*Int. J. of Med. Sc. & Pharm. Res., 2017*

International Journal of Medical Sciences and Pharma Research

*ISSN 2394-8973 www.ijmspr.com*
*Vol. 3, No. 1, February 2017*

***Research Paper***

# RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF COBICISTAT AND DARUNAVIR IN BULK AND PHARMACEUTICAL DOSAGE FORM

**T Sankarshana[1]* and Md. Musthafa[2]**

**Corresponding Author:* ***T Sankarshana*** ✉ *tsankarshana@yahoo.com*

---

A stability indicating RP-HPLC method has been developed and subsequently validated for the simultaneous estimation of cobicistat and darunavir in API and tablet dosage form. Optimized chromatographic condition were achieved by Eclipse XBD-C18(250mm x 4.6mm, 5µ) column, mobile phase as a mixture of buffer (0.01M potassium dihydrogen phosphate, pH-3.0): acetonitril in the ratio of 40:60v/v with a flow rate of 1ml/min., UV detection was performed at 240nm and the sample temperature was maintained ambient which was injected manually. This developed method for simultaneous estimation of cobicistat and darunavir is linear over a range of 40 – 120 % and 40 -120 % respectively. The validation parameters as per ICH guide lines show good precision having 0.066 and 0.039 %RSD for cobicistat and darunavir which shows nice repeatability, limit of detection and limit of quantification for cobicistat and darunavir was found to be 0.02 and 0.06 and 0.02 and 0.06 % respectively. The developed method is easy to perform assay for indicating quality control determinations in bulk and formulated form with rapid, selective and stability indicating.

***Keywords:*** RP-HPLC, Cobicistat, Darunavir, Prezcobix, Validation

## INTRODUCTION

### Cobicistat

Cobicistat Systematic (IUPAC) name was given as 1,3-Thiazole-5-ylmethy [(2R,5R)-5-{[(2S)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino}-4-(morpholin-4-yl)butanoyl]amino}-1,6-diphenylhexane-2-yl]carbamate. Its molecular formula is $C_{40}H_{53}N_7O_5S_2$ and molecular mass is 776.023 g/mol[1]. Cobicistat is adsorbed onto silicon dioxide. Cobicistat on silicon dioxide is a white to pale yellow solid with a solubility of 0.1 mg/ml in water

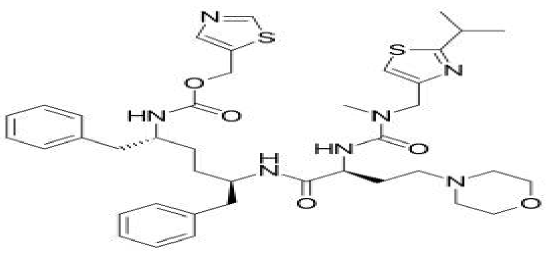
---

[1] Osmania University, Department of Chemistry, Hyderabad, Telangana.

[2] Osmania University, Faculty of pharmacy, University College of Pharmacy, Hyderabad.

at 20 °C drug shows reduced liability for drug interactions and may have potential improvements in tolerability over ritonavir. In addition it has high aqueous solubility and can be readily co formulated with other agents.

**Figure 1: Molecular Structure of Cobicistat**

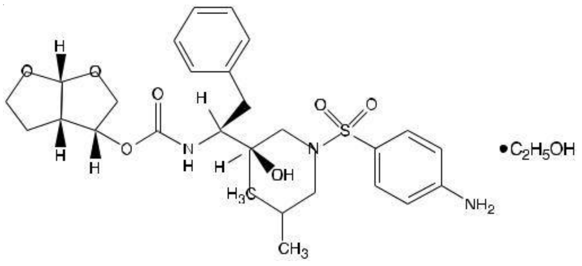


Cobisistat is an anti-retroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Cobisistat is frequently prescribed with highly active antiretroviral therapy, not for its antiretroviral action, but as it inhibits the same host enzyme that metabolizes other protease inhibitors. This inhibition leads to higher plasma concentrations of these latter drugs, allowing the clinician to lower their dose and frequency and improving their clinical efficacy. It also inhibits intestinal transport proteins, increasing the overall absorption of several HIV medications, including tenofovir and alafenamide. From the literature survey, it was found that Cobicistat was estimated by analytical methods such as spectrophotometric method, HPLC method and HPTLC method.

