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Original Article

Stability Indicating RP-HPLC Method for the Simultaneous Determination of Darunavir and Cobicistat in Bulk and Pharmaceutical Dosage form

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ABSTRACT

A simple, sensitive and precise stability indicating RP-HPLC method has been developed and validated for the simultaneous estimation of darunavir and cobicistat in combined dosage form. The column used was Inertsil ODS (4.6 x 150mm, 5µm) column. The mobile phase used was Phosphate buffer: Methanol: Acetonitrile (40:20:40). Quantification was carried out using PDA Detector at 239 nm. Linearity was found to be 50-250 mcg/ml for darunavir and 10-50 mcg/ml for cobicistat, respectively. The method was validated for system suitability, precision, accuracy, ruggedness, robustness, LOD & LOQ. Darunavir and cobicistat were also subjected to acid degradation, alkali degradation, oxidative degradation, thermal degradation and photo degradation. The degradation products obtained were well resolved from the darunavir and cobicistat with different retention times. Since the method can effectively separate darunavir and cobicistat in their combined dosage form, it can be used for the routine determination of darunavir and cobicistat.

Keywords: Darunavir, Cobicistat, Stability indicating RP-HPLC

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INTRODUCTION

Darunavir is an HIV protease inhibitor, having the molecular structure as follows;

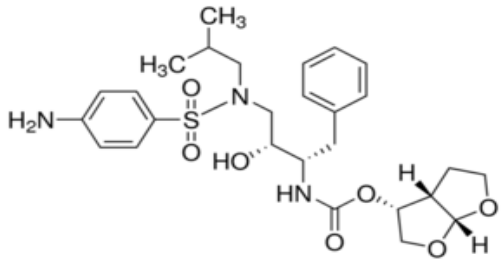

Figure 1. Chemical structure of darunavir

Chemically Darunavir is (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate. Cobicistat is a potent inhibitor of Cytochrome P450 3A, having the molecular structure as follows;

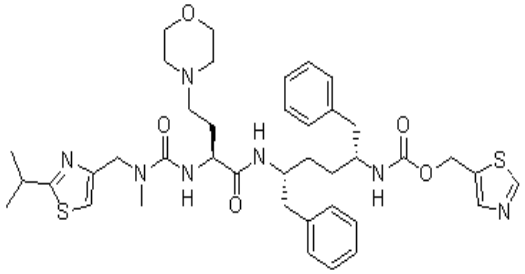

Figure 2. Chemical structure of cobicistat

Chemically cobicistat is 1,3-thiazol-5-ylmethylN-[(2R,5R)-5-[[(2S)-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]-4-morpholin-4-ylbutanoyl]amino]-1,6 diphenylhexan-2-yl] carbamate.

Literature survey reveals that few analytical methods have been reported for the simultaneous estimation of Darunavir and cobicistat in their combined dosage form. In the present investigation a stability indicating RP-HPLC method was described using Inertsil ODS (4.6 x 150mm, 5µm) column. The mobile phase used was Phosphate buffer: Methanol: Acetonitrile (40:20:40), with a flow rate of 1.0 mL/min. Quantification was carried out using PDA Detector at 239 nm. In the proposed method the low values of % RSD, LOD and LOQ indicates that the developed method is more precise and sensitive than the reported methods. The use of phosphate buffer in the preparation of mobile phase makes the method more economical than the reported methods.

MATERIALS AND METHODS

Instrumentation: Chromatography was carried out using Waters HPLC system, with Empower 2 software, 2695 separation module. Detector used was PDA detector.

Chemicals and solvents: Reference standards darunavir and cobicistat were obtained from Pharmatrain Laboratory. Solvents used were of HPLC grade. Other chemicals used were of analytical grade. Commercial tablets (Prezcobix**,** labeled to contain 800 mg and 150 mg of darunavir and cobicistat, respectively**)** were procured from local pharmacy.

Chromatographic conditions: Instrument used was Waters HPLC with auto sampler. The column used was Inertsil ODS (4.6 x 150mm, 5µm) column. The mobile phase used was Phosphate buffer: Methanol: Acetonitrile (40:20:40). Quantification was carried out using PDA Detector at 239 nm.

Preparation of standard solutions: The stock and working standard solutions were prepared with the mobile phase. The standard stock solutions of darunavir (8 mg/ mL) and cobicistat (1.5 mg/mL) were prepared by transferring accurately weighed amounts (80 mg of darunavir and 15 mg of cobicistat) into different 10 mL volumetric flasks. The drugs were dissolved by shaking gently with 5 mL of mobile phase and made upto the mark with the same solvent.

The working standard solutions (darunavir - 160 µg/mL and cobicistat - 30 µg/mL) were prepared by transferring 2 mL of stock standard solution into 100 mL volumetric flask and the volume was made upto the mark with the mobile phase. All the solutions were filtered through 0.45 µm membrane filters before use.

Calibration curves: Standard calibration curves were prepared with five calibrators over a concentration range of 50-250 µg/mL for darunavir and 10-50 µg/mL for cobicistat. 20 µL of solutions were injected in triplicate and chromatographed under the optimized conditions as described above. The peak areas measured were plotted against the concentration of the corresponding drug and the regression equation was derived.

