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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DARUNAVIR AND COBICISTAT IN COMBINED TABLET DOSAGE FORMS

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**ABSTRACT**

The study describes method development and subsequent validation of RP-HPLC method for simultaneous estimation of darunavir and cobicistat in combined tablet dosage forms. Chromatographic separation was achieved Inertsil ODS C18 column (150 mm x 4.6 mm, 5 µm)using a mobile phase consisting of (30:70 v/v) 0.1% TEA: methanol at a flow rate of 1.2 mL/min. The detection wavelength is 242 nm. The retention times of darunavir and cobicistatwere found to be 3.237 min and 4.805 min respectively. The developed method was validated as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of darunavir and cobicistat in tablet dosage forms.

**Keywords:** RP-HPLC, Darunavir, Cobicistat and validation.

## INTRODUCTION

Darunavir chemically,[(3aS,4R,6aR)-2,3,3a,4,5,6a-hexahydrofuro[2,3 b]furan-4-yl]N-[(2S,3R)-4-[(4-aminophenyl)sulfonyl- (2-methylpropyl)amino]-3-hydroxy-1-phenylbutan-2- yl] carbamate and its molecular formula is $C_{17}H_{19}N_3O_3S$. It is a protease inhibitor used to treat HIV. It acts on the HIV aspartyl protease which the virus needs to cleave the HIV polyprotein into its functional fragments (Figure-1). Cobicistat chemically, (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 (Figure-2) and its molecular formula is $C_{40}H_{53}N_7O_5S_2$. It is 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.A few chromatographic[1-7] methods have been reported for the simultaneous determination of darunavir and cobicistat in combined dosage forms. The present work is aimed to develop and validate simple, sensitive and more precise RP- HPLC method for simultaneous estimation of darunavir and cobicistat in bulk and pharmaceutical tablet dosage form.

## MATERIALS AND METHODS

### Equipment

Separation was carried out by using Shimadzu LC20A system equipped with LC20AT pump. SPD 20A Prominence UV-Visible detector and the peak areas were integrated by using spinchrome software CFR. Analysis was carried out on Inertsil C18 (150 mm x 4.6 mm, 5 µm) column.

**CHEMICALS AND REAGENTS**

HPLC grade methanol, acetonitrile and analytical grade dipotassium hydrogen phosphate, triethylamine and o-phosphoric acid were obtained from M/s. Rankem Chemicals Ltd, Mumbai, India. Milli-Q water dispensed through a 0.22 µ filter of the Milli-Q water purification system (Millipore, Merck KGaA, Darmstadt, Germany) was used throughout the study.

**Preparation of solutions**

**Preparation of 0.1% triethylamine**

Pipette out 1ml of TEA into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water.

**Preparation of Mobile phase**

A mixture of 300 mL of 0.1% of TEA and 700 mL of methanol in the ratio of 30:70 v/v/v was prepared and used as the mobile phase.

**Preparation of mixed standard solution of darunavir and cobicistat**

About 80 mg of darunavir and 15 mg of cobicistat were accurately weighed and transferred into a 100 mL clean dry volumetric flask containing 25 mL of the mobile phase.The solution was sonicated for 5 min and then volume was made up to the mark with a further quantity of the diluent to get a concentration of 8000 µg/mL of darunavirand 1500 µg/mL of cobicistat (Stock solution). A mixed working standard solution was further prepared by diluting the above stock solution to obtain a concentration of 800 µg/mL of darunavir and 150 µg/mL of cobicistat.

**Preparation of the tablet solution**

Twenty tablets of the commercial sample of **'PREZCOBIX'** were weighed and finely powdered. An accurately weighed portion of powdered sample equivalent to 80 mg of darunavir and 15 mg of cobicistat was transferred into a 100 mL volumetric flask containing 25 mL of the methanol. The contents of the flask were sonicated for about 10 min for complete solubility of the drugs and the volume made up with a further quantity of the diluent. Then, this mixture was filtered through a 0.45 µ membrane filter. This filtrate was used for further analysis.

**Chromatographic conditions**

A reverse phase column Inertsil C18 column (150 mm x 4.6 mm, 5 µm particle size), equilibrated with mobile phase (0.1% TEA and methanolin the ratio of 30:70 v/v) was used. Mobile phase flow rate was maintained at 1 mL/min and effluents were monitored at 242 nm. The sample was injected using 20 micro litre manual sample injector and run time was 9min (Figure 3and 4).

**METHOD VALIDATION**

**System suitability**

The system suitability studies were done for parameters like theoretical plates, tailing factor, retention time, resolution by injecting the standard solution in to the optimized chromatographic system for six times and the results are given in the Table 1.

**Accuracy**

The standard addition method was used to demonstrate the accuracy of the proposed method. For this purpose, known quantities of darunavir and cobicistat were supplemented to the previously analysed sample solution and then experimental and true values compared. Three levels of solutions were made corresponding to 50, 100 and 150 % of nominal analytical concentration. Standard preparation & sample preparation was injected into the HPLC and % RSD fordarunavir and cobicistat peaks in standard preparation was calculated and tabulated in Table 2 and figure 5-7. The mean recovery values of darunavir and cobicistatwere found to be 99.47-100.16 %, 99-99.6 % respectively.

**Linearity**

Linear calibrations plots of the proposed method were obtained over concentration ranges of 40-200 µg/mL for darunavir, (40, 80, 120, 160, 200µg/mL) and 7.5-45µg/mL for cobicistat (7.5, 15, 22.5, 30, 37.5 µg/mL). Each solution was prepared in triplicate. Regression coefficient was found to be 0.999 and 0.999 for both the drugs (Figure 8 - 14). Standard curve had a reliable reproducible over the standard concentrations across the calibration range. All back calculated concentrations did not differ from the theoretical value as no single calibration standard point was dropped during the validation.

**Precision**

For precision same concentration solution was injected 5 times and observed for any peculiar change in the areas and % RSD was calculated for each drug.

**Method precision**

Five replicate injections of the same dilution were analysed on two different days by different analyst for verifying the variation in the precision. The % RSD of the results for darunavir and cobicistat were found to be 0.97 and 0.52 respectively, which are within acceptable limit of ≤2. Hence, the method is

reproducible on different days with different analyst. This indicates that the method is precise. The results are shown in the Table 3 and figure 15-20.

**Intermediate Precision**

For system precision study the standard solution replicates was injected repeatedly for six times and was observed. The standard deviation values were found to be 338473 and 110173for darunavir and cobicistat and the % RSD values were 1.21 and 0.84 for darunavir and cobicistat and the results are tabulated in the Table 4 and figure 21-26.

**Limit of detection (LOD)**

The LOD for this method was found to be 0.78 µg/mL and 0.09 µg/mL for darunavir and cobicistat respectively.

**Limit of quantitation (LOQ)**

The LOQ for this method was found to be 2.37 µg/mL and 1.16µg/mL for darunavir and cobicistat respectively.

**Robustness**

Robustness is generally done by changing the parameters like flow rate, organic phase of the mobile phase. The results are shown in the following data is given in the Table 5.

