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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DARUNAVIR AND COBICISTAT BY RP- HPLC METHOD

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ABSTRACT

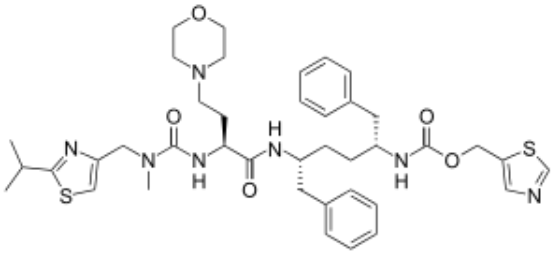
A new method was established for simultaneous estimation of Cobicistat and Darunavir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Cobicistat and Darunavir by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Cobicistat and Darunavir was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively.

Keywords: Cobicistat, Darunavir, RP-HPLC, Phosphate buffer and ACN.

INTRODUCTION

COBICISTAT

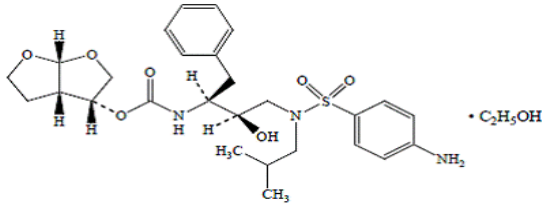
Cobicistat is a licensed drug for use in the treatment of infection with the human immunodeficiency virus (HIV). cobicistat is of interest for its ability to inhibit liver enzymes that metabolize other medications used to treat HIV. Cobicistat is a potent inhibitor of cytochrome P450 3A enzymes, including the important CYP3A4 subtype. It also inhibits intestinal transport proteins, increasing the overall absorption of several HIV medications,including gatazanavir, darunavir and tenofovir alafenamide fumarate. [1]



IUPAC Name
Thiazol-5-ylmethyl N-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl)methyl-methyl-carbamoyl]amino]-4-morpholino-butanoyl]amino]-5-phenyl pentyl]carbamate
Chemical formula : $C_{40}H_{53}N_7O_5S_2$.

DARUNAVIR

Darunavir (brand name **Prezista**, formerly known as TMC114) is a protease inhibitor drug used to treat HIV infection. Prezista is an OARAC recommended treatment option for treatment-naïve and treatment-experienced adults and adolescents. Developed by pharmaceutical company Tibotec. Darunavir is a second-generation protease inhibitor (PIs), designed specifically to overcome problems with the older agents in this class, such as indinavir. [3]



IUPAC Name
[(1S,2R)-3-[[(4aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3R,3aS,6aR)hexahydrofuro[2,3-b]furan-3-yl ester monoethanolate6-

Chemical formula
$C_{27}H_{37}N_3O_7S \bullet C_2H_5OH$

MATERIALS AND METHODS

i. Instrument
HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower

ii. Chemicals and Solvents
All the chemicals and solvents used were of analytical grade. Milli Q water was used throughout the experiment.

iii. Solutions
Preparation of Phosphate buffer (PH: 4.6)
Weighed 6.8 grams of KH2PO4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase
A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
Mobile phase is used as Diluent.

Preparation of the individual Cobicistat standard preparation
10mg of Cobicistat working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluent (Stock solution). Further 10 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent**.**

Preparation of the individual Darunavir standard preparation
10mg of Darunavir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume upto the mark with the diluant (Stock solution). Further 10 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted upto the mark with diluent**.**

Preparation of Sample Solution (Tablet)
Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Darunavir and Cobicistat (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume upto the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10ml volumetric flask and diluted upto the mark with diluent.