## Darunavir

Darunavir[3-5], in the form of darunavir ethanolate, has the following chemical name: [(1S,2R)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ester monoethanolate. Its molecular formula is C27H37N3O7S • C2H5OH and its molecular weight is 593.73.

**Figure 2: Molecular Structure of Darunavir**



A few spectroscopic and liquid chromatographic procedures have been reported for the estimation of Darunavir and Cobicistat individually, but there is no method for simultaneous estimation by RP-HPLC. Therefore there is a need to develop rapid and reliable method and validated for simultaneous estimation of combined dosage form and API (Active Pharmaceutical Ingredient) [6-11].

## EXPERIMENTAL METHODOLOGY

Instrumentation: Waters Alliance 2695 separation module (Waters Corporation, Milford, USA) equipped with 2998 UV detector with Empower-2 software was used for the analysis. The HPLC system was equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower-2 chromatography software. Eclipse XDB-C18 (250 mm x 4.6 mm I.D; 5 µm) was used as stationary phase. Solubility of the compound was enhanced by sonication. All the weights in the experiments were done with Essea model: AJ220 Digital Electronic Balance.

Chemicals and Reagents: The reference samples of cobicistat & darunavir were obtained from rainbow laboratories Ltd., Hyderabad. Purified water was prepared by using Milli-Q water purification system. HPLC grade methanol (Merck, Mumbai), which was used for preparing dilutions and mobile phase. Analytical grade potassium di-hydrogen phosphate 0.01M, pH 3.0 was adjusted with dilute orthophosphoric acid (buffer) was obtained from Rankem Fine Chemicals Ltd., New-Delhi. Prezcobix, a formulation containing 150 mg of cobicistat and 800 mg darunavir was purchased from local market.

Preparation of standard solution: The standard solution was prepared by dissolving 150mg of cobicistat and 800mg of darunavir of working standard into a 100ml volumetric flask, dissolve and dilute to volume with diluent (water : acetonitril in ratio of 50:50). Pipette out 5ml into 50ml volumetric flask with and dissolve with diluents to get the working prepared standard solutions as 150 µg/ml and 800 µg/ml respectively. This prepared standard was injected and chromatogram was recorded at 240nm.

Preparation of sample solution: Twenty tablets were weighed and average weight was determined. Tablet powdered equivalent to cobicistat 150mg and darunavir 800mg (Avg. wt is 1100.5mg) of Prezcobix formulation transferred into a 100 ml standard volumetric flask, dissolve with diluent. Solution was ultra-sonicated for 15min., filtered through Whatman filter paper No. 42. Filtrate was diluted upto the mark with diluent to obtain final concentration pipette out 5 ml into 50 ml standard volumetric flask dissolve with diluent, this prepared sample (20 µl) was injected and chromatogram was recorded at 240nm. Content of drugs in sample solution was calculated by comparing mean peak area of sample with that of the standard. The typical chromatogram of cobicistat and darunavir in tablet dosage form.