**Assay**

Twenty micro liters of standard and sample solutions were injected separately in to the chromatographic system and the peak areas for the analyte peaks were measured. The % content of each drug was calculated as shown in below table format.

| Brand name | Drug | Labeled amount (mg) | Amount found (mg) | % assay |
|---|---|---|---|---|
| PREZCOBIX | darunavir | 800 | 798.84 | 99.85 |
| | cobicistat | 150 | 149.16 | 99.44 |

## RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation with good peak symmetry was obtained with zodiac.In the present study, a new simple, precise and accurate HPLC method was developed and validated for the simultaneous estimation of darunavir and cobicistatin tablet dosage forms. In this method, an Inertsil C18 (150 x 4.6 mm; 5 µm) column using mobile phase containing 0.1% TEA: methanol (30:70 v/v) at a flow rate of 1.2 mL/min. Quantification was achieved with UV detection at 242 nm based on peak area. The retention time for darunavir and cobicistat were found to be 3.237 min and 4.805respectively. The optimized method was validated as per ICH guidelines. The System suitability parameters observed by using this optimized conditions were reported. A linearity range of 40-200µg/mL with correlation coefficient 0.999 was established for darunavirand 7.5-37.5 µg/mL with correlation coefficient 0.999was established for cobicistat. The precision of the proposed method was carried in terms of the method precision and intermediate precision of the % RSD values of darunavir were found to be 0.97 %, 0.52 % and cobicistat were found to be 1.21 %, 0.84 % and reveal that the proposed method is precise. The LOD and LOQ values for darunavir were 0.78µg/mL and 2.37µg/mL and for cobicistat were found to be 0.09 and 1.16µg/mL receptively. The results of analysis of commercial formulation indicated that there is no interference due to common formulation excipients with the developed method. Therefore, the proposed method can be used for routine analysis of these two drugs in their combined pharmaceutical dosage form.

## CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of darunavir and cobicistat from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of darunavir and cobicistat in pure form and its dosage form and also can be used for dissolution or similar studies.

