Research Article

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Novel method development and validation rp-hplc for simultaneous determination of darunavir and cobicistat in bulk and pharmaceutical formulation

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Abstract

A stability indicating reverse phase High performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the simultaneous determination of Darunavir and Cobicistat in bulk and pharmaceutical formulation. Separation was achieved in isocratic mode with a Kinetex C18 100 A (250 mm x 4.6 mm, 5µ) column and mixture consisting of 0.1% OPA(pH 3) and methanol in 80:20 v/v was used as mobile phase with a flow rate of 1 ml/min, column temperature at 25°C and the run time as 10 mins. UV detection was performed at 239 nm and the sample temperature was maintained ambient. The described method for simultaneous determination of Darunavir and Cobicistat is linear over a range of 8 µg/ml to 120 µg/ml and 5 µg/ml to 60 µg/ml respectively. The method shows good precision results which were below 2.0%RSD. Limit of Detection (LOD) and Limit of Quantification (LOQ) of Darunavir and Cobicistat was established and found to be 1.49 and 4.97 µg/ml and 1.13 and 3.77 µg/ ml respectively. The developed method was validated according to ICH guidelines for various parameters. The method is simple, rapid, selective and stability indicating method which would be used for regular stability indicating quality control determinations.

Keywords: RP-HPLC, Darunavir, Cobicistat, ART.

Introduction

Darunavir (DRV)

Darunavir (DRV) is a protease inhibitor, HIV-1 protease inhibitor (PI). The compound selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells, thus preventing formation of mature virions. DRV has the chemical name [(3aS,4R,6aR)-2,3,3a,4,5,6a-hexahydrofuro[2,3-b]furan-4-yl] N- [(2S,3R)-4-[(4-aminophenyl)sulfonyl-(2-methylpropyl)amino]-3-hydroxy-1-phenylbutan-2-yl]carbamate. DRV is a white to pale-yellow crystalline powder with a molecular formula of C8H14N2O2and a molecular weight of Salt form -802. and Free form - 704. DRV is slightly soluble in water (4-5 mg/ml, free base equivalent) with the pH of a saturated solution in water being about 1.9 at 24 ± 3°C. Its chemical structure is given in Fig 1.

Cobicistat (COBI)

Cobicistat (COBI) is a potent inhibitor of cytochrome P450 3A (CYP3A) which acts as a pharmaco-enhancing or "boosting" agent for antiviral drugs used in the treatment of HIV infection.Chemically COBI is 1, 3-thiazol-5-ylmethyl [(2R, 5R)-5-{[(2S)-2- [(methyl {[2-(propan-2-yl)-1,3-thiazol-4-yl] methyl} carbamoyl) amino] -4- (morpholin-4-yl) butanoyl] amino}-1, 6-diphenylhexan-2-yl] carbamate. It is adsorbed onto silicon dioxide and is a white to pale yellow solid powder with a molecular formula of C40H53N7O5S2 and a molecular weight of 776.0. COBI

solubility is 0.1 mg/ml in water at 20°C. Its Chemical structure is given in Fig 2.

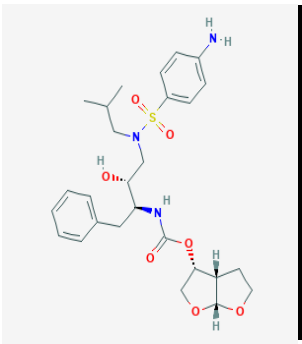


Fig.1 Stucture of Darunavir (DRV)

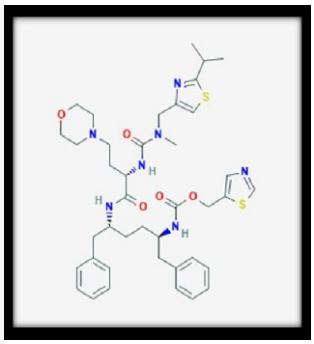
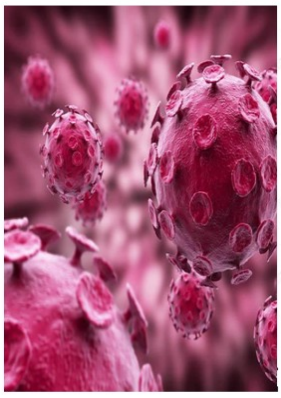
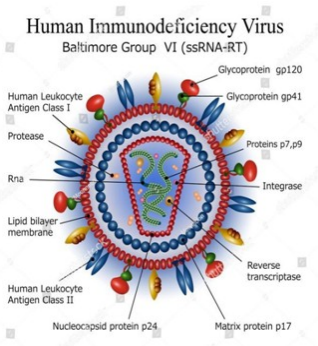