Chromatographic Conditions

Column	: Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio	: Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Detection wavelength	: 255nm
Flow rate	: 1ml/min
Injection volume	: 20µL
Column temperature	: Ambient
Rt	: 2.399 , 3.907

RESULTS AND DISCUSSION

Method Development
The chromatographic method development for the simultaneous estimation of Cobicistat and Darunavir were optimized by several trials for various parameters as different column, flow rate and mobile phase, finally the optimized chromatographic method was selected for the separation and quantification of Cobicistat and Darunavir in API and pharmaceutical dosage form by RP-HPLC method.[9]

Optimized Chromatographic Conditions
Column : Inertsil C18 5µm (4.6*250mm)
Mobile phase ratio : Phosphate buffer (0.05M) pH 4.6: ACN (30:70%v/v)
Detection wavelength : 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient
Rt : 2.399, 3.907

Fig 1: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Method Validation [8, 10]
Linearity
The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Results were shown in Table 1 and Linearity plot was shown in Fig 2 & Fig 3

Accuracy
The accuracy study was performed for 50%, 100% and 150 % for Cobicistat and Darunavir. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery and Results were shown in Table 2 and chromatograms were shown in Fig 4-6.

Precision
The precision study was performed for five injections of Cobicistat and Darunavir. Each standard injection was injected in to chromatographic system. The area of each Standard injection was used for calculation of % RSD. Results were shown in Table 3 and chromatogram was shown in fig 7.

Limit of Detection and Limit of Quantification
LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations,

LOD = 3.3 × σ / s
and
LOQ=10×σ/S.,

The results were presented in Table 5.
Where
σ = Standard deviation of the response
S = Slope of the calibration curve

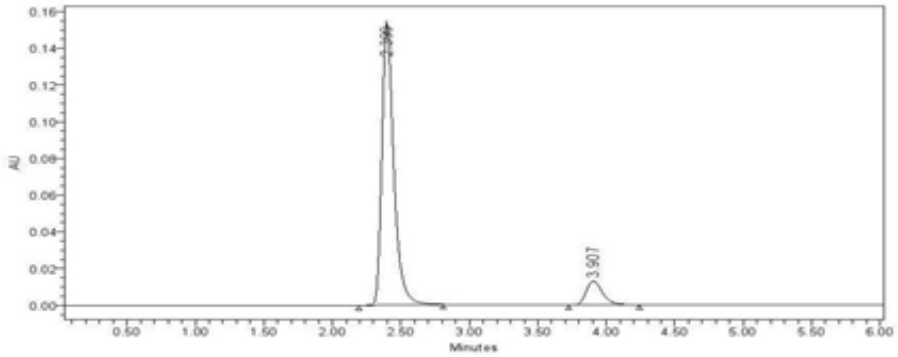


Fig. 1: Optimized chromatogram

Table 1: Linearity results of Cobicistat and Darunavir

	SampleName	Name	RT	Area	Height (µV)
1	Linearty 1	Cobicistat	2.309	1810101	145957
2	Linearty 1	Darunavir	4.307	1164173	75128
3	Linearty 2	Cobicistat	2.322	2044287	176935
4	Linearty 2	Darunavir	4.317	1342535	87703
5	Linearty 3	Cobicistat	2.324	2367133	206622
6	Linearty 3	Darunavir	4.323	1555931	101999
7	Linearty 4	Cobicistat	2.336	2602279	228576
8	Linearty 4	Darunavir	4.340	1777973	117084
9	Linearty 5	Cobicistat	2.345	2869778	259346
10	Linearty 5	Darunavir	4.340	1942319	129409