Preparation of tablet sample solution: Ten tablets were weighed and their average weight was deterrmined. The tablets were crushed to a homogenous powder and an amount equivalent to 800 mg of darunavir and 150 mg of cobicistat was accurately weighed and transferred into a 100 mL volumetric flask to which 30 mL of mobile phase was added. After sonication for 15 min, the mixture in the flask was diluted to the mark with mobile phase and mixed. An aliquot of 2 mL was transferred to a 100 mL flask and filled to the mark

with mobile phase. The solution was filtered through 0.45 µm membrane filter before use. 20 µL of solution was injected under the optimized conditions as described above. The contents of the analytes were obtained from the corresponding regression equation/corresponding calibration curve.

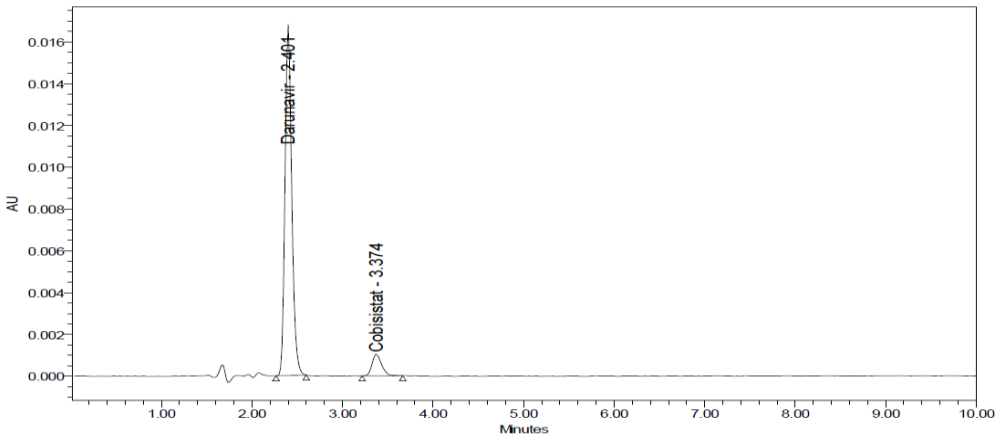

Figure 3. Chromatogram of darunavir and cobicistat under optimized chromatographic conditions

Method Validation

After development, the method was subjected to validation as per ICH guidelines

System suitability: The system suitability parameters were evaluated by injecting standard solution of 160 µg/mL darunavir and 30 µg/mL cobicistat. The results are presented in Table 1. The system was found to be suitable, as the parameters are within the acceptable limits.

Linearity: The linearity of the method was evaluated by analyzing a series of solutions containing darunavir and cobicistat in the concentration range of 50-250 µg/mL and 10-50 µg/ mL, respectively. The calibration curves were constructed. The regression coefficients of the curves were found to be ≥ 0.9990 for the two drugs, enabling the linear behavior of the method in the established concentration range. Darunavir and cobicistat showed linearity in the range of 50-250 µg/mL and 10-50 µg/mL, respectively. Linear regression equations and correlation coefficient are presented in Table 2.

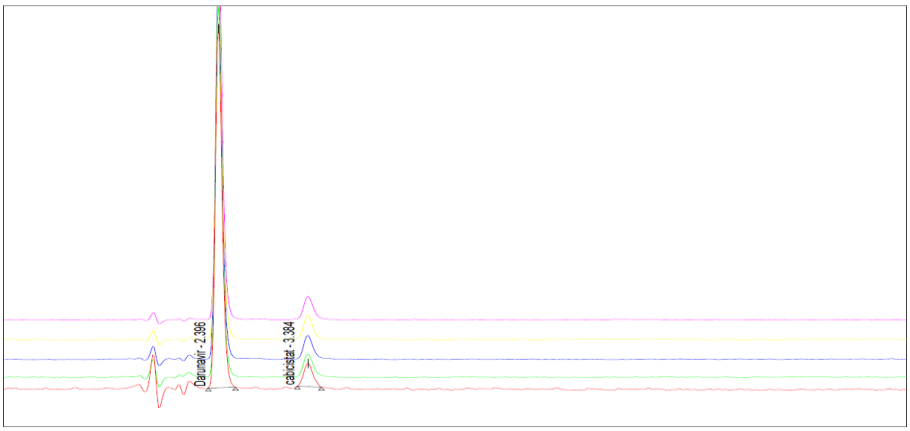

Figure 4. Chromatogram of Linearity studies

Precision: The precision of the method was evaluated by analyzing standard solutions of darunavir and cobicistat with a concentration of 160 µg/mL and 30 µg/mL, respectively. Six replicates were analyzed to determine the precision. The % RSD of peak areas was calculated and was found to be below 2.0 %. This indicates the precision of the method for the simultaneous estimation of darunavir and cobicistat. The results are shown in Table 3.