## METHOD DEVELOPMENT

Binary mixture of potassium di-hydrogen phosphate (0.001M) in water adjust pH 3.0 with dilute ortho phosphoric acid (solvent-A): acetonitrile (solvent –B) in 40:60 v/v proportions in isocratic mode of elution was used as mobile phase. The resultant solution was thoroughly mixed and filtered (poly-tetra-fluoro ethanol (PTFE) filter of 0.45 µm pore size) using vacuum pump and degassed by sonication to expel the dissolved gases in solvent system. The flow rate of mobile phase was adjusted at 1.0 mL/min and 20 ìL solutions as injection volume were maintained. The eluted compounds were monitored at 240 nm by using UV detector. The column oven temperature was maintained at 30$^{\%}$C. Data acquisition, analysis, and reporting was performed by LC solution Software found to be an efficient system for elution of drug with good peak shape as well as retention time 2.979 min. and 4.260 min. for cobicistat and darunavir respectively with baseline stability.

**Table 1: Results of Assay from Tablet Dosage Form**

| S.No. | Drug | Label Claim (mg) | Amount Found (mg) | Recovery (%) |
|---|---|---|---|---|
| 1. | Cobicistat | 150 | 150.09 | 100.06 |
| 2. | Darunavir | 800 | 780.08 | 97.60 |

## METHOD VALIDATION

As per (ICH) International Conference on Harmonization guidelines, the method validation parameters such as specificity, linearity, precision, accuracy, LOD, LOQ and robustness were optimized[13&14].

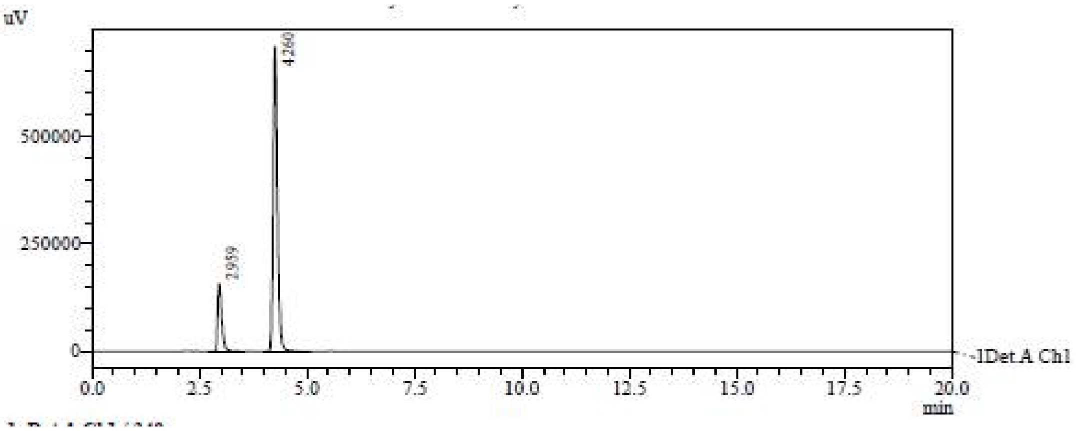
a) Specificity: Specificity is the extension to which the procedure applies to analyte of interest and is checked by examining the formulation sample for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in the formulation did not interfere with the drug peak and thus the method is specific. The HPLC chromatogram recorded for the drug matrix (mixture of the drug and excipients) showed almost no interfering peaks within retention time ranges. Figure 2 showed the representative chromatograms for standard and the dosage form. The figure describes that the selected drug was clearly separated and the proposed HPLC method is selective.

b) Linearity: To establish linearity, the stock solutions were prepared as 1000 µg/ml using mobile phase, from the stock solution further dilutions were prepared in the concentration range of 600 - 100 µg/ml, elution's are made on HPLC by injecting 20 µg/ml of each concentration repeats it for two times. The coefficient of determination and regression coefficient ($R^2$) was obtained and shown in the table 2&3 and Figures 5 and 6.

