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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DARUNAVIR AND COBICISTAT IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple stability indicating high performance liquid chromatographic method has been developed for the simultaneous determination of Darunavir in combination with Cobicistat using reverse phase Inertsil ODS C_{18} (4.6 x 150mm, 5µm) with UV detection at 242 nm. The mobile phase consisting of 0.1% TEA: Methanol in a ratio of (30:70, v/v) and at a flow rate of 1.2 mL/min. The method was linear over the concentration 40 -200 µg/ml for Darunavir and 7.5-37.5 µg/ml for Cobicistat. The recoveries of active pharmaceutical ingredient (API) Darunavir and Cobicistat were found to be in the range of 98.59 -99.68% and 99.87 - 101.67% respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing amlodipine besylate and metoprolol succinate in combined tablet dosage form.

KEYWORDS: Darunavir, Cobicistat, HPLC, Validation.

INTRODUCTION

Darunavir[1] is a Antiretroviral agent. Darunavir is chemically (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-ylN-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate (Fig. 1).

Cobicistat is chemically Thiazol-5-ylmethyl*N*-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl)methyl-methyl-carbamoyl]amino]-4-morpholino-butanoyl]amino]-5-phenyl-pentyl]carbamate (Fig. 2).

Darunavir/Cobicistat (Prezcobix), manufactured by Janssen Pharmaceuticals, is a combination antiretroviral agent approved by the FDA as a complete regimen for the treatment of HIV-1 infection in adults. As this a fixed-dose combination product containing 800mg of Darunavir and 150mg of Cobicistat. The recommended dosage regimen of Prezcobix in adults is one tablet once daily orally with or without food.[2] Numerous analytical methods employed for the quantitative determination of individual or multi-component combinations assay in pharmaceutical dosage forms. Various HPLC assay methods were reported in the literature for the estimation of Darunavir, Cobicistat and some other anti-retroviral drugs individually and in-combination with other drugs. These methods include[3-9] HPLC and[10] UV method On the contrary to the best of our knowledge, there is no official method for the stability-indicating simultaneous estimation of Darunavir and Cobicistat by RP-HPLC in tablet dosage form. Hence, we planned to develop and validate a new method for stability-indicating simultaneous determination of Darunavir and Cobicistat in pharmaceutical dosage form. The new method is capable of separating all three active analytes present in the dosage form.

EXPERIMENTAL

Chemicals and reagents

Water HPLC Grade, Methanol HPLC grade, TEA buffer, Darunavir and Cobicistat Working Standards and tablet dosage form. The commercial Pharmaceutical tablets of Prezcobix containing 800mg of Darunavir and 150 mg of Cobicistat respectively (manufactured by Janssen Pharmaceuticals) were procured from local pharmacy.

Apparatus and chromatographic condition

The chromatographic separation was performed on a Waters Alliance HPLC, integrated with Auto Sampler and UV detector. The Inertsil ODS C_{18} (4.6 x 150mm, 5µm) was used for the separation. The mobile phase consisted of 0.1% TEA: Methanol in a ratio of 30:70(v/v). The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.2 mL/min and the detector wavelength was set at 242 nm. The injection volume was 20 µL. The mobile phase was used as diluent.

Preparation of Darunavir and Cobicistat standard & sample solution

Standard solution preparation

Accurately weighed and transferred 80 mg of Darunavir and & 15mg of Cobicistat working standard into a 10ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1.0 ml of Darunavir & Cobicistat of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.5ml of Darunavir & Cobicistat of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation

Weigh and finely powder not fewer than 20 tablets. Accurately weighed and transferred tablet powder equivalent to 80 mg of Darunavir & 15 mg of Cobicistat was Standards into an 10 ml clean dry volumetric flasks, add diluent, sonicated for 10 minutes and make up to the final volume with diluent. From the above stock solution 1 ml was pipetted out in to a 10 ml volumetric flask and then make up to the final volume with diluent. From above stock solution 1.5ml was pipetted out into a 10 mL volumetric flask and then make up the final volume with diluent.

Procedure

Inject 20 µL of the standard, sample solution into the chromatographic system and measure the peak areas for amlodipine and metoprolol and calculate the % assay value.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to ICH guidelines[12]. Obtained validation parameters are presented in Table 1.

Linearity

The linearity for HPLC method was determined at five concentration levels ranging from 40 µg/mL -200 µg/mL for Darunavir and 7.5 µg/mL -37.5 µg/mL for Cobicistat. The calibration curve was constructed by plotting response factor against respective concentration of Darunavir and Cobicistat. The plots of peak area Vs respective concentration of Cobicistat were found to be linear in the range of 40-200 µg/mL and 7.5 µg/mL -37.5 µg/mL with coefficient of correlation (r^2) 0.999 and 0.999 for Darunavir and Cobicistat respectively. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Darunavir and Cobicistat were given in Fig. 3 and Fig. 4.