Fig.2 Stucture of Cobicistat (COBI)

COBI is a mechanism-based inhibitor of cytochrome P450 3A (CYP3A). Inhibition of CYP3Amediated metabolism by COBI increases the systemic exposure of CYP3A substrates to DRV. A few spectroscopic and liquid chromatographic procedures 4-7 have been reported for the determination of DRV and COBI individually but there is no method for stability indicating and simultaneous estimation of both the drugs. Therefore there is need to develop rapid and reliable Stability indicating liquid chromatographic method for simultaneous determination of DRV and





COBI in bulk and pharmaceutical dosage forms.

Fig.3: Structure of HIV

Experimental

Reagent and Materials

All the reagents in this assay along with triple distilled water were of analytical grade. Darunavir and Cobicistat were obtained as a gift sample from Hetro Ltd, Hyderabad & Macleod Pharmaceutical Pvt. Ltd. Gujrat, India. The marketed tablets used were obtained from ART center in Civil Hospital, Ahemadnagar. Brand Name: EVOTAZ- 3641 (Mfg by: Allergan India PVT LTD Karnataka). Methanol (HPLC Grade- Lobachemie), Orthophosphoricacid (OPA) (HPLC Grade- Fisher scientific) and HPLC Grade water - Merck.

Instrumentation

The analysis of the drug was carried out on a Chemito LC6600, SPD M20A prominanace DAD detector, Rheodyne universal injector 7725 port and Hamilton 50 µl manual injector. Data processing was performed with Chemito LC Solutions software version 1.25 for LC peak integration. Column details mfg by Cosmosil Code No, 38156-81 having the size 4.6x250mm.

Method Development

Chromatographic Condition: Chromatographic separation was achieved by using Kinetex C18 100 A (250 mm x 4.6 mm, 5µ) column as stationary phase and mixture consisting of 0.1% OPA(pH 3) and methanol in 80:20 v/v was used as mobile phase with a flow rate of 1 ml/min, column temperature at 25°C and the run time as 10 mins. UV detection was performed at 239 nm and the sample temperature was maintained ambient. standard and sample solutions were diluted with diluent filtered through Whattman filter paper (0.45µm) and degassed before use. Typical chromatogram of standard drug and sample were shown in Fig. 3 & 4.

Preparation of Mobile Phase: A 80:20 v/v mixture of 0.1% OPA (pH 3) and methanol was prepared by mixing 800mL 0.1% OPA (pH 3) and 200mL of methanol in a 1000 ml volumetric flask. The mixture was filtered through 0.45 µ membrane filter and sonicated before use. The same mixture was used as diluent for preparing working standard solutions of the drugs. Preparation of stock and working standard solution of DRV and COBI About 30 mg of DRV and 15 mg of COBI was weighed accurately and transferred into a 100 ml volumetric flask and dissolved with adequate amount of mobile phase. The solution was sonicated for 15 min and then the volume made up with a further quantity of the mobile phase. This solution was suitably diluted with the mobile phase to get a working standard solution of 30 µg/ml of DRV and 15 µg/ml of COBI.

Preparation of Sample Solutions

10 tablets were weighed and crushed to powder and then powder equivalent to 5 tablets sample was weighed and transferred to 250 mL volumetric flask. 200 mL of diluent was added, sonicated to dissolve and diluted to final volume with diluent. The contents are filtered through 0.45µ Nylon syringe filter. Further diluted 5 mL of filtrate to 100 mL with diluent.

Result

Assay of Drug Formulation (Tablet Dosage Form) Procedure 10µL of standard preparation and sample preparation were injected five times in the Chromatograph. Chromatograms were recorded and the peak responses for DRV and COBI were measured. The System suitability parameters should be met. From the peak responses, the content of ATV and COBI in the sample was calculated. Assay results were shown in Table No.1.