***Acceptance Criteria:*** Correlation coefficient ($r^2$) should be not less than 0.999.

c) Precision: The intraday and inter-day precision was determined by analyzing cobicistat (150 µg/ml) and darunavir (300 µg/ml) for six times on same day (intra-day) and repeated on the second day (inter-day) studies were given the

**Figure 2: Chromatogram of Standard Solution of Cobicistat and Darunavir**



| Peak | Rt (min.) | Name | Area | % Area | Theoretical Plate | Tailing Factor | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | 2.959 | Cobicistat | 1080796 | 17.647 | 4011.857 | 1.659 | 0.000 |
| 2 | 4.260 | Darunavir | 5043880 | 82.353 | 7641.953 | 1.321 | 6.814 |
| **Total** | | | **6124676** | **100.00** | | | |

**Table 2: Linearity Results for Cobicistat**

| Concentration of Drug (µg/mL) | Retention Time (min.) | Peak Area |
|---|---|---|
| 40 | 2.993 | 453186 |
| 60 | 2.989 | 644079 |
| 80 | 2.982 | 853653 |
| 100 | 2.978 | 1092304 |
| 120 | 2.968 | 1331124 |

**Table 3: Linearity Results for Darunavir**

| Concentration of Drug (µg/mL) | Retention Time (min.) | Peak Area |
|---|---|---|
| 40 | 4.266 | 2251396 |
| 60 | 4.268 | 3155929 |
| 80 | 4.267 | 4062455 |
| 100 | 4.271 | 5086904 |
| 120 | 4.269 | 6089876 |

**Table 4: Inter-day Precision for Cobicistat**

| Inj | Retention Time (min) | Peak Area |
|---|---|---|
| 1 | 2.967 | 1067096 |
| 2 | 2.965 | 1069882 |
| 3 | 2.966 | 1068209 |
| 4 | 2.967 | 1067920 |
| 5 | 2.966 | 1068921 |
| 6 | 2.971 | 1068140 |
| Mean | 2.967 | 1068361 |
| Std.Dev | 0.002 | 948 |
| %RSD | 0.066 | 0.089 |

Tables 4 and 5 for Cobicistat and 6 and 7 for Darunavir.

**Figure 5: Calibration Curve of Cobicistat**

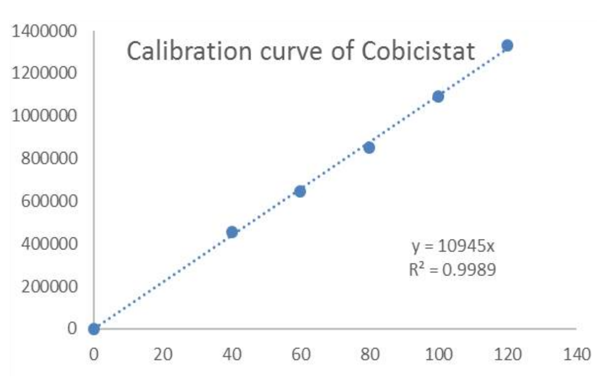


**Figure 6: Calibration Curve of Darunavir**

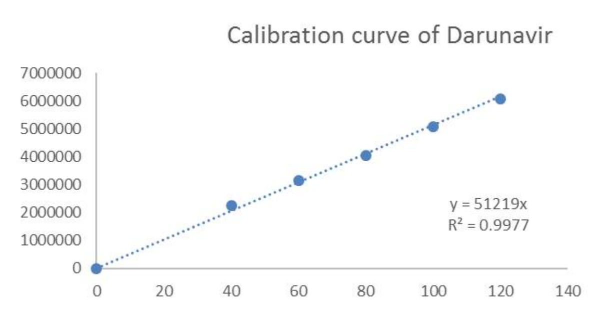


**Table 5: Intra-day Precision for Cobicistat**

| Inj | Retention Time (min) | Peak Area |
|---|---|---|
| 1 | 2.965 | 1075615 |
| 2 | 2.966 | 1068499 |
| 3 | 2.964 | 1070863 |
| 4 | 2.966 | 1069530 |
| 5 | 2.967 | 1044376 |
| 6 | 2.967 | 1069655 |
| Mean | 2.966 | 1066423 |
| Std.Dev | 0.001 | 11087 |
| %RSD | 0.039 | 1.040 |

d) Accuracy: The accuracy of the method shall be demonstrated through determination on samples in three concentrations from 120% , 100%, and 80% three replicates of each of