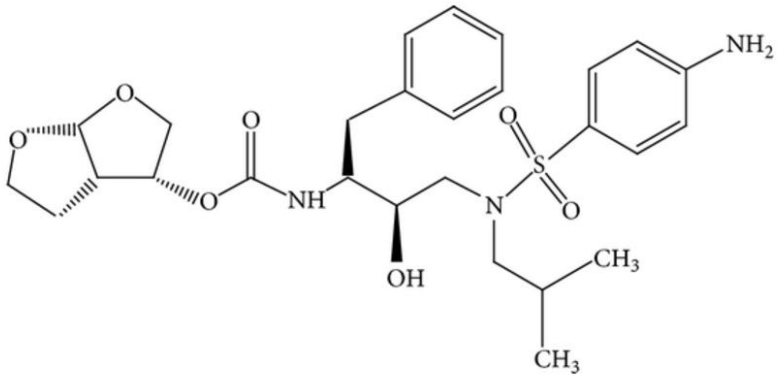


**Fig. 1: Chemical structure of Darunavir**

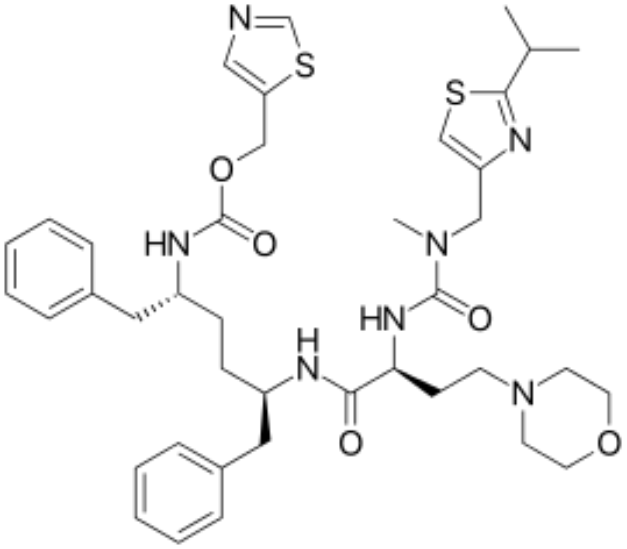


**Fig. 2: Chemical structure of Cobicistat**

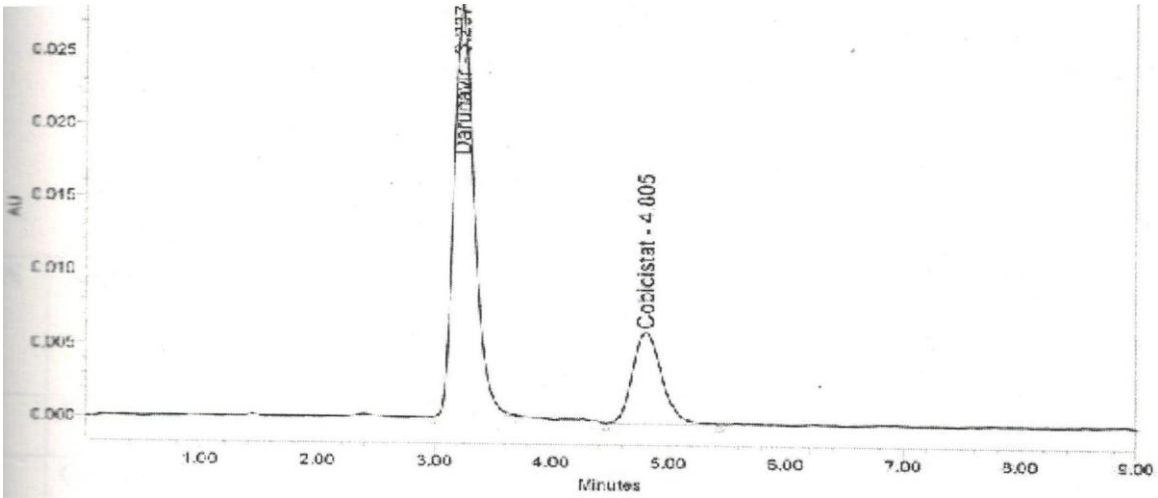


**Fig. 3: A typical chromatogram showing the separation of darunavir and cobicistat standard**

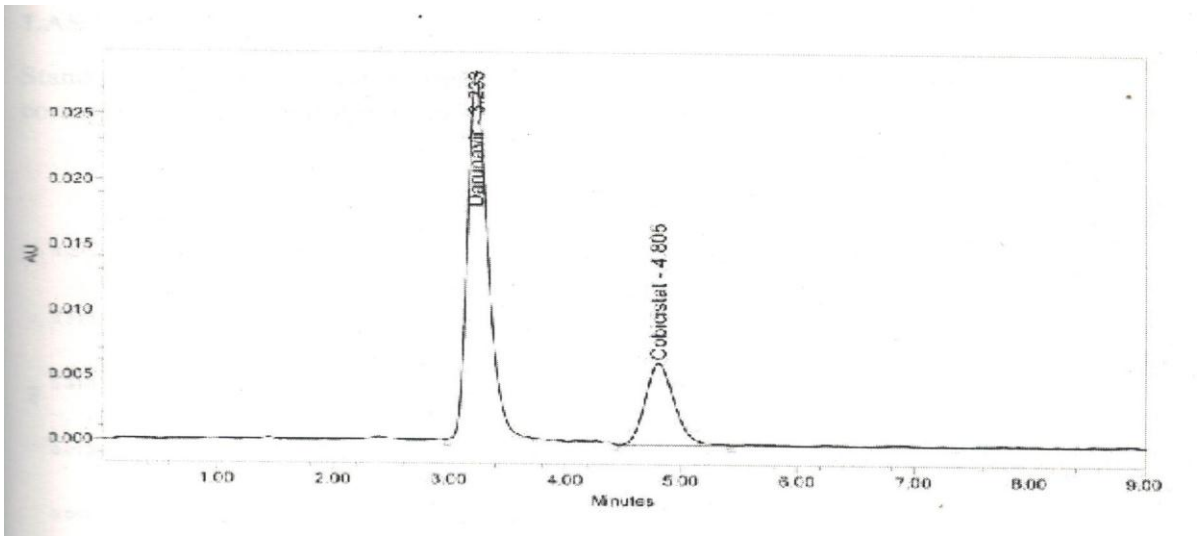


**Fig. 4: A typical chromatogram showing the separation of darunavir and cobicistat sample**

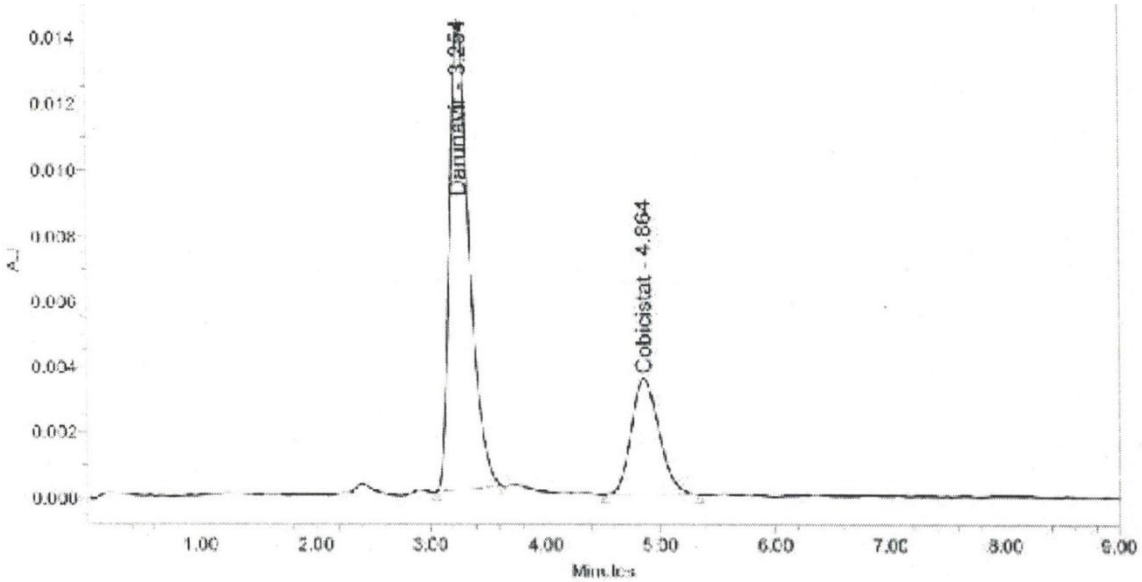


**Fig. 5: Chromatogram for accuracy at 50% level**

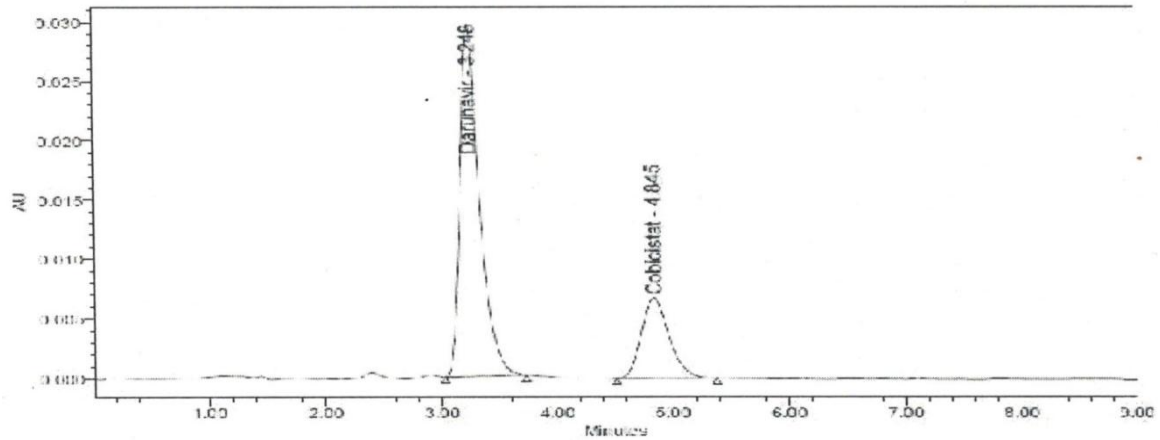


**Fig. 6: Chromatogram for accuracy at 100% level**

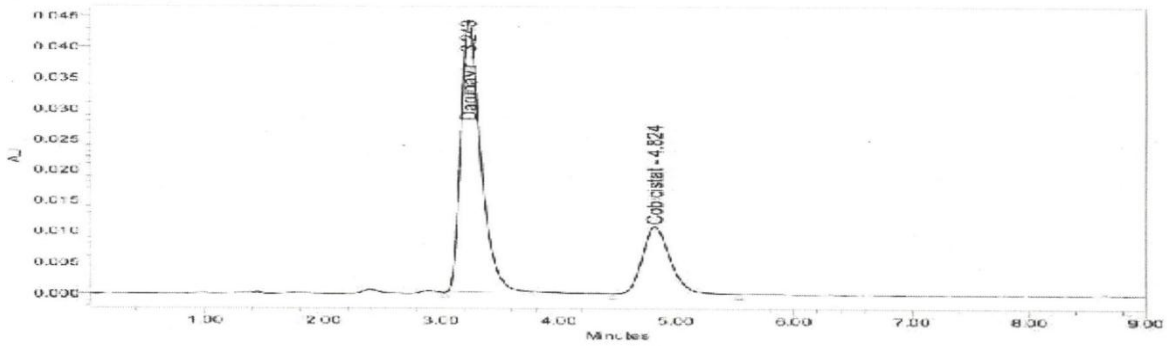


**Fig. 7: Chromatogram for accuracy at 150% level**

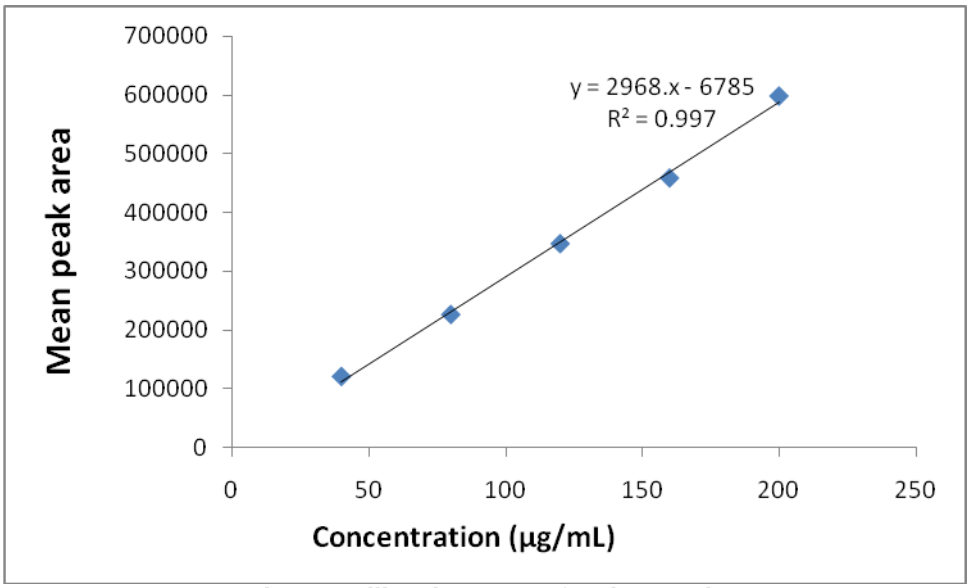


**Fig. 8: Calibration curve for darunavir**

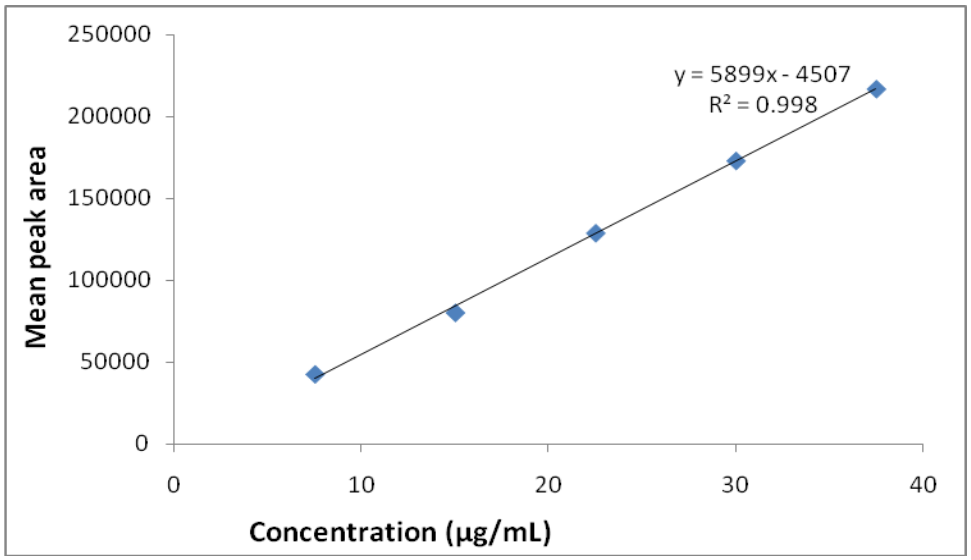