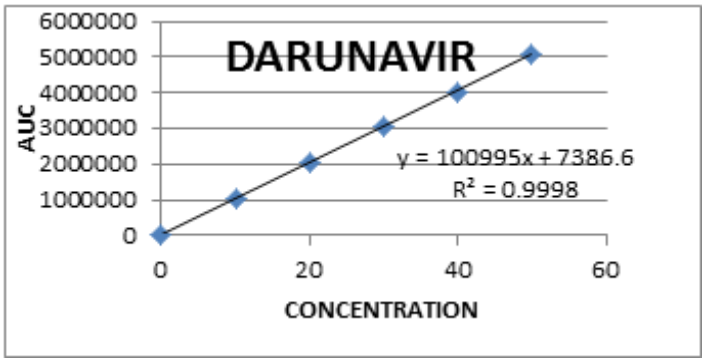


Fig. 2: Calibration curve of Darunavir

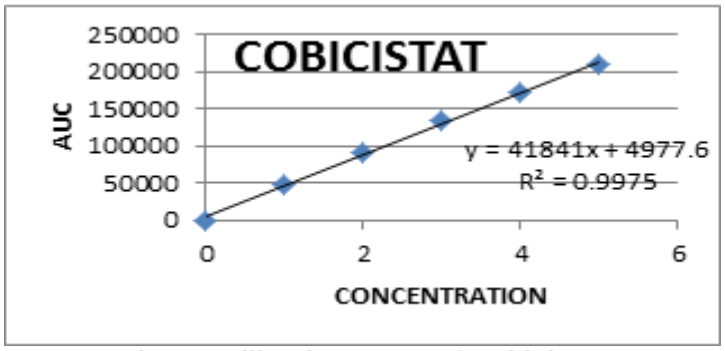


Fig. 3: Calibration curve of Cobicistat

Table 2: Accuracy data of Cobicistat and Darunavir

%Concentration (at specification Level)	Cobicistat			Darunavir		
	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Amount Added (ppm)	Amount Found (ppm)	% Recovery
50	5	5.0	101.3%	5	5.10	101.8%
100	10	9.94	99.4%	10	9.99	99.9%
150	15	14.8	99.2%	15	14.9	99.1%
Mean % Recovery			**100 %**			**100.5 %**

Acceptance Criteria

- The % Recovery for each level should be between 98.0 to 102.0%.