**Table 6: Inter-day Precision for Darunavir**

| Inj | Retention Time (min) | Peak Area |
|---|---|---|
| 1 | 4.258 | 5073355 |
| 2 | 4.259 | 5073719 |
| 3 | 4.261 | 5086606 |
| 4 | 4.265 | 5093012 |
| 5 | 4.264 | 5100941 |
| 6 | 4.271 | 5106060 |
| Mean | 4.263 | 5088949 |
| Std.Dev | 0.005 | 13666 |
| %RSD | 0.108 | 0.269 |

**Table 7: Intra-day Precision for Darunavir**

| Inj | Retention Time (min) | Peak Area |
|---|---|---|
| 1 | 4.265 | 5094551 |
| 2 | 4.267 | 5123695 |
| 3 | 4.265 | 5098612 |
| 4 | 4.266 | 5102669 |
| 5 | 4.266 | 5090259 |
| 6 | 4.265 | 5112281 |
| Mean | 4.266 | 5103678 |
| Std.Dev | 0.001 | 12367 |
| %RSD | 0.017 | 0.242 |

the theoretical concentrations employed as per the usual procedure and the results are summarized in Tables 8 and 9.

***Acceptance Criteria:*** The mean % recovery at each level should be NLT 98.0% & NMT 102.0%.

e) **Limit of detection (LOD) and limit of quantification (LOQ):** A series of 11 replicate concentrations were analyzed and quantified. Set up the described chromatographic conditions and allow the system to equilibrate. Starting with concentration 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02% and 0.01% peak area values were given in the Table 10 for Cobicistat and darunavir.

e) Robustness: The robustness of the method was determined as per USP guidelines, under different conditions including change in flow rate, different column, pH of buffer, and buffer concentration. The results obtained by deliberately variation in method parameters and data are summarized in Table 11.

**Table 8: Accuracy Results for Cobicistat**

| S.No. | Recovery at 120 µg/ml (80%) Dilution Level Peak Areas | | Recovery at 150 µg/ml (100%) Dilution Level Peak Areas | | Recovery at 180 µg/ml (120%) Dilution Level Peak Areas | |
|---|---|---|---|---|---|---|
| | Standard | Spiked (10%) | Standard | Spiked (10%) | Standard | Spiked (10%) |
| 1 | 844435 | 971235 | 1087081 | 1197395 | 1322505 | 1444310 |
| 2 | 849927 | 971232 | 1091089 | 1199754 | 1323229 | 1442036 |
| 3 | 849915 | 969436 | 1090560 | 1197458 | 1322153 | 1441607 |
| Mean | 848092 | 970634 | 1089577 | 1198202 | 1322629 | 1442651 |
| Std.Dev | 3167 | 1037.79 | 2177 | 1344 | 549 | 1452.66 |
| %RSD | 0.373 | 0.107 | 0.200 | 0.112 | 0.041 | 0.101 |
| **% Recovery** | **116.20** | | **100.6** | | **109.10** | |
| **Average % Recovery -108.63** | | | | | | |

**Table 9: Accuracy Results for Darunavir**

| S.No. | Recovery at 80% Dilution Level Peak areas | | Recovery at 100% Dilution Level Peak areas | | Recovery at 120% Dilution Level Peak areas | |
|---|---|---|---|---|---|---|
| | **Standard** | **Spiked (10%)** | **Standard** | **Spiked (10%)** | **Standard** | **Spiked (10%)** |
| 1 | 4061180 | 4580420 | 5084776 | 5575802 | 6062373 | 6572822 |
| 2 | 4055456 | 4580106 | 5076211 | 5578826 | 6061037 | 6564472 |
| 3 | 4056214 | 4576846 | 5085511 | 5584140 | 6053013 | 6564525 |
| Mean | 4057617 | 4579124 | 5082166 | 5579589 | 6058807.7 | 6567273 |
| Std.Dev | 3109 | 1979 | 5170 | 4221 | 5063 | 4806 |
| %RSD | 0.077 | 0.043 | 0.102 | 0.076 | 0.084 | 0.073 |
| % Recovery | 102.80 | 97.60 | 100.30 | | | |
| **Average % Recovery -100.23** | | | | | | |