Recovery

Three different samples of known concentration ranging from 40 µg/mL -200 µg/mL for Darunavir and 7.5 µg/mL -37.5 µg/mL for Cobicistat were prepared and these are analyzed against standard solution**.** The result of recovery analysis of Darunavir and Cobicistat was found to be in the range of 98.59-99.68% and 99.87-101.67% respectively. The obtained results are presented in Table 2.

Sensitivity

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the proposed RP-HPLC method. The LOD and LOQ showed that the method is sensitive for Darunavir and Cobicistat.

System suitability test

The specificity of this method was determined by complete separation of Darunavir and Cobicistat as shown in Fig. 5 with parameters like retention time, resolution and tailing factor. Here tailing factor for peaks of Darunavir and Cobicistat was less than 2% and resolution was satisfactory. The average retention time for Darunavir and Cobicistat were found to be 3.2 and 4.8 respectively, for five replicates. The peaks obtained for Darunavir and Cobicistat were sharp and have clear baseline separation. Hence the developed method was specific for the analysis of this product.

Precision

The method precision study was performed for five sample preparations of marketed formulations. A study was carried out for intermediate precision with the same analyst on the different day for five sample preparations of marketed formulations. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust. The Intra-day and Inter-day precision results are presented in Table 3. The assay results of tablet dosage formulation by the proposed method are presented in Table 4.

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results show that for both solutions, the retention time and peak area of Darunavir and Cobicistat remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 hr, which was sufficient to complete the whole analytical process. Further forced degradation studies were conducted indicating the stability of the method developed. The results of the degradation studies are presented in Table 5.

Hydrolytic degradation under acidic condition

Pipette 1.5 ml of above solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then

neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Hydrolytic degradation under alkaline condition
Pipette 1.5 ml of above solution into a 10ml volumetric flask into and add 3 ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

Thermal induced degradation
Darunavir and Cobisistat sample was taken in petridish and kept in Hot air oven at 110^0 C for 24 hours. Then the sample was taken and diluted with diluents and injected into HPLC and analysed.

Oxidative degradation
Pipette 1.5 ml above stock solution-2 into a 10ml volumetric flask, 1 ml of 3% w/v of hydrogen peroxide added and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

The chromatograms were recorded as shown in **Fig 6-9** and results are shown in **Table 5.**