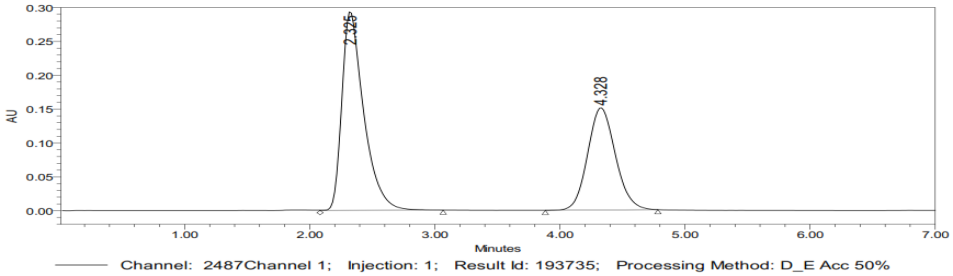


Fig. 4: Accuracy 50% Chromatogram

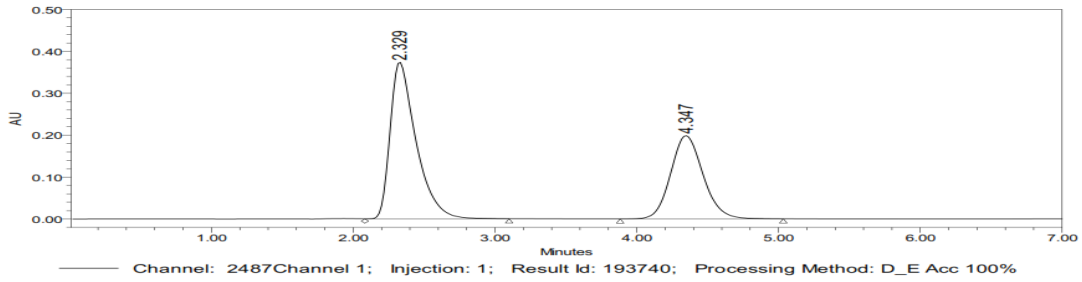


Fig. 5: Accuracy 100% Chromatogram

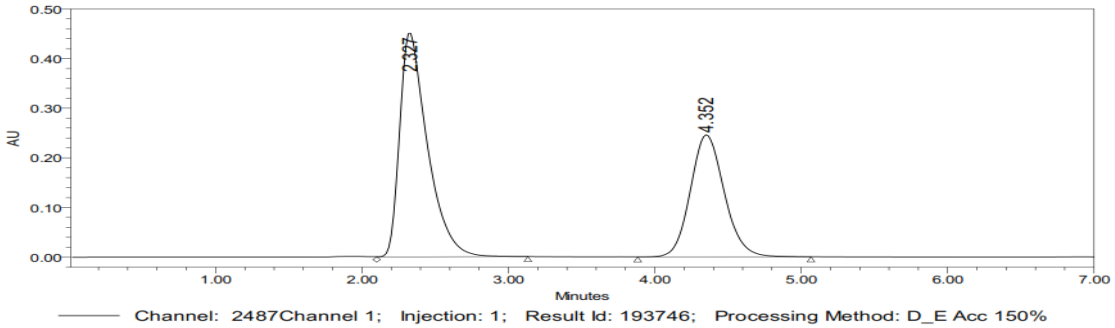


Fig. 6: Accuracy 150% Chromatogram

Table 3: Precession results of Cobicistat& Darunavir

Injection No.	Cobicistat		Darunavir	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	2.321	2235319	4.304	1501417
2	2.317	2240678	4.300	1486940
3	2.323	2249490	4.308	1490656
4	2.322	2245822	4.310	1487329
5	2.324	2251694	4.314	1490384
Mean		2244601		1491345
SD		6656.8		5881.4
%RSD		0.3		0.39

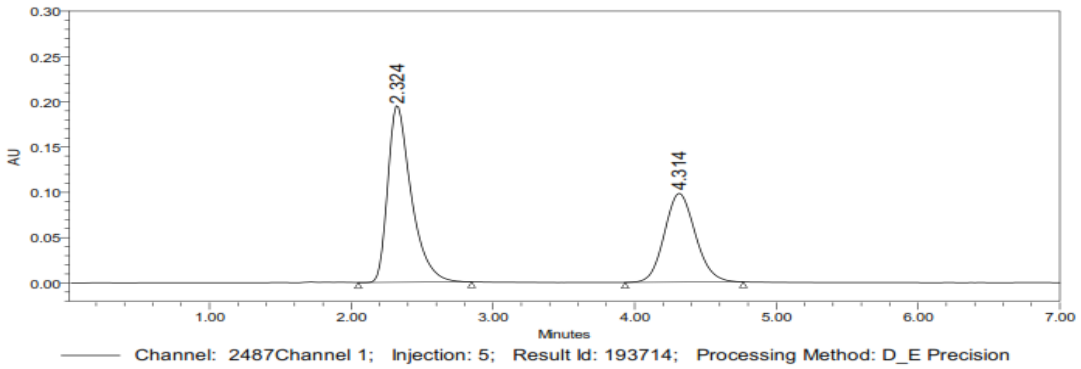


Fig. 7: Chromatogram for Precession

Acceptance Criteria

The % RSD for the area of five standard injections results should not be more than 2%

The Method precision study was performed for the %RSD of Cobicistat and Darunavir was found to be 0.3 and 0.3 (NMT 2).

Table: 4. Data table of LOD and LOQ for Cobicistat& Darunavir

Drug	LOD (µg/ml)	LOQ (µg/ml
Cobicistat	2.95	10.0
Darunavir	3.04	9.87

CONCLUSION

The retention times were found to be 2.399mins and 3.907mins. The % purity of Cobicistat and Darunavir was found to be 100.7% and 101.4% respectively. The system suitability parameters for Cobicistat and Darunavir such as theoretical plates and tailing factor were found to be 1.3, 5117.5and 1.4, 3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Cobicistat and Darunavir was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was 0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.95 and 3.04, and LOQ value was 9.87 and 10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Cobicistat and Darunavir in API and Pharmaceutical dosage form.

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