**Table 10: LOD and LOQ Values for Prezcobix**

| S.No. | % Concentration | Peak Area | |
|---|---|---|---|
| | | **Cobicistat** | **Darunavir** |
| 1 | 20 | 222098 | 1161716 |
| 2 | 10 | 113777 | 597142 |
| 3 | 05 | 56285 | 310533 |
| 4 | 02 | 23274 | 125347 |
| 5 | 01 | 11490 | 62394 |
| 6 | 0.5 | 6109 | 31271 |
| 7 | 0.2 | 2565 | 12288 |
| 8 | 0.1 | 1451 | 6178 |
| 9 | 0.05 | 718 | 3264 |
| 10 | 0.02 | 298 | 1508 |
| 11 | 0.01 | ND | ND |
| Limit of Detection (LOD): | | 0.02% | 0.02% |
| Limit of Quantification (LOQ): | | 0.06% | 0.06% |
| **Note:** ND* - Not Detected. | | | |

***Acceptance Criteria:*** There should be no significant effect on the result by doing small deliberate changes in the system as well as in method parameters.

g) System suitability: For system suitability, six replicates of the working standard samples were injected and the parameters like – plate number (N), retention time (Rt), and peak asymmetry of samples were calculated for Prezcobix and given in Table 12.

***Acceptance Criteria:*** The % RSD for the retention times of principal peak from 5 replicate injections of each standard solution should be not more than 2.0%.

## RESULTS AND DISCUSSION

The aim of the present study was to develop a simple, sensitive, precise, and accurate RP-HPLC method for the analysis of Cobicistat & Darunavir in bulk and tablet formulation. To optimize the mobile phase, various combinations of buffer and acetonitrile solvents were studied, on a column Eclipse XBD-C18 (250 mm x 4.6 mm, 5 µ). Finally by using mixture of 0.01M potassium di-hydrogen phosphate pH 3.0 (adjusted with ortho phosphoric acid) as buffer and acetonitrile in the ratio of (40:60 v/v) found to be an efficient system for elution of drug with good peak shape as well as retention time 2.959 and 4.260 min., for Cobicistat and Darunavir respectively, flow rate 1.0 mL/min. at UV

**Table 11a: Robustness Results for Cobicistat**

| Parameter | Peak Areas for Flow Rate | | Peak Areas for Variable Column | |
|---|---|---|---|---|
| | Flow Rate 1.2 ml | Flow Rate 0.8 ml | Zobrax Eclipse XBD-C18 | Inertsil ODS - C18 |
| Injection-1 | 1321967 | 1329137 | 1089359 | 1099621 |
| Injection-2 | 1319868 | 1327695 | 1093130 | 1092293 |
| Injection-3 | 1327966 | 1327069 | 1089006 | 1091219 |
| Mean | 1323267 | 1327967 | 1090498 | 1094378 |
| Std. dev | 4203 | 1060.49 | 2285.91 | 4572.5 |
| % RSD | 0.318 | 0.080 | 0.210 | 0.418 |