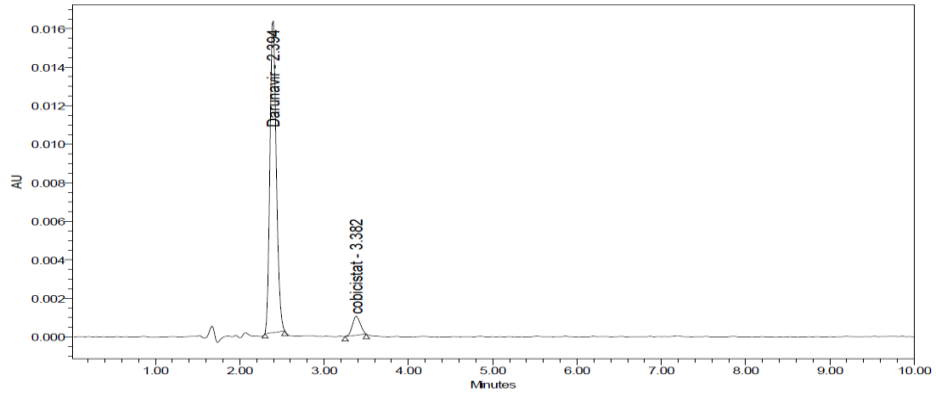

Figure 5. Chromatogram of Precision studies

Accuracy: To determine the accuracy of the method, recovery studies were carried out by application of the standard addition method. Known amounts of the darunavir and cobicistat at three different concentration levels (50 %, 100 % and 150 %) were added to a pre-analyzed tablet sample; the prepared samples were then analyzed by the proposed method and the percentage recoveries were then calculated. Good percentage recoveries were obtained, confirming the accuracy of the proposed method. The results are shown in Table 4.

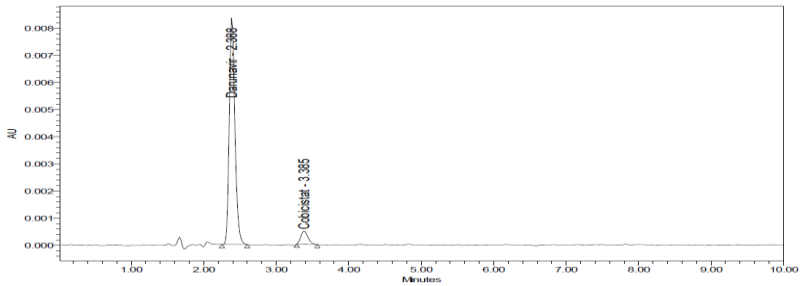

Figure 6. Chromatogram of Accuracy studies – 50% spiked level

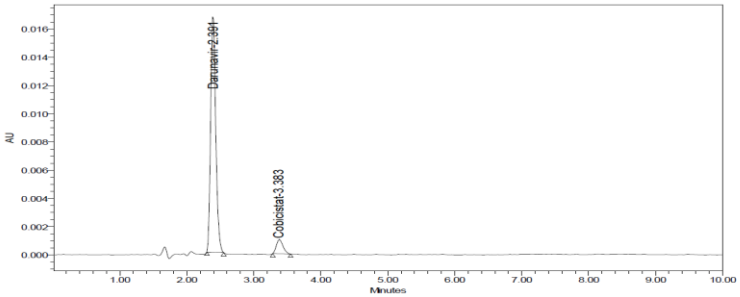

Figure 7. Chromatogram of Accuracy studies – 100% spiked level

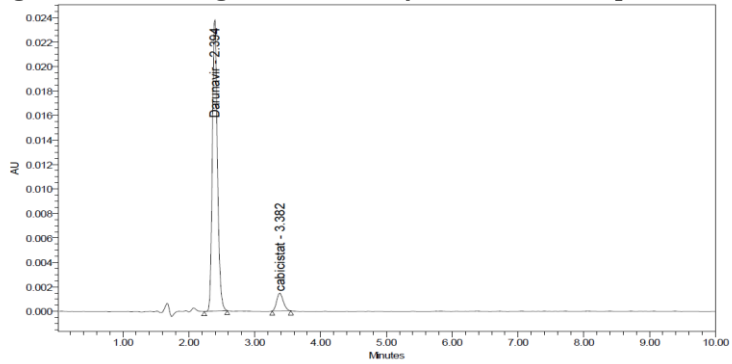

Figure 8. Chromatogram of Accuracy studies – 150% spiked level

Ruggedness: To evaluate the intermediate precision of the method, analysis was carried out using a different analyst. The precision of the method was evaluated by analyzing standard solutions of darunavir and cobicistat with a concentration of 160 µg/mL and 30 µg/mL, respectively. Six replicates were analyzed to determine the precision. The % RSD of peak areas was calculated and was found to be below 2.0 %. This indicates the precision of the method for the simultaneous estimation of darunavir and cobicistat.