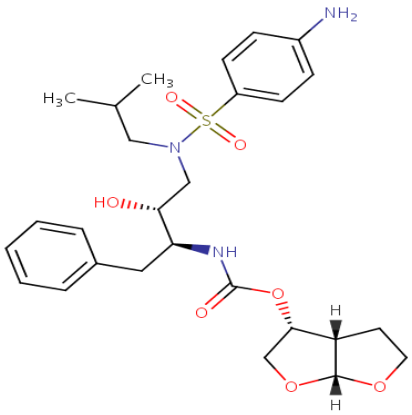

Fig. 1: Chemical structure of Darunavir

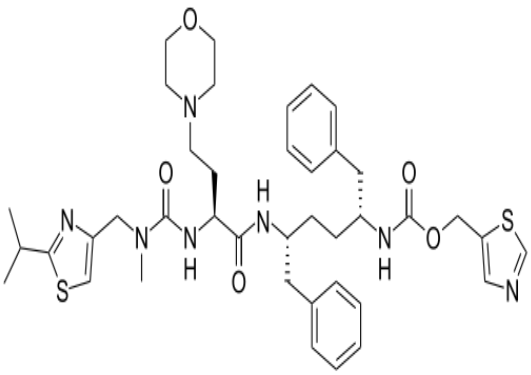

Fig. 2: Chemical structure of Cobicistat

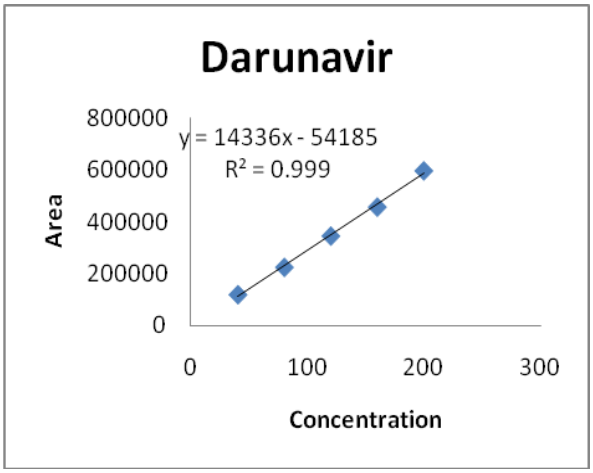

Fig. 3: Calibration curve for Darunavir

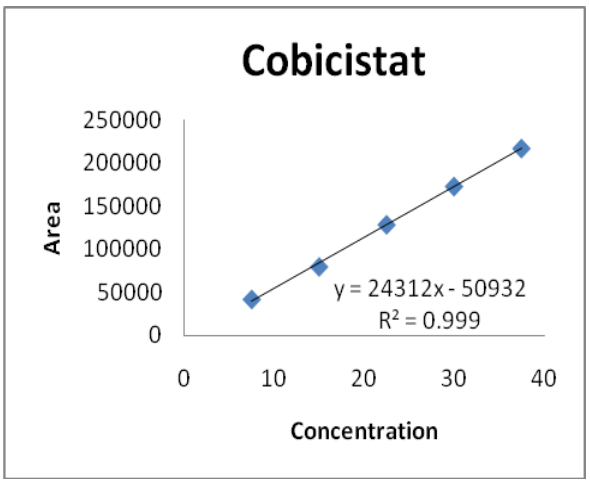

Fig. 4: Calibration curve for Cobicistat

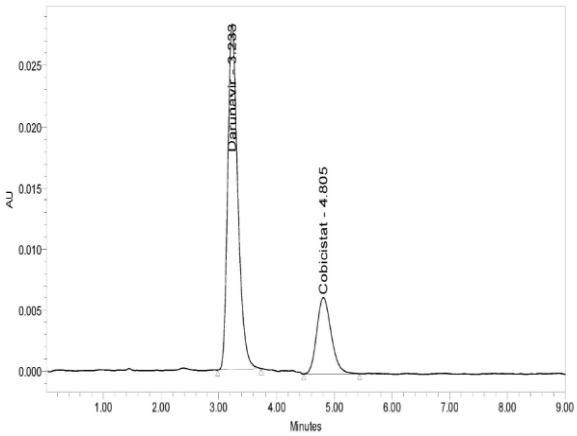

Fig. 5: Typical chromatogram of Darunavir and Cobicistat

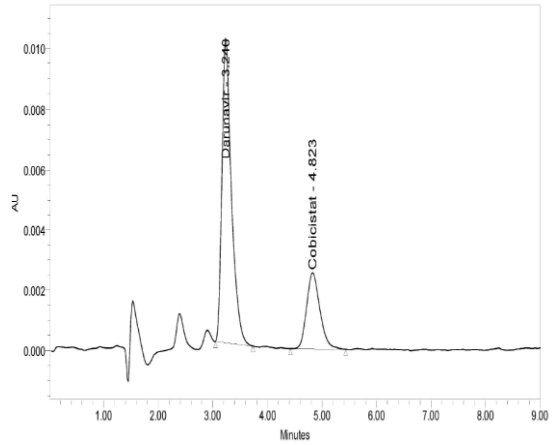

Fig. 6: Acid degradation chromatogram of Darunavir and Cobicistat

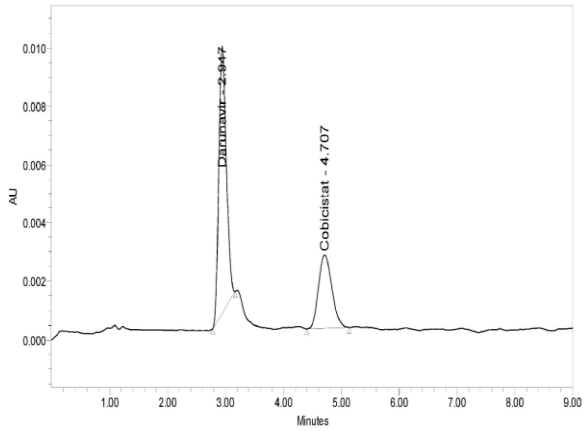

Fig. 7: Base degradation chromatogram of Darunavir and Cobicistat

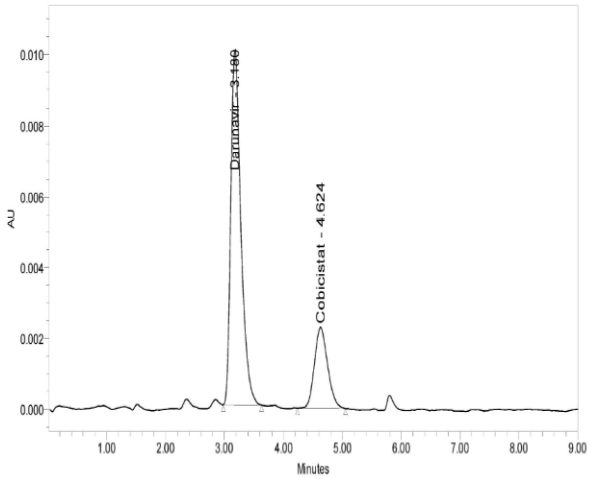

Fig. 8: Thermal degradation chromatogram of Darunavir and Cobicistat

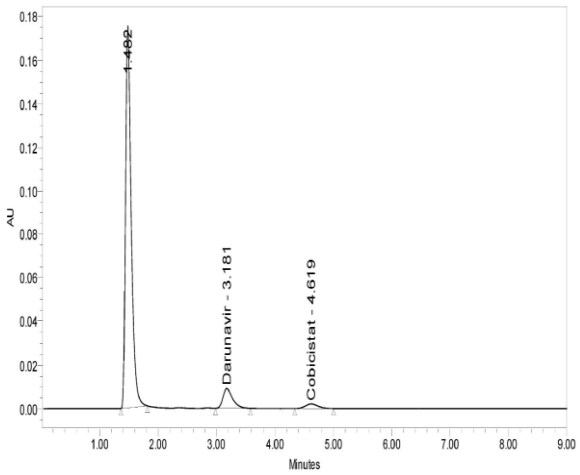

Fig. 9: Peroxide degradation chromatogram of Darunavir and Cobicistat

Table 1: Analytical validation parameters (System suitability and Linearity)

Parameter	DARUNAVIR	COBICISTAT
Retention time (min)	3.247	4.836
Tailing factor	1.33	1.20
USP Resolution	-	4.12
Plate count	2644.98	3766.53
Linearity range (µg/ml)	40-200	7.5-37.5
Correlation coefficient	0.9999	0.9999
LOD(µg/ml)	0.78	0.09
LOQ(µg/ml)	2.37	1.16
Intermediate precision (%RSD)	1.27	0.35
Method precision (%RSD)	0.97	0.52

Table 2A: Accuracy (recovery) data for Darunavir

%Concentration (at specification Level)	Amount spiked (µg)	Amount Found (µg)	% Recovery	Mean Recovery
50%	60.0	59.75	99.59%	98.94%
100%	120.0	119.47	99.68%	
150%	180.0	178.75	98.59%	

*Average of three determinations.

Table 2B: Accuracy (recovery) data for Cobicistat

%Concentration (at specification Level)	Amount spiked (µg)	Amount Found (µg)	% Recovery	Mean Recovery
50%	22.5	22.20	99.87%	101.81%