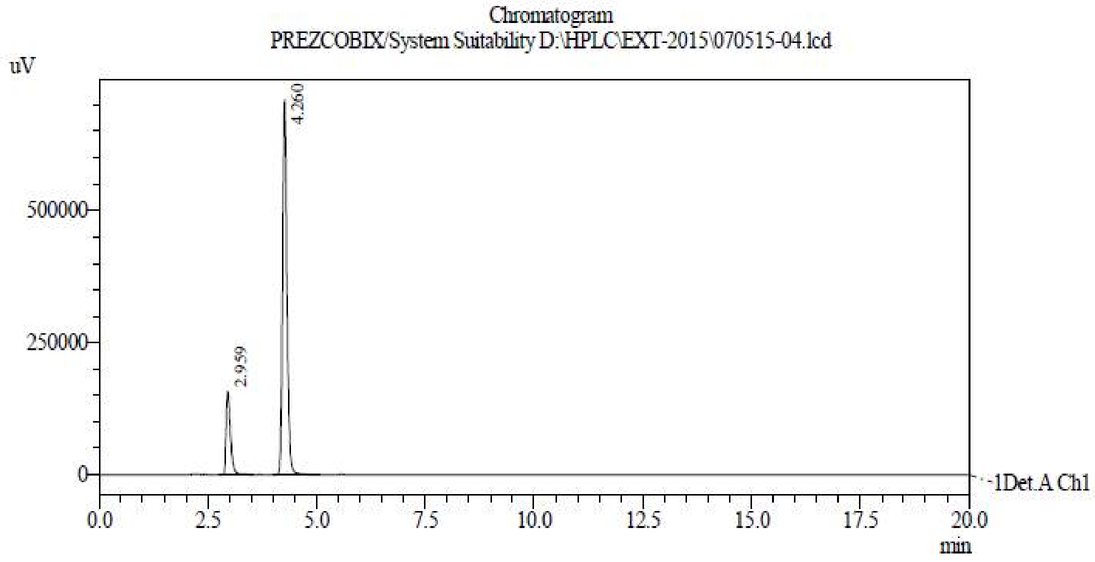
**Table 11b: Robustness Results for Darunavir**

| Parameter | Peak Areas for Flow Rate | | Peak Areas for Variable Column | |
|---|---|---|---|---|
| | Flow Rate 1.2 ml | Flow Rate 0.8 ml | Zobrax Eclipse XBD-C18 | Inertsil ODS - C18 |
| Injection-1 | 6087482 | 6097145 | 5061257 | 5045599 |
| Injection-2 | 6093124 | 6097369 | 5060817 | 5038877 |
| Injection-3 | 6108172 | 6091574 | 5064823 | 5045098 |
| Mean | 6096259 | 6095363 | 5062299 | 5043191 |
| Std. dev | 10695 | 3283 | 2197 | 3745 |
| % RSD | 0.175 | 0.054 | 0.043 | 0.074 |

**Table 12: System Suitability Parameters**

| Parameter | Results of the Proposed HPLC Method | |
|---|---|---|
| | Cobicistat | Darunavir |
| Retention time (min) | 2.959 | 4.260 |
| Theoretical plates (n) | 4011.872 | 7641.953 |
| Peak Area | 1080796 | 5043880 |
| Peak asymmetry (Tailing) | 1.659 | 1.321 |
| Linearity range (%) | 40-120 | 40-120 |
| Regression coefficient (R2) | 0.9989 | 0.9977 |
| Limit of Detection (% ) | 0.02 | 0.02 |
| Limit of Quantification (%) | 0.06 | 0.06 |

**Figure 7: System Suitability Spectrum with Data for PREZCOBIX**



**Detector A Ch1 240 nm**

**Peak Table**

| Peak | Retention Time (min) | Name | Peak Area | % Peak Area | Theoretical Plate | Tailing Factor | Resolution |
|---|---|---|---|---|---|---|---|
| 1 | 2.959 | COBICISTAT | 1080796 | 17.647 | 4011.872 | 1.659 | 0.000 |
| 2 | 4.260 | DARUNAVIR | 5043880 | 82.353 | 7641.953 | 1.321 | 6.814 |
| Total | | | 6124676 | 100.00 | | | |