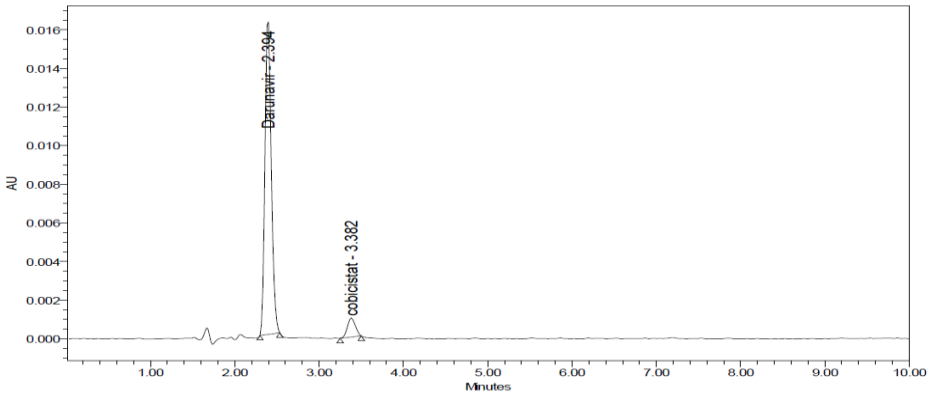

Figure 9. Chromatogram of Ruggedness studies

Robustness: The robustness of the method was studied by varying the chromatographic conditions with respect to the flow rate of the mobile phase and column temperature. The study was conducted at three different flow rates (0.9 mL/min, 1.0 mL/min and 1.1 mL/min) and at three different column temperatures (28 °C, 30 °C, 32 °C). The effect of these changes on the different chromatographic parameters was studied. The results are summarized in Table 3. Negligible difference was found in system suitability parameters for darunavir and cobicistat such as USP plate count, resolution and the USP tailing factor, therefore the method found to be robust.

