Research Article

RP-HPLC Method Development and Validation for the Simultaneous Estimation of Darunavir and Cobicistat in Bulk and in Formulation

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Received: 18-05-2022; **Revised:** 23-07-2022; **Accepted:** 01-08-2022; **Published on:** 15-08-2022.

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Darunavir and Cobicistat in Tablet dosage form. Chromatogram was run through Std BDS 250 x 4.6 mm, 5m. Mobile phase containing 40% KH2PO4 (0.01N): 60% Acetonitrile was pumped through column at a flow rate of 1.2 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 265 nm. Retention time of Darunavir and Cobicistat were found to be 2.091min and 2.516. %RSD of the Darunavir and Cobicistat was found to be within limits. %Recovery was obtained as 99.69% and 99.32% for Darunavir and Cobicistat respectively. LOD, LOQ values obtained from regression equations of Darunavir and Cobicistat were 0.24, 0.73 and 0.23, 0.69 respectively. Regression equation of Darunavir is y = 4484x+23981, and y = 12802x + 2205 of Cobicistat. So, the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Darunavir, Cobicistat, RP-HPLC, validation, Assay.

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DOI: 10.47583/ijpsrr.2022.v75i02.015

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v75i02.015

INTRODUCTION

Cobicistat is used in the treatment of 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 co administered agents that are metabolized by CYP3A enzymes. More specifically, cobicistat is indicated to increase systemic exposure of Darunavir 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]

Darunavir compound belongs to the class of organic compounds known as aminobenzene sulfonamides. These are organic compounds containing a benzene sulfonamide moiety with an amino group attached to the benzene ring.[2]

MATERIALS AND METHODS

Materials:

- Cobicistat and Darunavir pure drugs (API), Combination Cobicistat and Darunavir tablets, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid.

Instruments:

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultra sonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Cobicistat and Darunavir solutions.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 7.5 mg of Cobicistat, 40mg of Darunavir and transferred to 25ml volumetric flask and 3/4th of diluent was added to this flask and sonicated for 10 minutes. Flask was made up with diluent and labeled as Standard stock solution. (300µg/ml of Cobicistat and 1600µg/ml of Daruna)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (30µg/ml Cobicistat of and 160µg/ml of Daruna)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1250 tablet was transferred into a 500 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (300µg/ml of Cobicistat and 1600µg/ml of Darunavir)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (30µg/ml of Cobicistat and 160µg/ml of Darunavir)

RESULTS AND DISCUSSIONS

Optimized conditions

Chromatographic conditions:

Mobile phase: 40% KH2PO4 (0.01N): 60% Acetonitrile

Flow rate: 1.2 ml/min

Column: BDS C18 (4.6 x 250mm, 5µm)

Detector wave length: 265nm

Column temperature: 30°C

Injection volume: 10µL

Run time: **5** min

Diluent: Water and Acetonitrile in the ratio 50:50

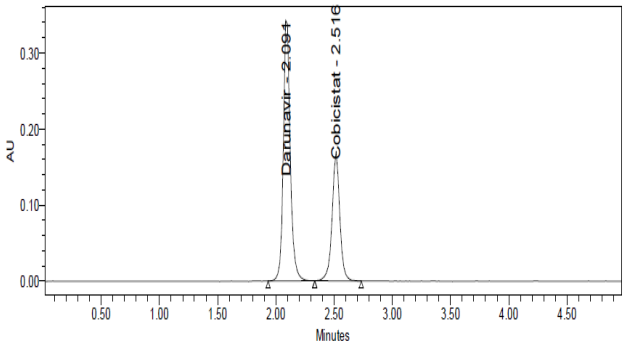

Figure 1: RP-HPLC Peak elution at 265nm

Observation: Darunavir and Cobicistat were eluted at 2.091 min and 2.516 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines

Table 1: System suitability parameters for Darunavir and Cobicistat

S no	Darunavir			Cobicistat			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.091	6224	1.16	2.516	7623	0.97	3.6
2	2.091	6267	1.17	2.516	7711	0.97	3.7
3	2.095	6419	1.12	2.517	7833	0.98	3.7
4	2.096	6475	1.11	2.520	7503	0.99	3.7
5	2.097	6628	1.12	2.521	7185	1.00	3.7
6	2.102	7099	1.13	2.523	7148	1.00	3.8

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Validation:

Specificity: Demonstration of specificity is done to confirm that the procedure is unaffected by the presence of impurities or excipients. This is performed by running blank concentration.

Discussion: Retention times of Darunavir and Cobicistat were 2.091 min and 2.516 min. respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.[3]

Linearity: Linearity is the property of a mathematical relationship or function which means that it can be graphically represented as a straight line. Linearity was studied by analyzing standard solutions covering the range of standard concentrations of sample solutions.