wavelength of 240 nm. Quantitative linearity was obeyed in the percentage range of 40 to 120% and 40 to 120% the regression equations of concentration over their peak areas were found to be Y= 10945.X , $r^2$=0.9989, and Y=51219.X, $r^2$=0.9977 for cobicistat and darunavir respectively where Y is the peak area and X is the concentration of drug. The number of theoretical plates obtained was 4011.872 for cobicistat and 7641.953 for darunavir which indicate the efficient performance of the column. The Limit of detection was 0.02% and 0.06% and limit of quantification was 0.02% and 0.06% for cobicistat and darunavir, which indicates the sensitivity of the method the high percentage recovery, indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method.

## CONCLUSION

In conclusion a new isocratic RP-HPLC method was developed and validated for the estimation of Cobicistat & Darunavir in bulk and combined tablet dosage form. The developed method is simple, precise and accurate and satisfactory results were obtained through the method validation data. The present method can be easily applicable for routine drug analysis in laboratories and pharmaceutical industry.

## ACKNOWLEDGMENT

Authors thank for providing the gift sample of standard Cobicistat & Darunavir from rainbow laboratories and carrying the research work at Osmania University, Hyderabad. The authors are also thankful to Director & Head of Nizam Institute of Pharmacy, Hyderabad, for providing equipment for the present work.

## REFERENCES

1. http://en.wikipedia.org/wiki/Cobicistat.
2. http://www.drugs.com/mtm/cobicistat.html.
3. https://en.wikipedia.org/wiki/Darunavir
4. https://www.drugs.com/cdi/darunavir.html
5. http://www.accessdata.fda.gov/drugsatfda_docs.pdf.
6. Eve-Irene Lepist *et al*. (2012), "Cobicistat Boosts the Intestinal Absorption of Transport Substrates, Including HIV Protease Inhibitors and GS-7340", *In Vitr,* Antimicrobial Agents Chemother., Vol. 56, No. 10, pp. 5409-5413.
7. Kavitha K, Geetha Y, Hariprasad G, Venkatnarayana R and Subramanian R (Jan, 2013) "Development and Validation of RP-HPLC Analytical Method For Simultaneous Estimation of Emtricitabine, Rilpivirine, Tenofovir Disoproxil Fumarate and Its Pharmaceutical Dosage Forms", *Academic Journal Pharmacie Globale*, [Online] Vol. 4, No. 1, p. 1.
8. Charushila H B, Shivan and N H (2013), "Stability Indicating RP-HPLC Method for the Determination of Atazanavir Sulphate in Bulk and Dosage Form", *Drug Invention Today,* Vol. 5, pp. 81-86.
9. Reddy B V, Jyothi G, Reddy B S, Raman N V, Reddy K S and Rambabu C (2013), "Stability-indicating HPLC Method for the Determination of Darunavir Ethanolate", *J Chromatogr Sci.,* Vol. 51, pp. 471-476.
10. Tulsidas Mishra and Pranav S S (2014), "Validation of Simultaneous Quantitative

Method of HIV Protease Inhibitors Atazanavir, Darunavir and Ritonavir in Human Plasma by UPLCMS/MS", *The Scientific World Journal,* pp. 1-12.

11. Urooj Fatima, Mamatha T and Rajesh Goud Gajula (2014), "A Novel RP-HPLC Method Development and Validation of Cobicistat in Bulk Drug and Tablet Dosage Form", *Der Pharmacia Sinica,* Vol. 5, pp. 99-105.
12. Sindu Priya D and Gowri Sankar D (2015), "Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Determination of Atazanavir and Cobicistat in Bulk and Pharmaceutical Formulation", *Am. J. PharmTech. Res*., Vol. 6, No. 1.
13. United States Pharmacopeia USP 34-NF 29, (2011).
14. ICH Guidelines on Validation of Analytical procedure: Text and Methodology Q 2 (R1), (2011).