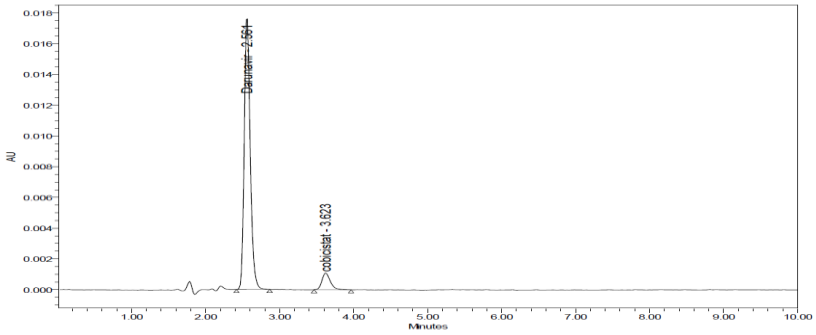

Figure 10.Chromatogram of Robustness studies – Flow rate 0.9 mL/min

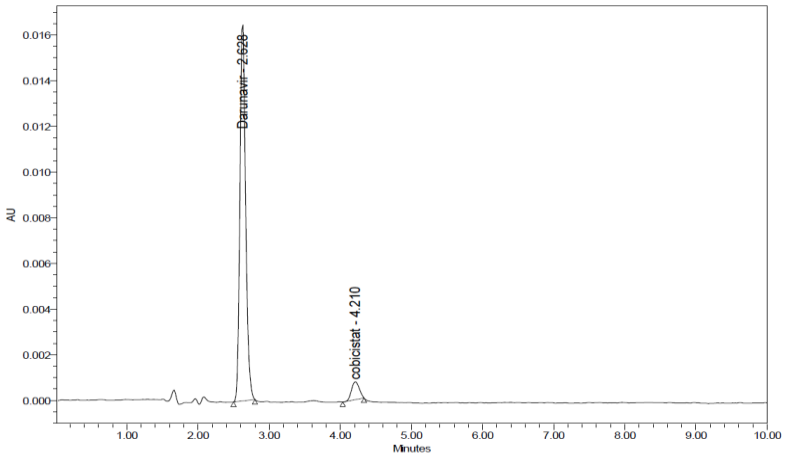

Figure 11.Chromatogram of Robustness studies – Column Temperature 28 °C

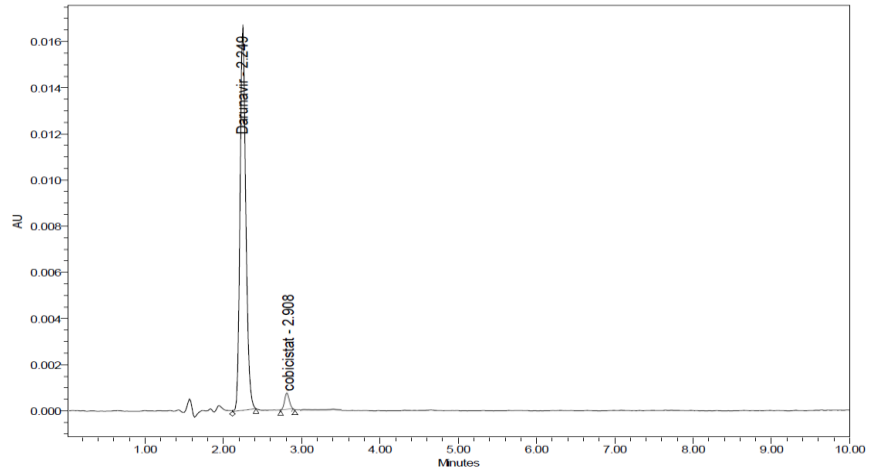

Figure 12.Chromatogram of Robustness studies – Flow rate 1.1 mL/min

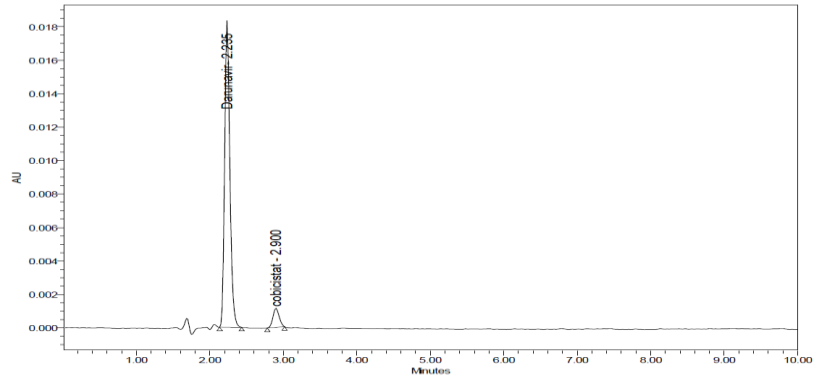

Figure 13. ***Chromatogram of Robustness studies – Column Temperature*** 32 °C

Limit of detection (LOD) and Limit of quantification (LOQ): The limits of detection and quantification were evaluated based on residual standard deviation of the response and the slope. The LOD and LOQ values for darunavir and cobicistat are presented in Table 2. The values indicate the adequate sensitivity of the method.

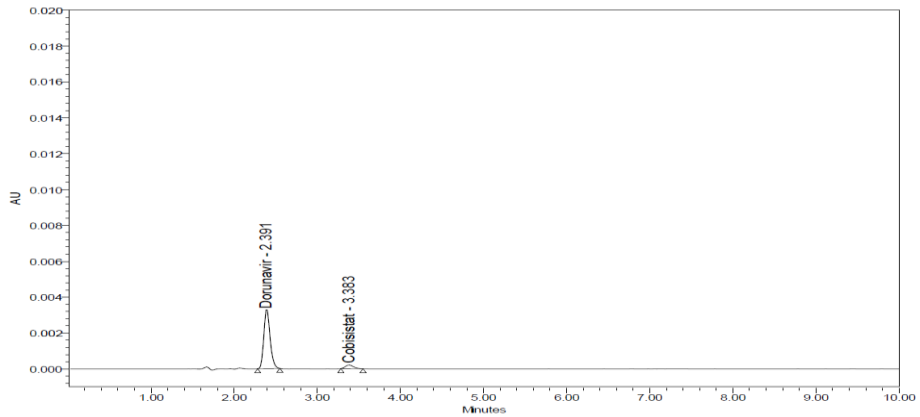

Figure 14. Chromatogram of LOD

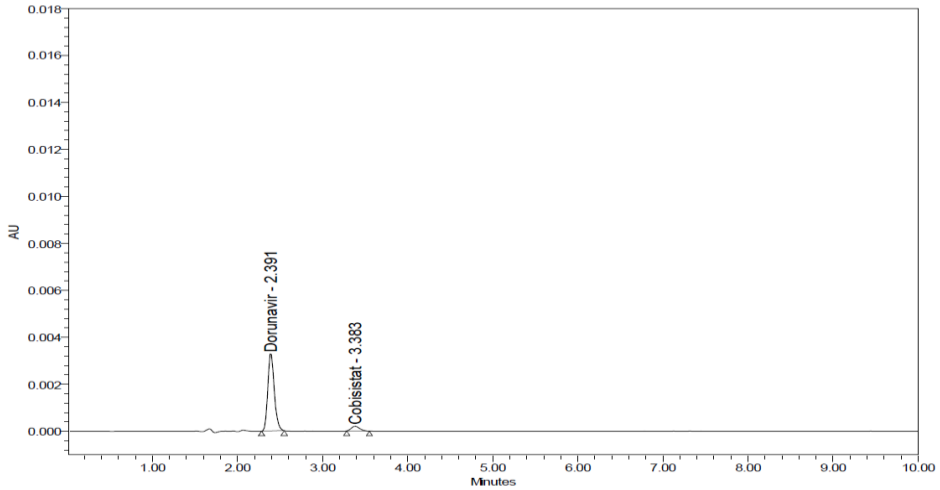

Figure 15. Chromatogram of LOQ

Specificity: The chromatograms of mobile phase blank, placebo blank, test sample (160 µg/mL darunavir and 30 µg/mL cobicistat) and standard (160 µg/mL darunavir and 30 µg/mL cobicistat) were compared to give reason for the specificity of the method. The method was specific & selective since excipients in the formulation and components of the mobile phase did not interfere in the simultaneous analysis of darunavir and cobicistat.

