Research Article

A New and Validated Stability Indicating RP-HPLC Analysis of Darunavir and Cobicistat in Bulk Drug and Tablet Dosage Form

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Accepted on: 29-11-2015; **Finalized on:** 31-12-2015.

ABSTRACT

A New, Simple, Accurate and Reproducible RP-HPLC method has been proposed for the simultaneous determination of Darunavir and Cobicistat in tablet dosage forms. The present formulation is a new combination for antiretroviral therapy requiring a simple RP-HPLC method development. Hence, Chromatography was carried out on Thermosil ODS C-18 Column (250x4.6mm) having 5 um particle size, using a mobile phase mixture of Phosphate buffer pH 7.0 and acetonitrile in the ratio 45:55. The Pump was calibrated to give flow rate of 1ml/min of the respective mobile phase. The detection was made at the lambda max of 253 nm. The elution time was observed at 2.3 and 3.5 mins for Darunavir and Cobicistat respectively. The calibration curves were linear over the range of 160-480 mcg/ml and 30-90 mcg/ml for both Darunavir and Cobicistat respectively. The proposed method was validated as per ICH guidelines and subsequently stress degradation studies were also performed on the API. The method was found to be suitable for routine quality control analysis of the drugs in bulk and tablet dosage forms.

Keywords: New, RP-HPLC, Darunavir and Cobicistat, Validated, Tablet Dosage form.

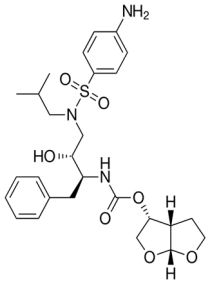
INTRODUCTION

Darunavir Ethanolate (DRV), (3R, 3AS, 6ar)-hexahydrofuro [2, 3-b] furan-3-yIN-((1S, 2R)-1-benzyl-2-hydroxy-3-(N (1)-isobutylsulfanilamido)propyl) carbamate is a protease inhibitor used to treat HIV infection Fig1(a). It is a second generation Protease inhibitor, designed specifically to overcome problems with the older agents in this class such as Indinavir. Early Protease inhibitors often have severe side effects and drug toxicities; also they require a higher therapeutic dose and are expensive in the making.

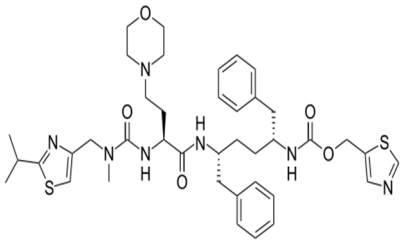
It was developed to increase interactions with HIV Protease and to be more resistant against HIV- 1 protease mutations. This drug is used in combination with other HIV medications to help control HIV infection so that the immune system can work better. This lowers your chance of getting HIV complications (such as new infections, cancer) and improves one's quality of life.

Cobicistat (CBST) also known as 1, 3-thiazol-5-ylmethyl N-[(2R, 5R)-5-[[(2S)-2-[[methyl-[(2-propan-2-yl-1, 3-thiazol-4yl) methyl] carbamoyl] amino]-4-morpholin-4-ylbutanoyl] amino] 1,6diphenylhexan2yl] carbamate. Fig 1(b). On the other hand is of interest due to its ability to inhibit liver enzymes that metabolize other medications used to treat HIV, notably Darunavir.

They are available as a fixed-dose combination of Cobicistat and Darunavir under the brand name Prezcobix, by Janssen Therapeutics. Thorough Literature survey has revealed that individual analysis of Darunavir using UV-Spectroscopy[11] and RP-HPLC[10-14], a bioanalytical[15] work employing RP-HPLC on Darunavir has been developed. In the case of Cobicistat several RP-HPLC methods on single[4-6] and Combination[7-8] have been reported. But no work on the present combination has been proposed. Hence the present method is an attempt towards developing a validated stability indicating RP-HPLC method for the combination of Darunavir and Cobicistat.



(a)



(b)

Figure 1: Structure of **(a)** Darunavir and **(b)** Cobicistat

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MATERIALS AND METHODS

Reagents and Chemicals

Darunavir Ethanolate (DRV) and Cobicistat (CBCST) pure powder with 99.6% and 99.2 % purity were kindly received as gift samples by Chandra Labs. Pvt. Ltd. All the reagents used were of Analytical grade purchased from S.D. Fine chemicals, Mumbai, India and used without further purification. Darunavir and Cobicistat tablets (800 mg and 150 mg) respectively are manufactured under the brand name of Prezcobix and were purchased from the local Market.