**Fig. 9: Calibration curve for cobicistat**

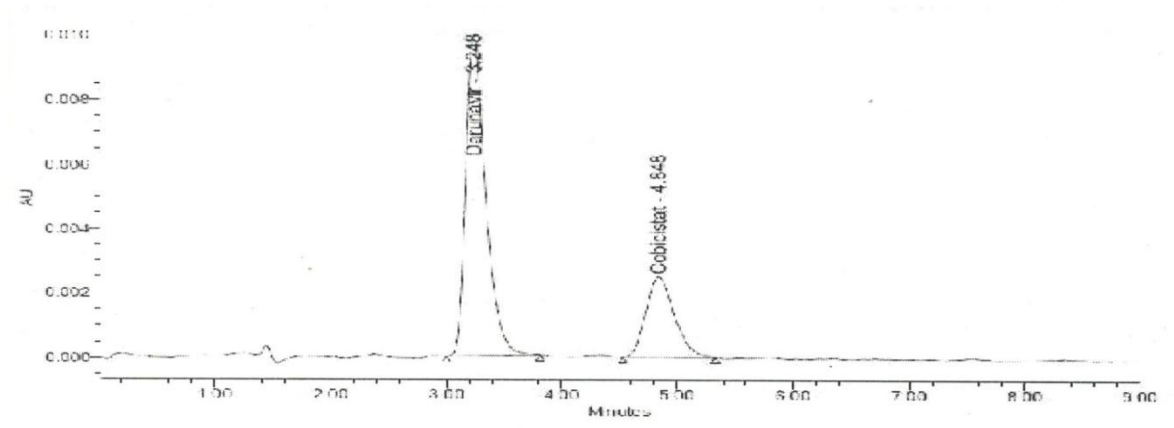


**Fig. 10: Chromatogram for linearity at level 1**

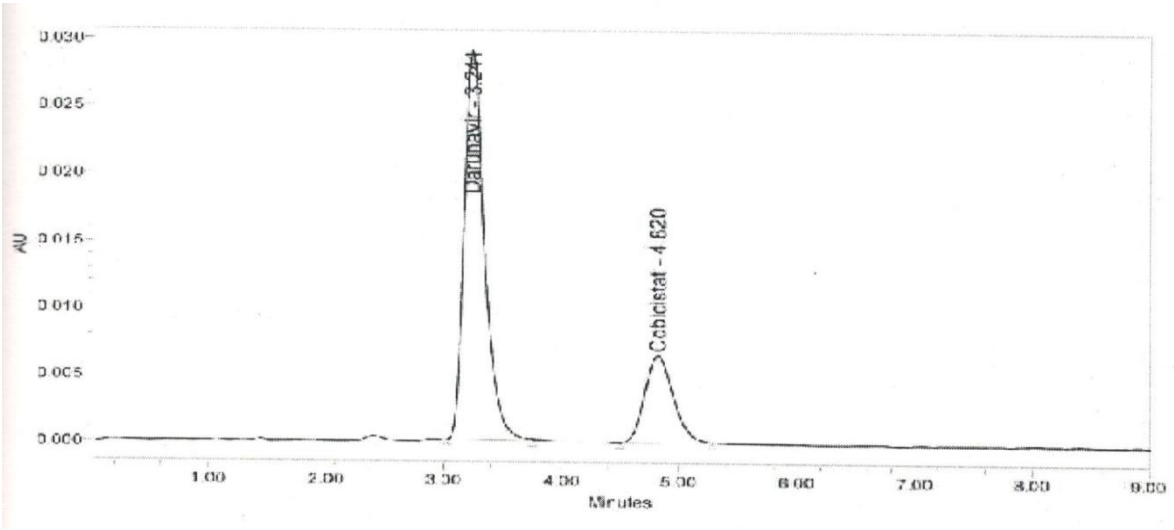


**Fig. 11: Chromatogram for linearity at level 2**

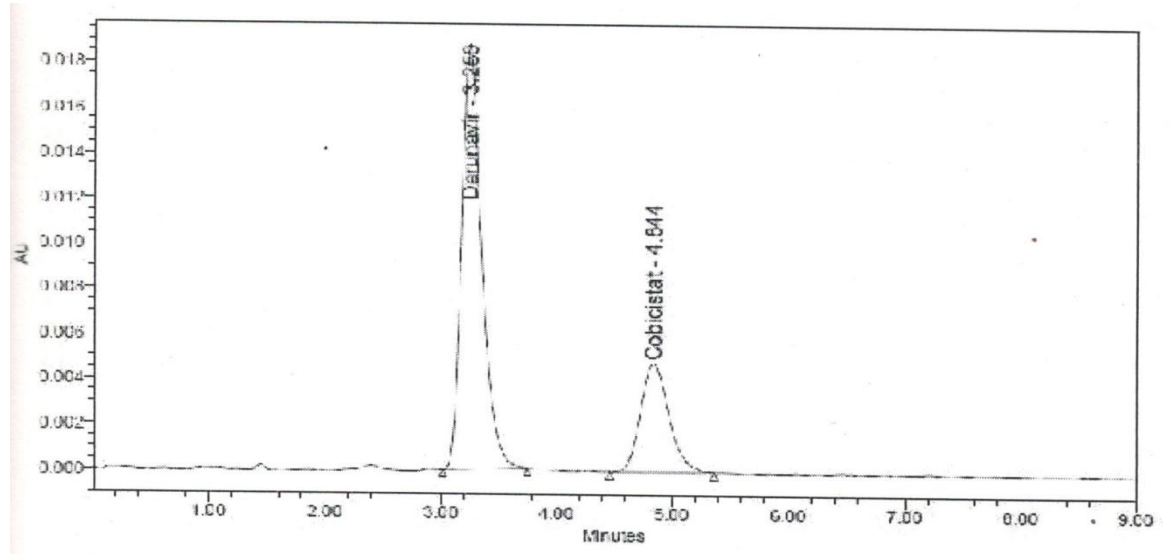


**Fig. 12: Chromatogram for linearity at level 3**

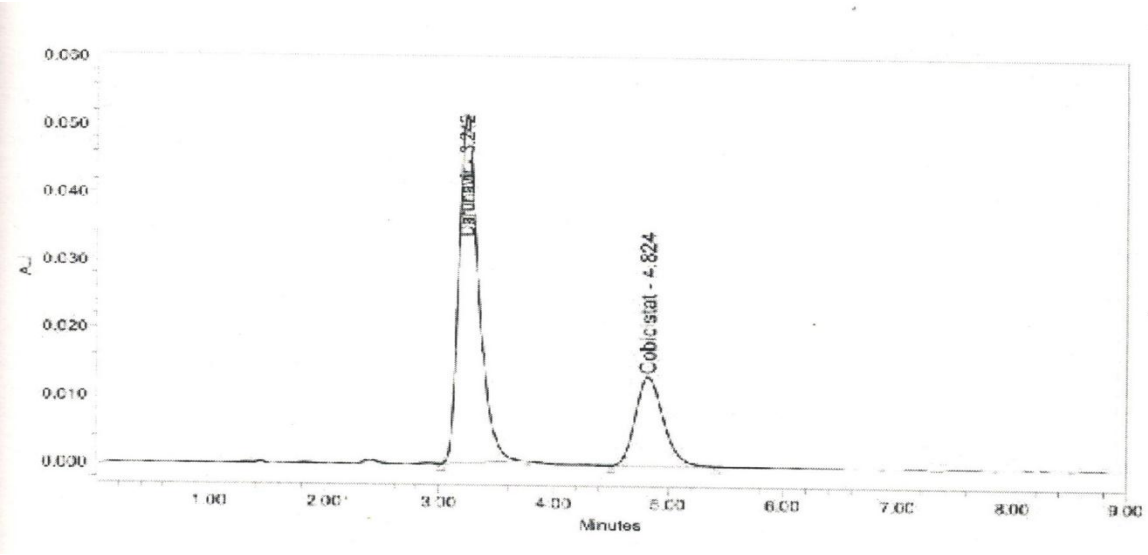


**Fig. 13: Chromatogram for linearity at level 4**

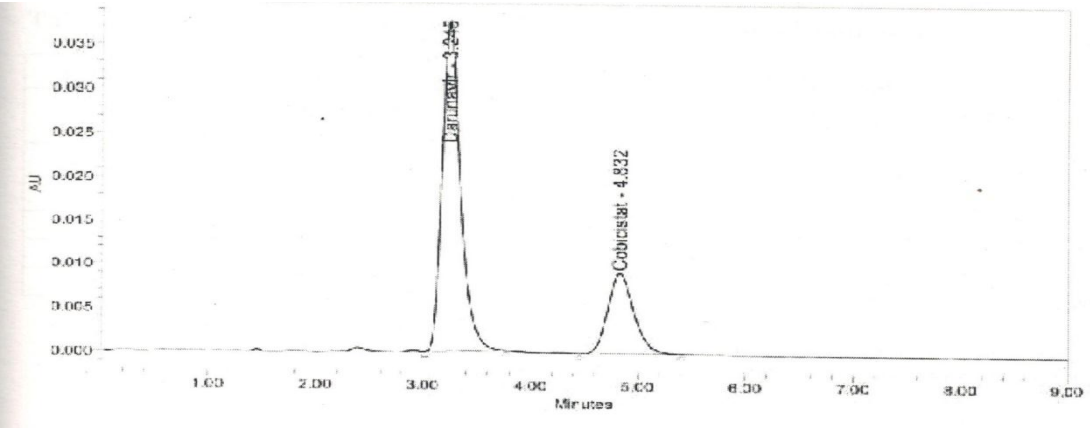


**Fig. 14: Chromatogram for linearity at level 5**

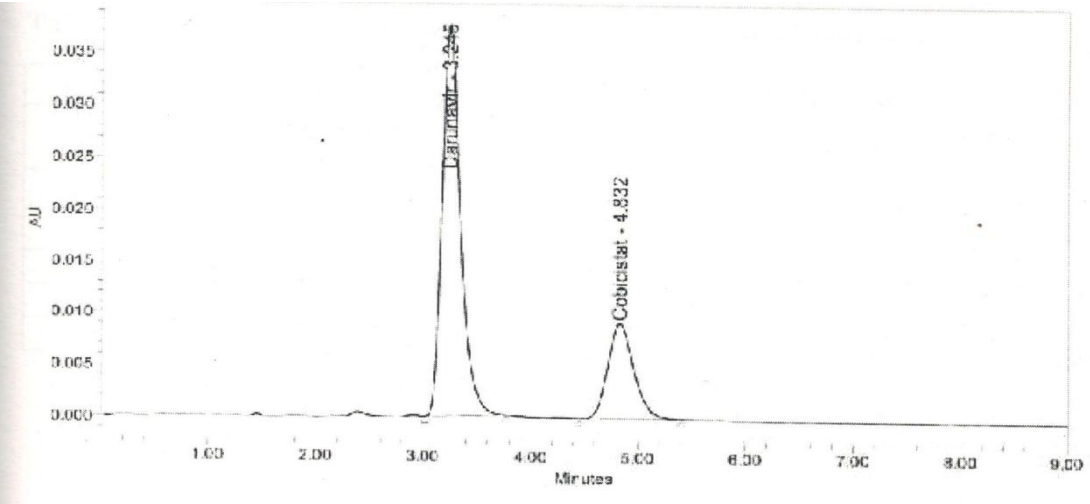


**Fig. 15: Chromatogram-1 for method precision of Darunavir and Cobicistat**

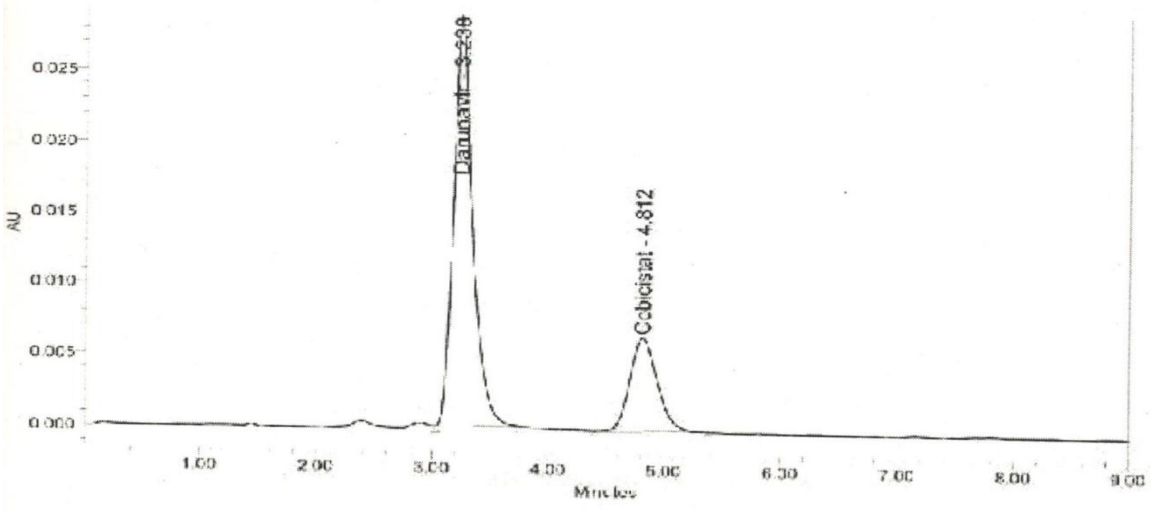


**Fig. 16: Chromatogram-2 for method precision of Darunavir and Cobicistat**

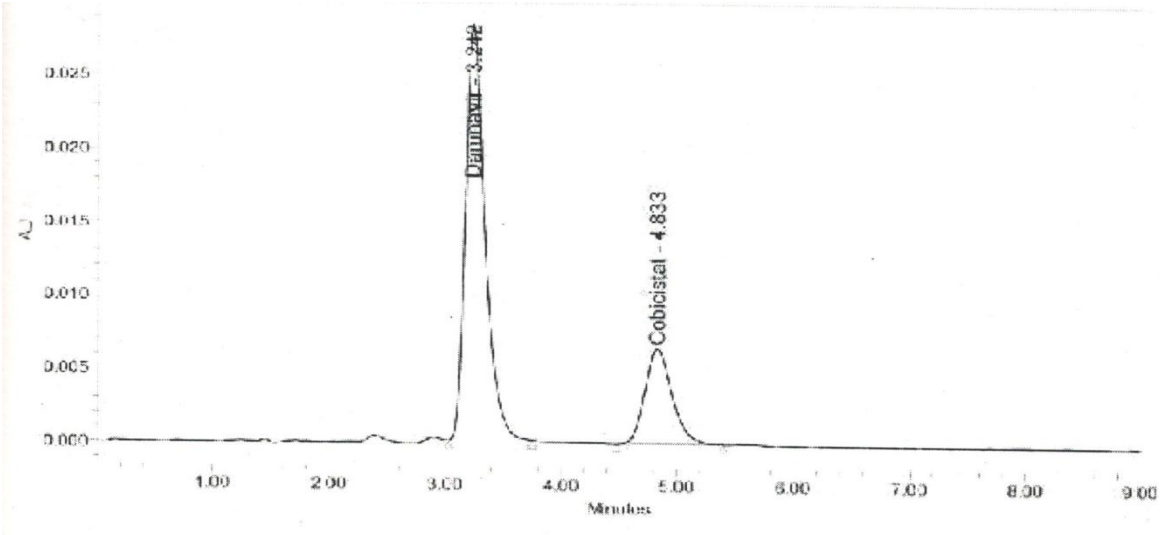


**Fig. 17: Chromatogram-3 for method precision of Darunavir and Cobicistat**

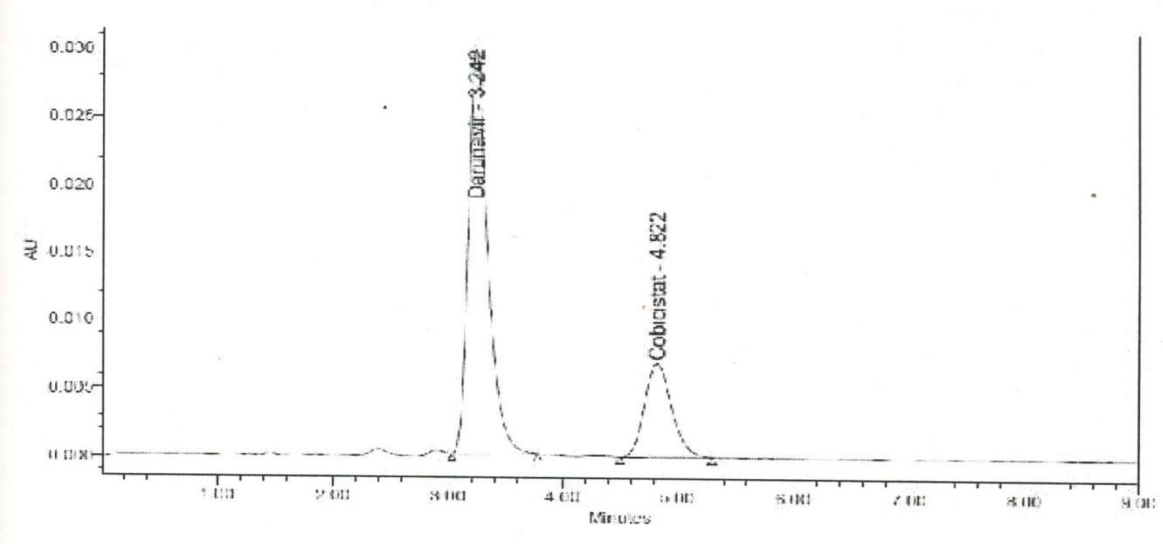