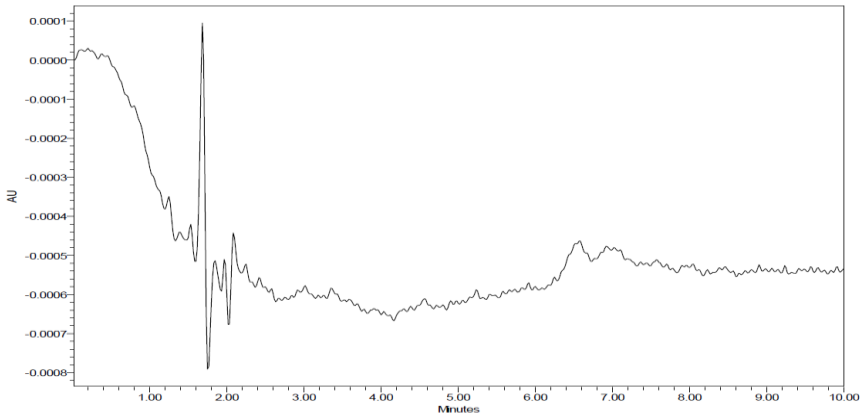

Figure 16. Mobile phase Blank

Forced degradation

Forced degradation studies were performed on tablet sample using different stress conditions such as acidic, basic, oxidative, thermal and photolytic stresses and then the samples are filtered through 0.45 µm membrane filter and subjected to HPLC analysis.

When darunavir and cobicistat was subjected to different forced degradation conditions (acid, base, oxidative, thermal, and photolytic), significant degradation was observed. The percentage of degradation and percent relative standard deviation values are summarized in Table 5. The degradants produced in all the forced degradations were well separated from darunavir and cobicistat. The method therefore proved to be stability-indicating.

Acidic degradation: Acidic degradation was carried out using 0.1 N HCl. For this, tablet powder equivalent to 800 mg of darunavir and 150 mg of cobicistat was taken in 100 mL volumetric flask. 10 mL of 0.1 N HCl was added and sonicated for 30 min. After completion of the stress, the solution was neutralized using 0.1N NaOH and filled up to the mark with mobile phase. The sample was injected into HPLC and analysed.

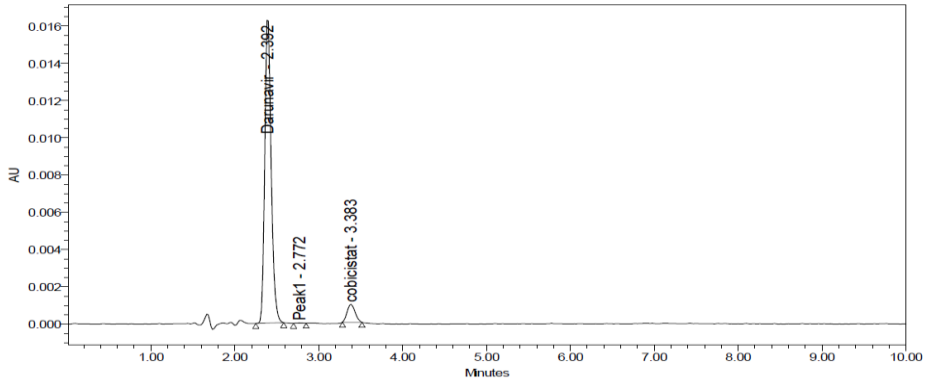

Figure 17. ***Acidic degradation***

Alkali degradation: Alkali degradation study was carried out using 0.1 N NaOH. For this, tablet powder equivalent to 800 mg of darunavir and 150 mg of cobicistat was taken in 100 mL volumetric flask. 10 mL of 0.1 N NaOH was added and sonicated for 30 min. After completion of the stress, the solution was neutralized by using 0.1 N HCl and filled upto the mark with mobile phase. The sample was injected into HPLC and analysed.

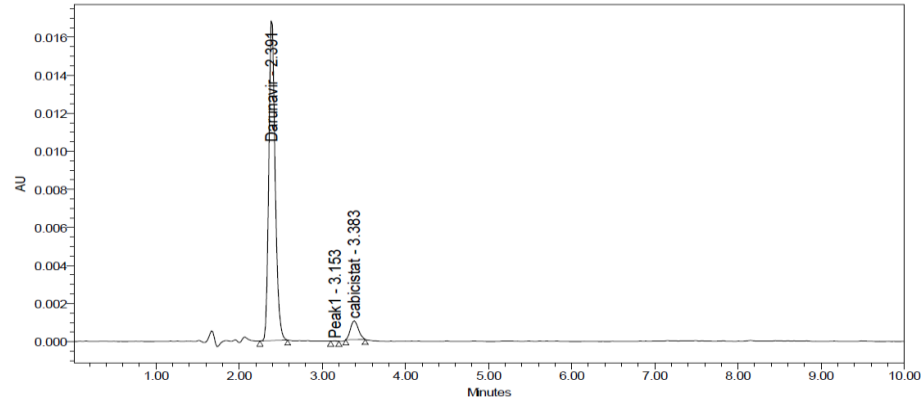

Figure 18. ***Alkali degradation***

Oxidative degradation: Oxidative degradation was carried out using 30 % H_2O_2. To perform this, tablet powder equivalent to 800 mg of darunavir and 150 mg of cobicistat was taken in 100 mL volumetric flask. 10 mL of 30 % H_2O_2 was added to it. The contents of the flask were sonicated for 30 min. After completion of the stress, the volume of the flask was made up to the mark with mobile phase. The sample was injected into HPLC and analysed.