100%	45	45.87	101.67%	
150%	67.5	67.87	101.67%	

*Average of three determinations.

Table 3: Intra-day and Method precision of Darunavir and Cobicistat

Drug	Concentration (µg/mL)	Method precision		Intra-day precision	
		SD	%RSD	SD	%RSD
Darunavir	120	3407.311	0.97	18375.8	0.57
Cobicistat	12.5	582.7475	0.52	2844.8	0.35

Table 4: Assay result of tablet dosage formulation

Drugs in Formulation	Label Claim	Assay %
Darunavir	**800 mg**	**101.9**
Cobicistat	**150 mg**	**98.1**

Table 5: Degradation Results for Darunavir and Cobicistat

	Cobisistat			
	Area	%degraded	Purity Angle	Purity Threshold
Standard	36838		--	--
Acid	34289	6.92	0.485	0.776
Base	32065	12.96	0.662	0.998
Peroxide	32260	12.43	0.405	0.811
Thermal	35574	3.44	0.523	0.907
	Darunavir			
	Area	**%degraded**	**Purity Angle**	**Purity Threshold**
Standard	118590		--	--
Acid	101843	13.13	0.568	0.879
Base	93978	20.76	0.495	0.951
Peroxide	104526	11.86	0.562	0.976
Thermal	102660	13.44	0.681	0.884

CONCLUSION

This study presents a simple and validated stability indicating HPLC method for simultaneous estimation of Darunavir and Cobicistat in the presence of degradation products. The developed method is specific, accurate, precise and robust. The method was linear response in stated range and is accurate and precise. All the degradation products formed during forced decomposition studies were well separated from the analyte peaks demonstrating that the developed method was specific and stability indicating. The method could be applied with success even to the analysis of marketed products of Darunavir and Cobicistat combined tablet formulation, as no interference was observed due to excipients or other components present.

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