**Fig. 18: Chromatogram-4 for method precision of Darunavir and Cobicistat**

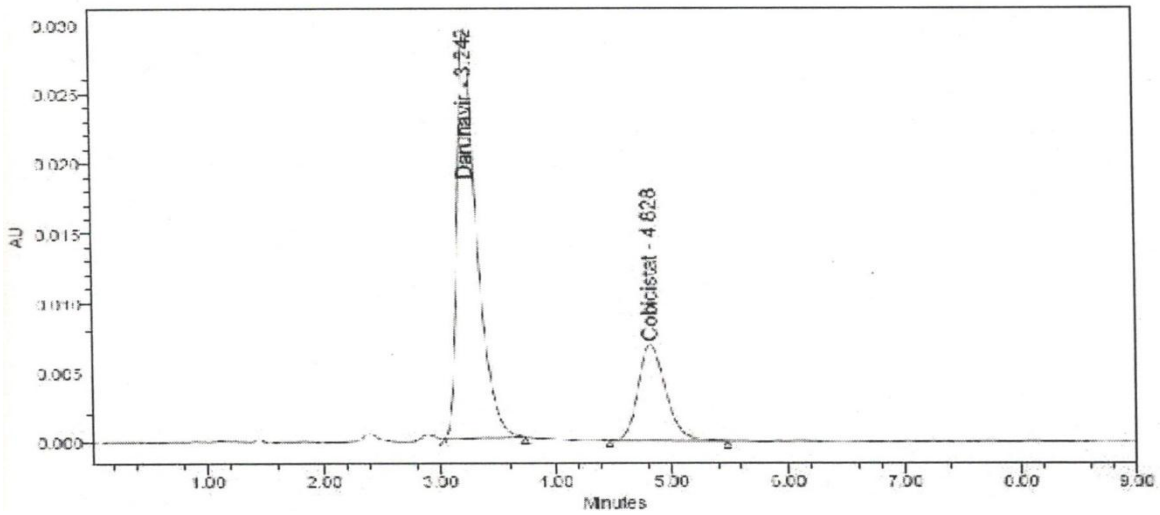


**Fig. 19: Chromatogram-5 for method precision of Darunavir and Cobicistat**

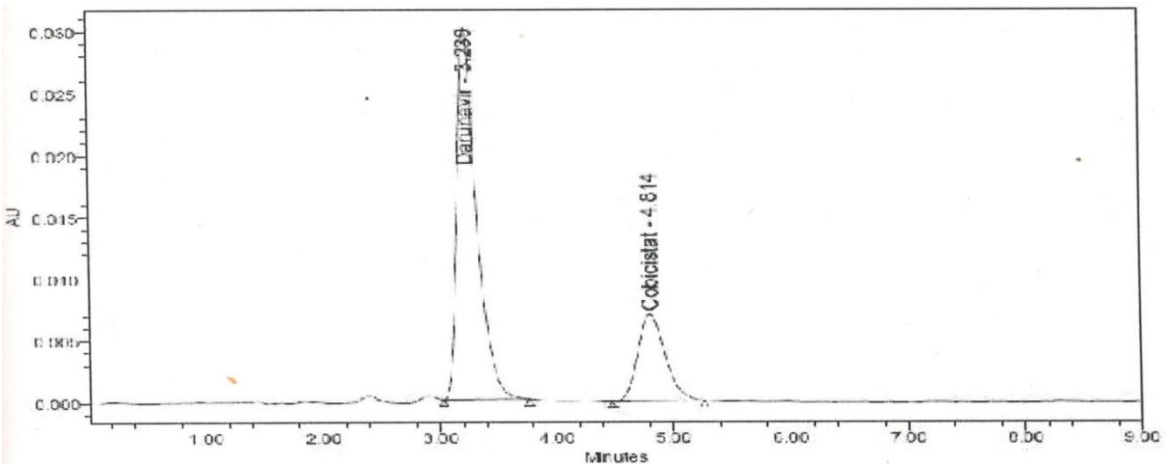


**Fig. 20: Chromatogram-6 for method precision of Darunavir and Cobicistat**

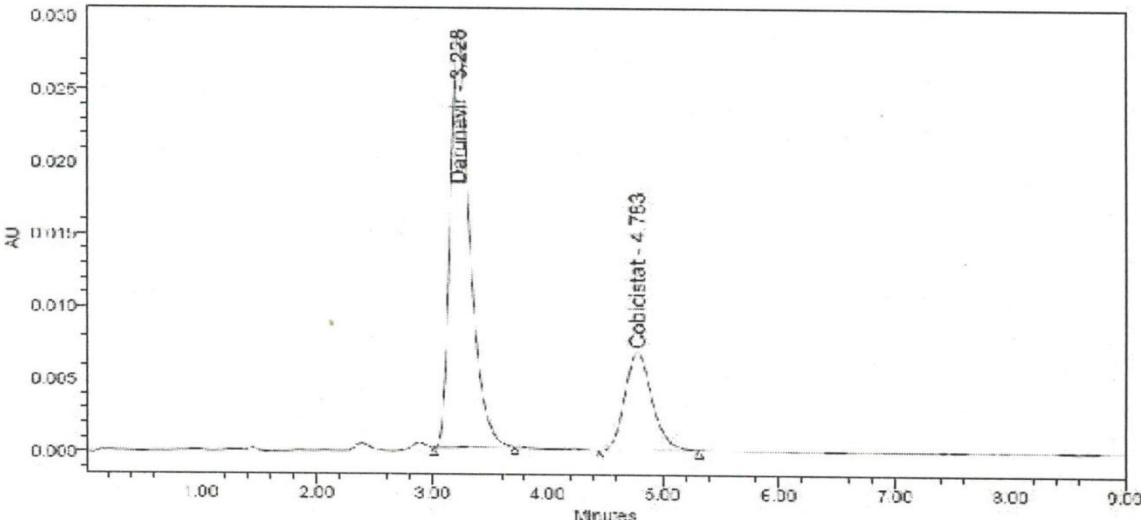


**Fig. 21: Chromatogram-1 for intermediate precision of Darunavir and Cobicistat**

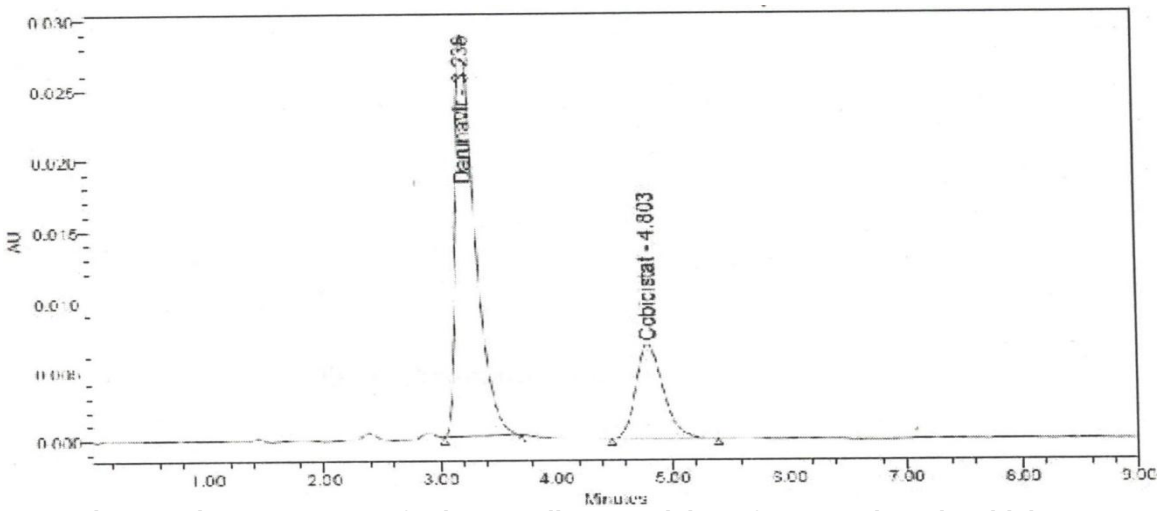


**Fig. 22: Chromatogram-2 for intermediate precision of Darunavir and Cobicistat**

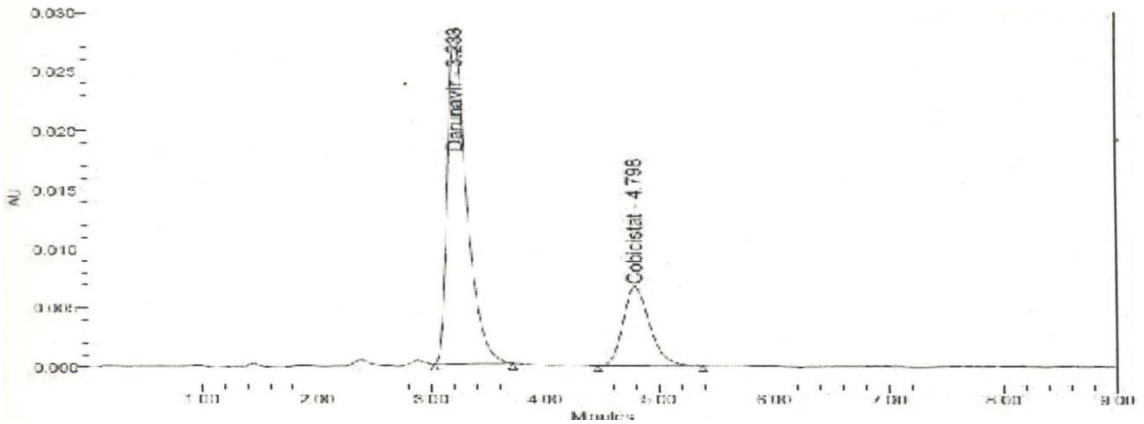


**Fig. 23: Chromatogram-3 for intermediate precision of Darunavir and Cobicistat**

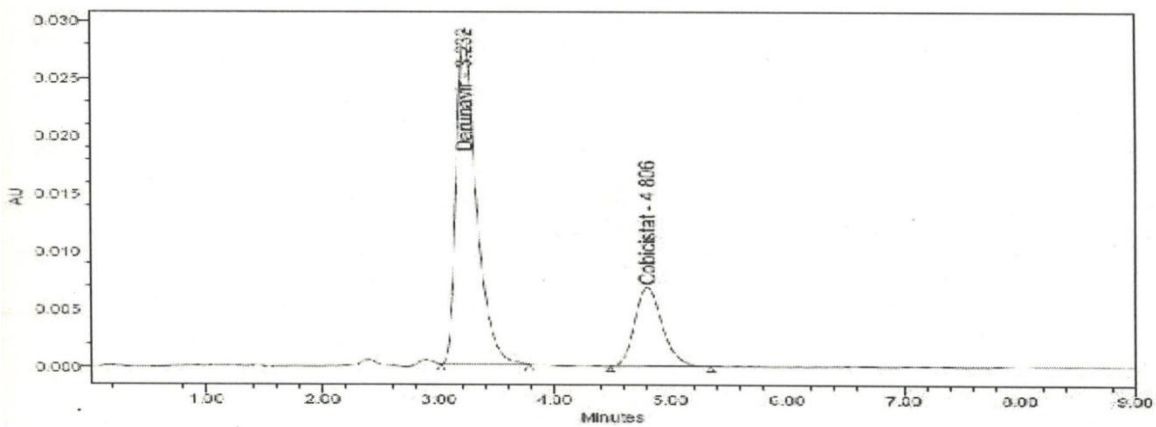


**Fig. 24: Chromatogram-4 for intermediate precision of Darunavir and Cobicistat**

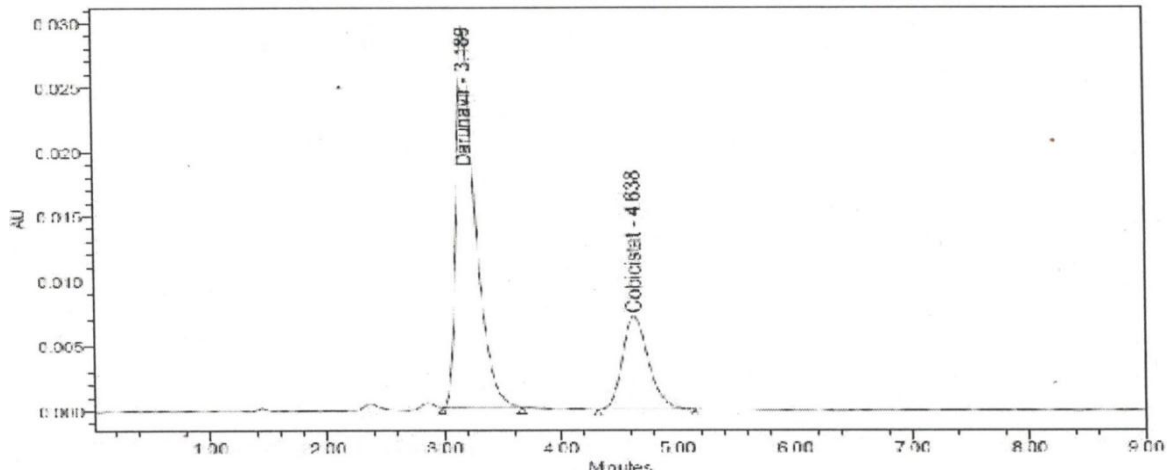