Table 2: Linearity table for Darunavir and Cobicistat.

Darunavir		Cobicistat	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
80	403628	15	196724
160	726942	30	385734
240	1134778	45	566905
320	1462711	60	778606
400	1804546	75	982654
480	2169227	90	1137306

Discussion: Six linear concentrations of Darunavir (80-480µg/ml) and Cobicistat (15-90µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Darunavir was y = 4484.x +23981 and of Cobicistat was y = 12802.x + 2205 Correlation coefficient obtained was 0.999 for the two drugs.[4]

Precision: Precision is a description of random errors, a measure of statistical variability. The Precision of the instrument was checked by repeated injection and measurement of peak areas and retention time of solution.

System Precision:

Table 3: System precision table of Darunavir and Cobicistat

S. No	Area of Darunavir	Area of Cobicistat
1.	1419216	770426
2.	1411865	774953
3.	1423920	774944
4.	1411456	775514
5.	1408184	770070
6.	1415089	773304
Mean	1414955	773202
S.D	5765.1	2407.15
%RSD	0.4	0.3

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.4% and 0.3% respectively for Darunavir and Cobicistat .As the limit of Precision was less than "2" the system precision was passed in this method.[5]

Intermediate precision (Day_ Precision):

Table 4: Intermediate precision table of Darunavir and Cobicistat

S. No	Area of Darunavir	Area of Cobicistat
1.	1419216	774953
2.	1404443	770376
3.	1418949	772061
4.	1413039	778923
5.	1404352	777687
6.	1428178	778147
Mean	1414696	775358
S.D	9329.0	3514.7
%RSD	0.7	0.5

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table.[6] Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% and 0.5% respectively for Darunavir and Cobicistat. As the limit of Precision was less than "2" the system precision was passed in this method.[7]

Accuracy: Accuracy is the degree of closeness of measurements of a quantity to that quantity's true value.

Discussion: Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.69% and 99.32% for Darunavir and Cobicistat respectively.[8,9]

Sensitivity:

a) LOD: It is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit.

$$LOD = (3.3 \times S.D.)/ slope$$

b) LOQ: It is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

$$LOQ = (10 \times S.D.)/ slope$$

Table 5: Sensitivity table of Darunavir and Cobicistat

Molecule	LOD	LOQ
Darunavir	0.24	0.73
Cobicistat	0.23	0.69

Robustness: It is the measure of a method remain unaffected by small deliberate changes in method parameters like flow rate and mobile phase composition ratio.

Table 6: Robustness data for Darunavir and Cobicistat.

S.no	Condition	%RSD of Darunavir	%RSD of Cobicistat
1	Flow rate (-) 1.1ml/min	0.3	0.4
2	Flow rate (+) 1.3ml/min	0.4	1.1
3	Mobile phase (-) 35B:65A	0.4	0.6
4	Mobile phase (+) 45B:55A	0.2	0.4
5	Temperature (-) 25°C	0.4	0.3
6	Temperature (+) 35°C	0.4	0.5

Discussion: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (35B:65A), mobile phase plus (45B:55A), temperature

minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Assay: Rhodes pharmaceuticals, bearing the label claim Darunavir 7.5mg, Cobicistat 325mg. Assay was performed with the above formulation. Average % Assay for Darunavir and Cobicistat obtained was 99.75 and 98.98% respectively

Table 7: Assay Data of Darunavir and Cobicistat

S.no	Standard Area Darunavir	Sample area Darunavir	% Assay Darunavir	Standard Area Cobicistat	Sample area Cobicistat	% Assay Cobicistat
1	1419216	1410823	99.61	770426	777852	100.50
2	1411865	1412031	99.69	774953	774355	100.05
3	1423920	1406703	99.32	774944	772076	99.75
4	1411456	1403104	99.06	775514	777687	100.48
5	1408184	1418178	100.13	770070	778147	100.54
6	1415089	1426370	100.71	773304	777454	100.45
Avg	1414955	1412868	99.75	773202	776262	100.30
SD	5765.1	8351.4	0.59	2407.15	2477.6	0.32
%RSD	0.4	0.6	0.59	0.3	0.3	0.32

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Darunavir and Cobicistat in Tablet dosage form. Retention time of Darunavir and Cobicistat were found to be 2.091min and 2.516. %RSD of the Darunavir and Cobicistat was found within limits. %Recovery was obtained as 99.69% and 99.32% for Darunavir and Cobicistat respectively. LOD, LOQ values obtained from regression equations of Darunavir and Cobicistat were 0.24, 0.73 and 0.23, 0.69 respectively. Regression equation of Darunavir is y = 4484x+23981, and y = 12802x + 2205 of Cobicistat. Retention times were decreased and that run time was decreased, so the method developed was simple, accurate and precise.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.