Instrumentation and Chromatographic Conditions

Analysis was performed with a Shimadzu LC-20 AT VP separation model equipped with Spinchrome software, and a loop of injection capacity of 20 ul with a Hamilton syringe and a UV-VIS detector. The wavelength was set at 253 nm. Compounds were eluted using a Thermosil ODS C-18 COLUMN (250x4.6mm) having 5 um particle size under reversed phase conditions. The mobile phase used was a mixture of 550 volumes of Phosphate buffer Ph 7.0 and 450 volumes of acetonitrile. The mobile phase was mixed and sonicated for 10 min to remove gases.

Preparation of Standard Solutions and Calibration Curve

Weigh accurately 8 mg of Darunavir and 1.5 mg of Cobicistat in 25 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. This solution contains 320 µg/ml of Darunavir and 60 µg/ml of Cobicistat. Further dilutions were prepared for the calibration curve.

Preparation of Test Solution

10 tablets each containing Darunavir 800 mg and Cobicistat 150 mg were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solution of Darunavir and Cobicistat was prepared by dissolving weight equivalent to 800 mg of Darunavir and 150mg of Cobicistat dissolved in sufficient mobile phase. The solution was sonicated for 5 mins and then filtered using 0.45-micron syringe filter and diluted to 25 ml with mobile phase. This solution contains 320 µg/mL of Darunavir and 60 µg/mL of Cobicistat. This solution is used for recording chromatogram.

Validation Parameters

The proposed method was validated as per ICH Q2 (R1) guidelines.

Degradation Studies

The Drug solutions in appropriate concentrations were evaluated for degradation studies. Standard procedures for stress degradation studies were referred and followed. After subsequent treatment of the sample solutions, a predestinated volume was injected into the system and the chromatograms were recorded to determine the sample stability.

Alkaline Hydrolysis

1 ml of working solution of Darunavir and Cobicistat was mixed with 1 ml of 1 N NaOH solution. This solution was refluxed at 80 °C for 5 hr. After alkaline treatment there was no degradation peak observed.

Acidic Hydrolysis

1 ml of working solution of Darunavir and Cobicistat was mixed with 1 N HCl acid and refluxed at 80 °C for 5 hr. The resultant solution was analyzed and no significant changes were observed.

Neutral Hydrolysis

1 ml of working solution of Darunavir and Cobicistat was mixed with 9 ml water and kept in dark place for 24 hrs. The resultant solution was analysed for degradation products.

Oxidation

1 ml of working solution of Darunavir and Cobicistat was mixed with 1ml of 30 % H_2O_2 and 8 ml of methanol. The solution was boiled at 80 °C for 5 hr and analysed for degradation products.

Degradation under Dry Heat

Dry heat studies were performed by keeping drug sample separately in Hot air oven for a period of 24 hours at a temp of 105 °C. A sample was withdrawn after 24 hours dissolved in methanol to get a solution of 100 mcg/ml and analysed for degradation products.

Photo-degradation Study

The photochemical stability of the drug was also studied by exposing the sample solution to UV light for 7 days at upto 200 watt hours/square meter and subsequently to cool fluorescent light to achieve an illumination of 1200 Lux Hrs. The solutions of Darunavir and Cobicistat were analysed for degradation studies.

RESULTS AND DISCUSSION

Method Development

Repeated trials employing different mobile phases and different compositions of mobile phase as per literature survey were tried and tested. The Ideal mobile phase was finally found to be that of Phosphate Buffer and Acetonitrile in the ratio of 55:45 respectively. The resulting mobile phase gave satisfactory elution results with good resolution for both Darunavir and Cobicistat. Increasing or decreasing the PH and flow rate of the mobile phase by 0.2±/ml/min, did not show significant change in the retention time of the analyte. The elution time for both the drugs i.e Darunavir and cobicistat was found to be 2.3 and 3.5 mins respectively, using a Thermosil ODS C-18 Column (250x4.6mm) having 5 µm particle size column. The Chromatographic device was maintained at a flow rate of 1ml/min. The injection Volume was 20 µl. The above optimized method was validated as per standard ICH and USP guidelines for

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routine analysis and quality control. Fig.2 represents a chromatogram of the standard drugs.