**Fig. 25: Chromatogram-5 for intermediate precision of Darunavir and Cobicistat**

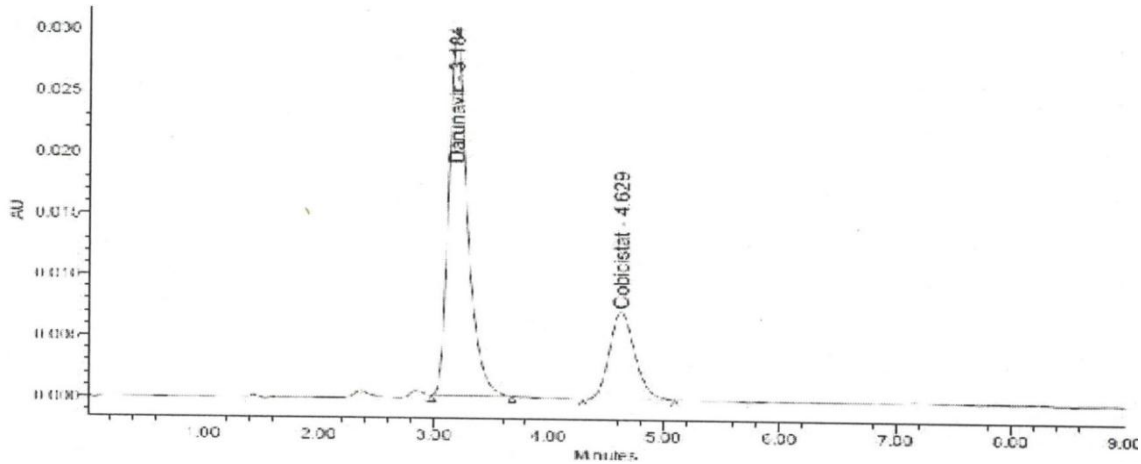


**Fig. 26: Chromatogram-6 for intermediate precision of Darunavir and Cobicistat**

**Table 1: System suitability studies of Darunavir and Cobicistat**

| Parameter | Darunavir | Cobicistat | Acceptance criteria |
|---|---|---|---|
| Retention time | 3.233 | 4.805 | --- |
| No. of theoretical plates | 2644.98 | 3766.98 | NLT 2000 |
| Tailing factor | 1.33 | 1.20 | NMT 2.0 |
| Resolution | 4.12 | | NLT 2.0 |

**Table 2: Results of recovery experiments of darunavir and cobicistat**

| Preanalysed amount (µg/mL) | | Spiked amount (µg/mL) | | % recovered | |
|---|---|---|---|---|---|
| Darunavir | Cobicistat | Darunavir | Cobicistat | Darunavir | Cobicistat |
| 120 | 45 | 60 | 22.5 | 99.59 | 99.87 |