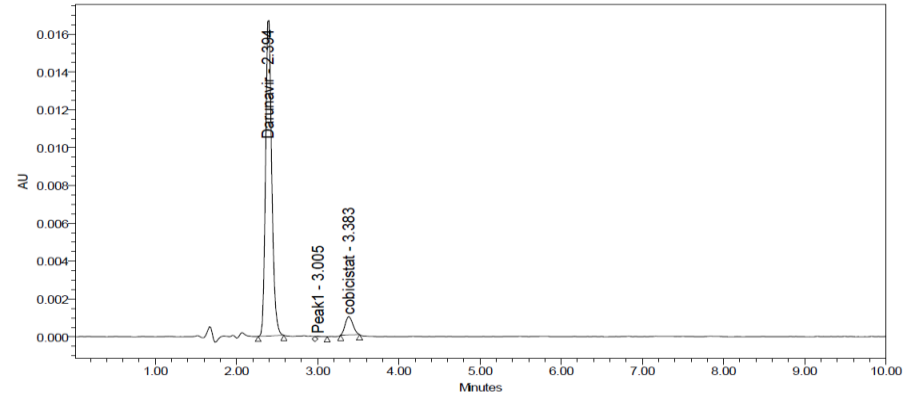

Figure 19. ***Oxidative degradation***

Thermal degradation: Thermal degradation was performed in hot air oven at 110°C. For this study, tablet powder equivalent to 800 mg of darunavir and 150 mg of cobicistat was taken in glass petri dish and placed in oven at 110 °C for 30 min. After specified time, the sample was cooled, transferred into a 100 mL volumetric flask and dissolved in 30 mL of mobile phase and the volume was made upto mark with mobile phase. The sample was injected into HPLC and analysed.

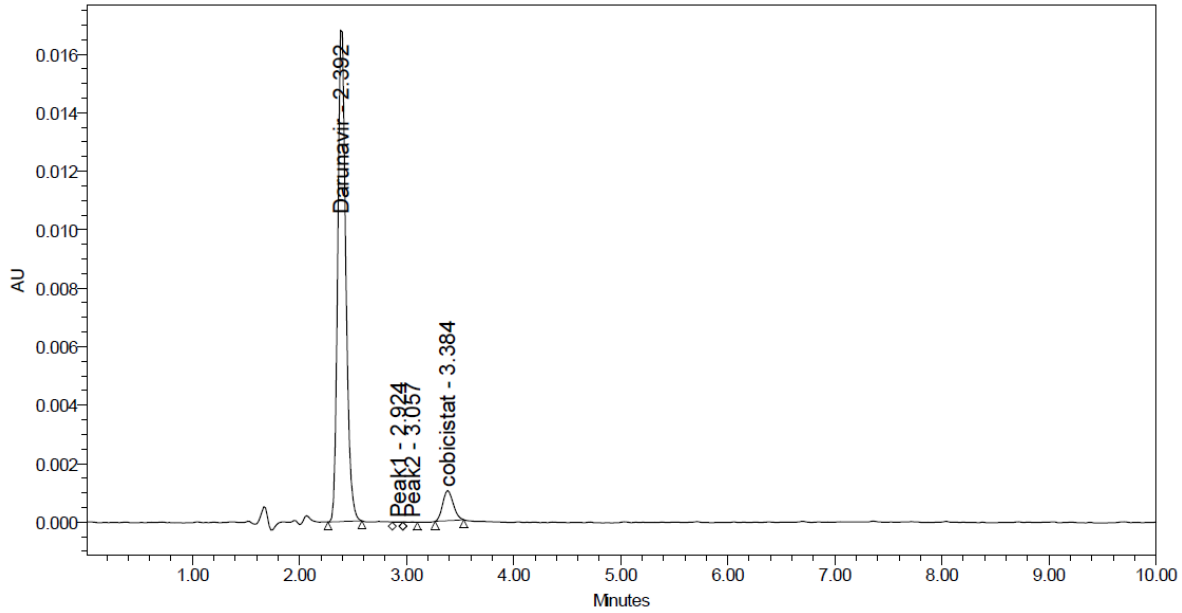

Figure 20. ***Thermal degradation***

Photolytic degradation: For photolytic degradation study, 800 mg of darunavir and 150 mg of cobicistat tablet powder was taken in glass petri dish and placed in the direct sunlight for 24 h. After completion of the stress, the drug sample was cooled, transferred into a 100 mL volumetric flask and dissolved in 30 mL of mobile phase and the volume was made upto mark with mobile phase. The sample was injected into HPLC and analysed.

