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Research Paper

RP-HPLC Method Development and Validation for The Simultaneous Estimation of Cobicistat and Darunavir in Bulk and Solid Dosage Forms

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ABSTRACT

A rapid, sensitive, and accurate RP-HPLC method was developed for the identification and quantification of Cobicistat and Darunavir using a Shimadzu HPLC system. Chromatographic separation was achieved on an Inertsil ODS-C18 column (250 × 4.6 mm, 5 μm particle size) with a flow rate of 1.0 mL/min. The mobile phase consisted of degassed and filtered Methanol: Water (68:32, v/v). Detection was performed at 245 nm, providing reliable resolution and measurement of both analytes

INTRODUCTION

Prezcobix is a fixed-dose prescription medication used in combination with other antiretroviral agents to treat HIV-1 infection in adults and pediatric patients. It combines two drugs into one once-daily tablet: **Darunavir** (a protease inhibitor that stops the virus from replicating) and **Cobicistat** (a CYP3A inhibitor that boosts darunavir's effectiveness)

Cobicistat (6-7) is a pharmacokinetic enhancer (or "booster") with no antiviral activity of its own. Its primary mechanism of action is the potent, mechanism-based inhibition of the cytochrome P450 3A (CYP3A) enzyme system in the liver and

intestines, which slows the metabolism of certain co-administered HIV medications. Cobicistat selectively and irreversibly binds to CYP3A enzymes (primarily CYP3A4). These enzymes are responsible for breaking down many antiretroviral drugs. By inhibiting them, cobicistat drastically reduces the breakdown of these drugs.

Darunavir (8) is an HIV-1 protease inhibitor that stops the virus from replicating. It selectively binds to the active site of the HIV protease enzyme and prevents its dimerization. This blocks the cleavage of viral polyproteins, resulting in the production of immature, non-infectious viral particles.

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II. MATERIALS AND METHODS

Preparation of Stock solution: 10 mg each of Cobicistat and Darunavir was accurately weighed and is transferred into neat and dry volumetric flasks of 10ml. About 7ml of methanol was added and allow to sonicate to remove the air bubbles formed in it, which is again make up to mark with methanol to give 1000 μ g/ml or 1mg/ml.

Preparation of working solution: From the stock solution 0.15ml of Cobicistat and 0.795ml of Darunavir are taken to the 10ml volumetric flask which is again diluted with methanol up to the mark to get 15 μ g/ml Cobicistat and 79.5 μ g/ml Darunavir.

III. RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (1-5).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Cobicistat (15 μ g/ml) and Darunavir (79.5 μ g/ml) were injected into the HPLC system. The peak purity data of Cobicistat and Darunavir were compared. There should not be any interference at the retention time of the main peaks.

Linearity: Linearity for the drugs Cobicistat and Darunavir (9-12) was determined by preparing the standard solutions at five concentrations levels in the range of 5-25 μ g/ml of Cobicistat and 26.23-131.90 μ g/ml of Darunavir from stock solution. The linearity charts of Cobicistat and Darunavir was shown in the figure no 2&3. The correlation coefficient was found to be 0.9998 and 0.9988 for

Cobicistat and Darunavir respectively. Linearity results were tabulated in table 2.

Accuracy: Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100% and 150%) and there by analyzed for %RSD which should not be more than 2.0. The % recovery was calculated and was found to be 99.67 and 99.6 for Cobicistat and Darunavir respectively. The results were reported in table no. 3 & 4.

Precision: The precision (13-16) of the analytical method was studied by injecting five replicates of standard containing 15 μ g/ml of Cobicistat and 79.5 μ g/ml Darunavir which were injected into HPLC system. The % RSD was calculated and the results were reported in the table no. 5.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection was defined as the concentration which yields a signal - to - noise ratio 3:1 where as the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to - noise ratio 10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 6.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no. 7.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 8.



Table 1: Optimised Chromatographic Conditions

Parameter	Conditions
Flow rate	1ml/min
Column	Inertsil – C18, ODS (250x4.6mm, 5μ)
Detector wave length	245nm
Column temperature	35°C
Mobile phase	Degassed methanol: water (68:32)
Injection volume	10μl
Run time	6 min
Retention time	2.121 min for Cobicistat 3.643 min for Darunavir

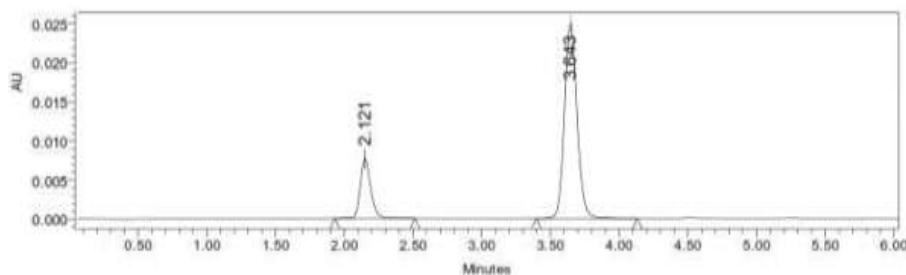


Figure 1: Optimized Chromatogram

Table 2: Linearity data of Cobicistat and Darunavir

Cobicistat		Darunavir	
Conc (μg/ml)	Peak area	Conc (μg/ml)	Peak area
0	0	0	0
5	135005	26.23	489094
10	277120	52.47	1149397
15	405128	79.50	1657592
20	534643	105.70	2150412
25	672357	131.90	2748444

