxide** | 96.62 | 3.38 | Passes |
| **Thermal** | 99.57 | 0.43 | Passes |
| **Photo** | 99.59 | 0.41 | Passes |

## CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Cobicistat and Atazanavir in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Cobicistat and Atazanavir in tablet dosage forms.

## REFERENCES


1. Lepist EI, Phan TK, Roy A, Tong L, Maclennan K, Murray B, Ray AS: Cobicistat boosts the intestinal absorption of transport substrates, including HIV protease inhibitors and GS-7340, in vitro. Antimicrob Agents Chemother, 2012; 56(10): 5409-13.
2. Dierynck I, De Wit M, Gustin E, Keuleers I, Vandersmissen J, Hallenberger S, Hertogs K: Binding kinetics of darunavir to human immunodeficiency virus type 1 protease explain the potent antiviral activity and high genetic barrier. J Virol, 2007; 81(24):13845-51. Epub 2007 Oct 10. [PubMed:17928344]
3. Dandache S, Sevigny G, Yelle J, Stranix BR, Parkin N, Schapiro JM, Wainberg MA, Wu JJ: In vitro antiviral activity and cross-resistance profile of PL-100, a novel protease inhibitor of human immunodeficiency virus type 1. Antimicrob Agents Chemother, 2007; 51(11): 4036-43. Epub 2007 Jul 16. [PubMed:17638694]
4. Wood R: Atazanavir: its role in HIV treatment. Expert Rev Anti Infect Ther, 2008; 6(6): 785-96.
5. Anupama A, Vishvanath A, Babu PS, Sasidhar R. Development of novel and simple analytical method for the estimation of atazanavir sulphate in pharmaceutical formulation by RP-HPLC; IJRPC, 2013; 3(3): 645-649.
6. Padmalata M, Prakash KV, Dopadally E. Validated, reversed phase high performance liquid chromatography method for the estimation of atazanavir sulfate in pharmaceutical formulations. Oriental Journal of Chemistry, 2010; 26(1): 123-127.
7. Konidala S, Sujana K, Rani AP. New validated RP-HPLC method for the determination of atazanavir sulphate in bulk and dosage form. Der Pharma Chemica, 2012; 4(3): 1305-1310.
8. Tiyyagura A, Gunda A, Chitturi A, Sai A, Laxmi A. Method development and validation for the simultaneous estimation of atazanavir and ritonavir in pharmaceutical dosage form by RP-HPLC. International Journal of Pharmaceutical, Chemical and Biological Sciences, 2012; 3(1): 44-54.
9. Venktesh J, Chowdhary S, Haritha D, Anuroop. Reverse phase high performance liquid chromatographic estimation of atazanavir and ritonavir in pharmaceutical dosage form. Pharmatutor-pharma infopedia.
10. Fatima U, Mahatma T, Gajula R. A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form. Der Pharmacia Sinica, 2014, 5(5):99-105
11. Ganji S, Satyavati D. Development and validation of RP-HPLC method for the analysis of Cobicistat and related impurities in bulk and pharmaceutical dosage forms. Asian Pharma Press, 2015; 5(1), 1-8.
12. Kalyani K, Anuradha V. A stability-indicating high performance liquid chromatographic method for the determination of Cobicistat. IJPDA, 2015; 4(3): 117-125.
13. Khaleel N, Rahman A. A validated stability indicating rp-hplc method for simultaneous estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir in bulk and pharmaceutical dosage form. International Journal of Pharmacy, 2015; 5(3): 991-1002
14. Jampala RR, Kumar VK, Neemala AR. Development and application of liquid chromatographic method for simultaneous determination of elvitegravir, tenofovir disoproxil fumarate, emtricitabine, and cobicistat in fixed dosage form. Pharmaceutical Methods, 2014; 5(1): 7-13.