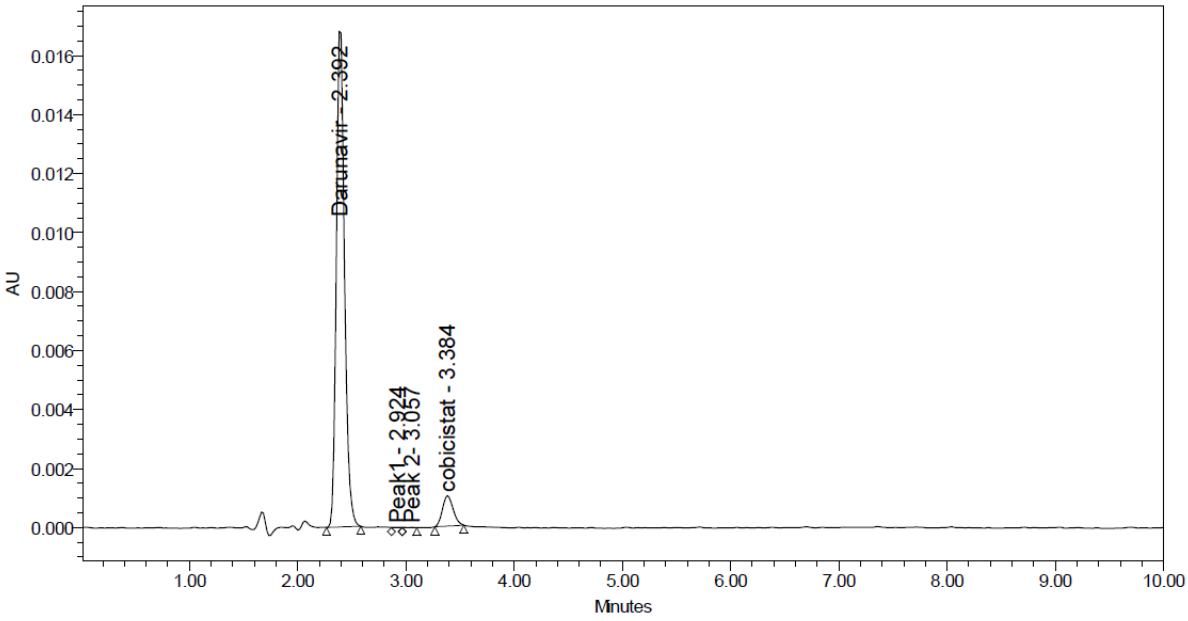

Figure 21. ***Photolytic degradation***

Table 1. Results of system suitability

Parameter	Darunavir	Cobicistat	Recommended Limits
Retention Time	2.401	3.374	----
% RSD	0.369	0.124	RSD ≤2
Tailing factor	1.17	1.32	≤ 2
Theoretical plates	3215	3468	> 2000

Table 2. Results of Linearity, LOD, LOQ and precision

Parameter	Darunavir	Cobicistat
Linearity (µg/mL)	50-250	10-50
Regression equation	y = 586.8x - 268.8	y = 249.9x + 37.4
Regression Coefficient	0.999	0.999
LOD (µg/mL)	0.220	0.097
LOQ (µg/mL)	0.733	0.323
RSD (%)	0.245	0.307

Table 3. Results of Robustness

Parameter	Investigated Value	Area	USP Plate Count	USP Tailing
		Darunavir		
Temperature	28	86017	4383.84	1.21
	30	86172	4560.55	1.22
	32	86652	4368.06	1.22
Flow rate (mL/min)	0.9	86680	4477.98	1.19
	1	86818	4487.83	1.18
	1.1	86585	4433.77	1.16
		Cobicistat		
Temperature	28	7508	5487.39	1.04
	30	7587	5495.89	1.02
	32	7576	5347.5	1.18
Flow rate (mL/min)	0.9	7534	5201.62	1.2
	1	7558	5746.69	1.08
	1.1	7517	5326.16	1.18

Table 4. Results of Accuracy studies

Spiked Level	Darunavir				Cobicistat			
	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean (%)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean (%)
50%	25	25.44	101.76		10	9.96	99.6	
50%	25	25.4	101.6	101.76	10	10	100	99.83
50%	25	25.48	101.92		10	9.99	99.9	
100%	50	50.46	100.92		20	19.99	99.9	
100%	50	50.61	101.22	101.14	20	19.96	99.8	99.81
100%	50	50.64	101.28		20	19.95	99.75	
150%	75	75.02	100.02		30	29.96	99.86	
150%	75	75.08	100.1	100.05	30	29.89	99.63	99.79
150%	75	75.03	100.04		30	29.97	99.9	

Table 5. Results of Degradation studies

Type of Degradation	Darunavir				Cobicistat			
	Peak area	Assay (%)	RSD (%)	Degradation (%)	Peak area	Assay (%)	RSD (%)	Degradation (%)
Undegraded	86056	100	0.23	0	7565.7	100	0.59	0
Acid	81872	94.9	0.32	5.1	7239	91.6	0.36	8.4
Base	81285	94.8	0.36	5.2	7298	93.6	0.51	6.4
Oxidative	82049	96	0.59	4	7267	92.6	0.24	7.4
Thermal	82411	95.4	0.42	4.6	7245	94.4	0.35	5.6
Photolytic	82185	96.3	0.36	3.7	7264	94.7	0.56	5.3

CONCLUSION

The developed stability indicating RP-HPLC method has been successfully applied for the simultaneous determination of darunavir and cobicistat in their combined dosage form. The method was found to be rapid, simple and accurate. When the developed method was completely validated, the results showed satisfactory data for all the method validation parameters. From the values percentage RSD, LOD and LOQ, it was found that the developed method is more precise and sensitive than the reported HPLC methods. So the proposed method can be easily and conveniently adopted for routine quality control analysis of darunavir and cobicistat.

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