| 120 | 45 | 60 | 22.5 | | |
| 120 | 45 | 60 | 22.5 | | |
| 120 | 45 | 120 | 45 | 99.68 | 101.67 |
| 120 | 45 | 120 | 45 | | |
| 120 | 45 | 120 | 45 | | |
| 120 | 45 | 180 | 67.5 | 98.59 | 101.67 |
| 120 | 45 | 180 | 67.5 | | |
| 120 | 45 | 180 | 67.5 | | |
| | | | **MEAN** | **99.28** | **101.07** |
| | | | **SD** | **0.605** | **1.039** |
| | | | **%RSD** | **0.61** | **1.03** |

**Table 3: Results of method precision of darunavir and cobicistat**

| Sample number | Method precision | |
|---|---|---|
| | Darunavir | Cobicistat |
| 1 | 352960 | 112771 |
| 2 | 350827 | 111383 |
| 3 | 352176 | 112738 |
| 4 | 350147 | 112476 |
| 5 | 344319 | 112658 |
| MEAN | 350085.8 | 112405.2 |
| SD | 3407.311 | 582.74 |
| % RSD | **0.97** | **0.52** |

**Table 4: Results of intermediate precision of darunavir and cobicistat**

| S.No. | Area | |
|---|---|---|
| | darunavir | Cobicistat |