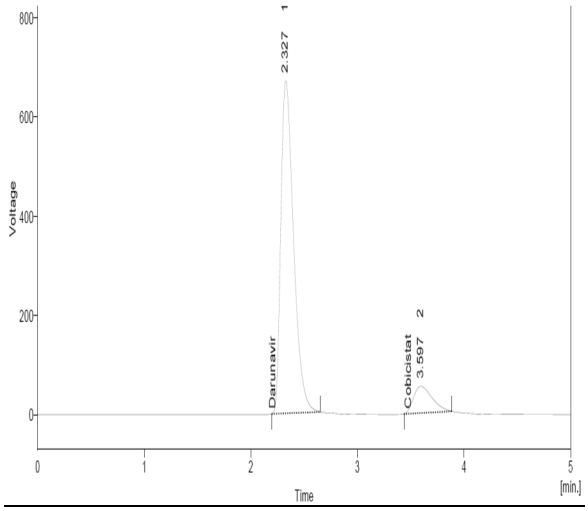

Figure 2: Chromatographic representation of Darunavir and Cobicistat standard.

System Suitability Parameters

System suitability was carried out with a solution of 100 % concentration having 100 µg/ml of Darunavir and Cobicistat into the chromatographic system. The solution was injected six times into the chromatographic system. Data for system suitability studies are given in Table 1 & Table 2.

Linearity

Calibration Curves were obtained from a graphical plot between peak area and concentration of the drug by subjecting to regression analysis and correlation coefficients. Table 3 represents the linearity of the proposed method.

Precision

Precision of the method was fixed by the repeated analysis of test sample six times. The % RSD values were found to be satisfactory. The acceptable % RSD values indicate that the drugs showed good agreement with the label claim, hence the method was precise.

Intraday and Interday Precision of the method was also performed by preparing six (n=6) replicate samples and analyzed on same day for intraday and on different days for interday precision. The peaks were recorded and % RSD was calculated for both the analytes under study.

The % RSD of Intraday and Inter day precision was calculated and the validation results are summarized in table 3.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to the pre-analyzed sample solution at three different levels 80%, 100%, 120%. The percentage recoveries were calculated, results of which are summarized in table 3.

LOD and LOQ

LOD and LOQ were calculated using the equation 3.3 σ/S and 10 σ/S respectively where **"σ"** is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD value for both Darunavir and Cobicistat was detrmined to be 19.07mcg/ml and 10mcg/ml respectively. The LOQ was found to be 57.78 mcg/ml and 2.47 mcg/ml for Darunavir and Cobicistat respectively.

Robustness

As defined by ICH, the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters in order to establish an indication of its reliability during routine usage of the method for quality control. Robustness was performed by injecting standard and sample solutions in the prescribed chromatographic conditions with minute variations in the volume of mobile phase composition and flow rate in the range of ± 2%. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method was robust in nature. Results, are presented in Table 6.

Specificity

Specificity was tested against standard compounds and against potential excipients. Specificity was determined by comparing the responses of standard and sample solution. No interferences in the form of extra peaks or alterations in retention times were observed.

Assay

10 Tablets were weighed and the average weight was calculated, the tablets were crushed into fine powder then the weight equivalent to 1 tablet was transferred into a 25 ml volumetric flask, Tablet stock solution of Darunavir and Cobicistat (150 µg/ml) was prepared by dissolving weight equivalent to 800 mg of Darunavir and 150 mg of Cobicistat dissolved in sufficient mobile phase. Later, the solution was sonicated for 10 mins and filtered using 0.45micron filter and the solution was diluted to 25 ml with mobile phase. The validated HPLC method was adopted for the quantification of Darunavir and cobicistat in their combined pharmaceutical dosage form and typical chromatograms of the formulation were recorded. The results of assay are given in Table.

The contents of the pharmaceutical dosage form were found to be within the range of 100 ±2% with RSD less than 2% which shows that the method can be used for routine analysis in Quality control labs.

The results for assay are shown in table 4 & 5.

Forced Degradation Studies

Forced degradation studies were performed to demonstrate the stability and sensitivity of the sample under stress conditions like hydrolysis, dry heat,

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oxidation, UV light and Photolysis. The stress degradation studies indicated that Darunavir and Cobicistat are susceptible to degradation conditions. Table No 7 presents the observations for stability study.