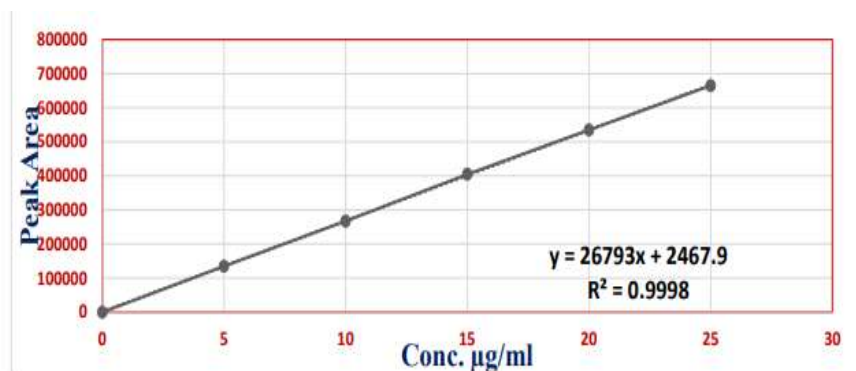
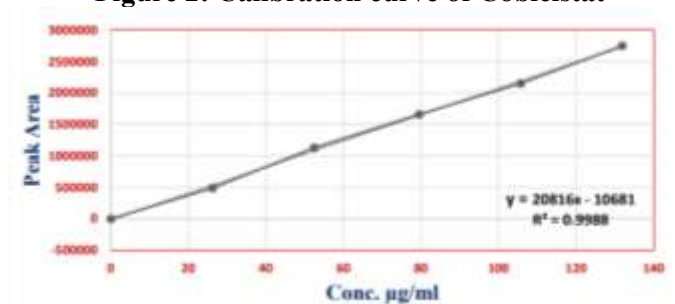


Figure 2: Calibration curve of Cobicistat**Figure 3: Calibration curve of Darunavir****Table 3: Accuracy Data of Cobicistat**

% of spiked level	Amount added (µg)	Amount found (µg)	% Recovery	Statistical Analysis of % Recovery
50% Injection - 1	7.5	7.477	99.70	MEAN=99.8 %RSD=0.66
50% Injection - 2		7.537	100.5	
50% Injection - 3		7.44	99.2	
100 %Injection - 1	15	14.91	99.4	MEAN=99.3 %RSD=0.21
100 %Injection - 2		14.86	99.1	
100%Injection - 3		14.92	99.5	
150%Injection - 1	22.5	22.39	99.53	MEAN=99.9 %RSD=0.35
150% Injection - 2		22.53	100.16	
150% Injection - 3		22.52	100.12	

Table 4: Accuracy Data of Darunavir

% of spiked level	Amount added(µg)	Amount found(µg)	% Recovery	Statistical Analysis of % Recovery
50% Injection - 1	39.73	39.71	100.75	MEAN=99.9 %RSD=0.92
50% Injection -2		39.65	99.31	
50% Injection - 3		39.60	99.02	
100 % Injection - 1	79.5	79.48	99.70	MEAN=99.0 %RSD=0.41
100 % Injection - 2		79.54	100.30	
100% Injection - 3		79.42	99.50	
150% Injection - 1	119.25	119.29	100.21	MEAN=99.9 %RSD=0.31
150% Injection - 2		119.23	99.61	
150% Injection - 3		119.27	100.10	

Table 5: Precision data of Cobicistat

Injection	Peak areas of Cobicistat	Peak areas of Darunavir
1	407673	1628262
2	406506	1615796
3	402118	1617864
4	400459	1618493
5	405412	1619626
Mean	404433.6	1620008.2
SD	3037.485	4819.422
% RSD	0.75	0.30

Table 6: LOD and LOQ data of Cobicistat and Darunavir

Drug Name	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
Cobicistat	0.5	1.5
Darunavir	9.1	27.7

Table 7: Robustness data of Cobicistat and Darunavir

S No	Drug Name	Condition	Peak area	Tailing factor
1	Cobicistat	Decreased Flow rate of 0.8 ml/min	398841	0.9
2		Increased Flow rate of 1.2 ml/min	389947	1.1
3	Darunavir	Decreased Flow rate of 0.8 ml/min	1613422	0.9
4		Increased Flow rate of 1.2 ml/min	1619138	0.8

Table 8: Assay data Cobicistat and Darunavir

S. No	Peak areas of Cobicistat	% Assay	Peak areas of Darunavir	% Assay
1	406538	99.12	1609924	99.73
2	409975		1601840	
3	402283		1602832	
4	406538		1609924	
5	409975		1601840	



6	402911		1603821	
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CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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