| 1 | 332365 | 108816 |
| 2 | 340012 | 110789 |
| 3 | 335294 | 109615 |
| 4 | 342701 | 110643 |
| 5 | 337945 | 109819 |
| 6 | 342525 | 111360 |
| MEAN | 1442223 | 815370.3 |
| SD | 18375.8 | 2844.8 |
| % RSD | **1.27** | **0.35** |

**Table 5: Robustness study for darunavir and cobicistat**

| Condition | darunavir | | cobicistat | |
|---|---|---|---|---|
| | Tailing factor | Theoretical plates | Tailing factor | Theoretical plates |
| Flow rate at 1.0 mL/min | 1.48 | 2860.09 | 1.18 | 3131.66 |
| Flow rate at 1.2 mL/min | 1.33 | 2644.98 | 1.20 | 3766.53 |
| Flow rate at 1.4 mL/min | 1.44 | 2847.37 | 1.20 | 3055.53 |
| Mobile phase: | | | | |
| 0.1% TEA:Methanol (40:60) | 1.5 | 2071 | 1.2 | 3266 |
| 0.1% TEA:Methanol (30:70) | 1.3 | 2644 | 1.2 | 3766 |
| 0.1% TEA:Methanol(20:80) | 1.4 | 2732 | 1.2 | 2894 |

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