Table 1: System Suitability Parameters for Darunavir

Injection	Retention Time	Peak Response	Theoretical Plates	Tailing Factor
1	2.320	5593.35	2775	1.58
2	2.327	5626.64	3373	1.56
3	2.320	5599.36	2764	1.50
4	2.323	5600.89	2769	1.60
5	2.322	5606.28	2776	1.60
AVG	2.3224	5605.304		
SD	0.00288	12.785		
% RSD	0.124	0.23		

Table 2: System Suitability parameters for Cobicistat

Injection	Retention Time	Peak Response	Theoretical Plates	Tailing Factor
1	3.529	707.341	2778	1.60
2	3.531	692.140	2836	1.53
3	3.537	687.020	2854	1.55
4	3.530	696.71	2847	1.56
5	3.540	700.09	2836	1.51
AVG	3.5334	696.66		
SD	0.0048	7.733		
% RSD	0.14	1.11		

Table 3: Summary of Validation Parameters

S.No	Validation Parameter	Darunavir	Cobicistat
1	***Linearity Equation***	Y=12.85X+1300	Y=8.989+100.4
2	***(r^2)***	0.992	0.992
3	***Range***	160-480 mcg	30-90 mcg
	Precision (%RSD)		
4	***Intraday***	0.20 *	0.86 *
5	***Interday***	0.22 *	1.00 *
6	***Accuracy (% recovery)***	99.30,100.53,100.24	100.94,100.04,100.88
7	***LOD***	19.07 mcg/ml	10 mcg/ml
8	***LOQ***	57.78 mcg/ml	2.47 mcg/ml
9	***Specificity***	Specific	Specific
10	***Robustness***	Robust	Robust

* RSD of six (n=6) replicate samples.

Table 4: Assay of Darunavir and Cobicistat by RP-HPLC

S.NO	Darunavir				Cobicistat			
	Standard Rt	Sample Rt	Standard Area	Sample Area	Standard Rt	Sample Rt	Standard Area	Sample Area
1.	2.327	2.317	5588.996	5592.200	3.523	3.530	680.249	672.536
2.	2.326	2.322	5600.893	5596.716	3.520	3.522	687.020	675.717
3.	2.323	2.324	5600.837	5599.365	3.537	3.533	678.819	678.130
Mean			5596.909	5596.093			682.029	675.461
Std.Dev			6.85263	3.622			4.380	2.805
%RSD			0.12	0.06			0.64	0.42

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Table 5: Assay of Darunavir and Cobicistat by RP-HPLC

S.NO	Darunavir		Cobicistat	
1.	Sample Area	5596.093	Sample Area	675.461
2.	Standard Area	5596.909	Standard Area	682.029
3.	Standard. WT	8 mg	Standard. WT	1.5 mg
4.	Sample.WT	9.92 mg	Sample.WT	9.92 mg
5.	Label Claim	800 mg	Label Claim	150 mg
6.	Avg.WT	992 mg	Avg.WT	992 mg
7.	Std.Purity	99.2	Std.Purity	99.3
8.	%Assay	99.24	%Assay	99.06

Table 6: Robustness testing of the method

Parameter	Measurement Value	%RSD
Flow rate	0.8	1.314
	1.0	1.595
	1.2	1.545
Detection Wavelength	251	1.517
	253	1.033
	255	1.898
pH	6.5	1.314
	7.0	1.595
	7.5	1.545

Table 7: Observations for Stress degradation studies

S.NO	Injection	% Assay	% Degradation	Purity Angle	Purity Threshold
1	**Acid Degradation**	96.4	3.6	0.133	0.246
2	**Base Degradation**	97.93	2.07	0.140	0.285
3	**Peroxide**	94.64	5.36	0.270	0.512
4	**Thermal Degradation**	98.14	1.86	0.208	0.552
5	**UV Degradation**	99.08	0.92	0.222	0.372

CONCLUSION

The proposed HPLC method is new, simple, precise, accurate and sensitive for the simultaneous estimation of Darunavir and Cobicistat in pharmaceutical dosage forms.

Degradation studies suggest that the developed method represented formation of impurities and can be a prospect for future analysis.

Hence, this method can be conveniently adopted for routine quality control analysis of Darunavir and Cobicistat in pure and formulation dosage forms.

Acknowledgement: The authors take this opportunity to thank the management of M/s. Chandra Labs. Kukatpally, Hyderabad for providing the necessary facilities for the successful completion of the above work.

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Source of Support: Nil, **Conflict of Interest:** None.

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