Picture No.1: Tablet brands containing DRV and COBI

Table No. 1: Assay Results of COBI & DRV

Sr. No.	Brand Name	Drug	Label Claim (mg/Tablet)	Amount Estimated (mg/Tablet)*	Percentage Label Claim (%)	% RSD
1	EVOTAZ-3641 TABLET Allergan India PVT LTD Karnataka	Cobicistat (COBI)	150	150	100	0.0899
		Darunavir (DRV)	300	399.90	99.99	1.456

*Mean of three reading

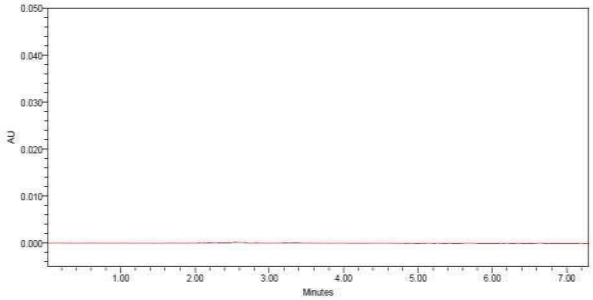


Fig. 4: Chromatogram of Blank

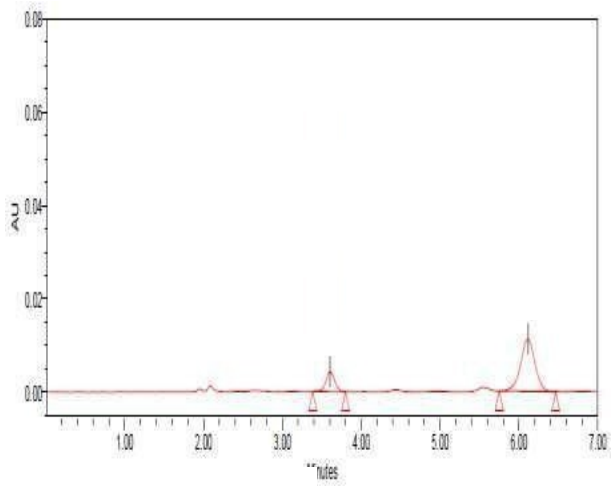


Fig. 5: Chromatam of Standard.

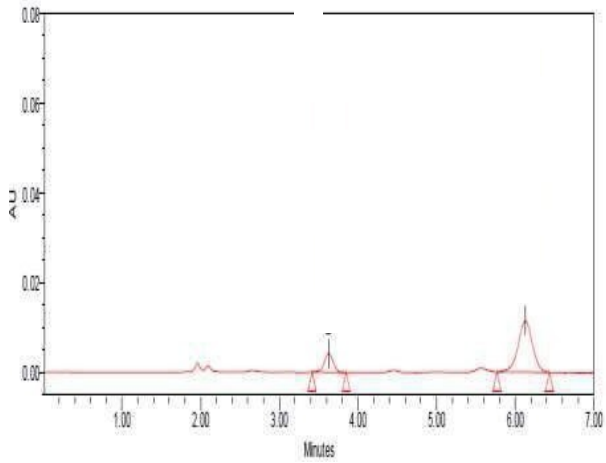


Fig.6: Chromatogram of Formulation

Method Validation

The method was validated for its linearity range, accuracy, precision, sensitivity and specificity.

Method validation is carried out as per ICH guidelines.

1. System Suitability

Table No. 2: System Suitability Results for COBI & DRV.

Sr. No.	Drug	Peak Area*	SD	% RSD
1	COMBI	1466117	3140.37	0.214
2	DRV	6241162	16967.46	0.272

*** Mean of Five Determinations**

2. Linearity

Table No.3: Linearity data of DRV and COBI.

Concentration of DRV(µg/mL)	Peak Area of DRV*	Concentration of COBI (µg/mL)	Peak Area of COBI*
8	88228	5	22508

16	2076 54	10	5465 7
30	3801 68	19	1046 52
40 48	506671 611524	23 30	134265 178606
78	9867 44	37	2178 91
120	1533 953	60	3589 20

*** Mean of Three Determinations**

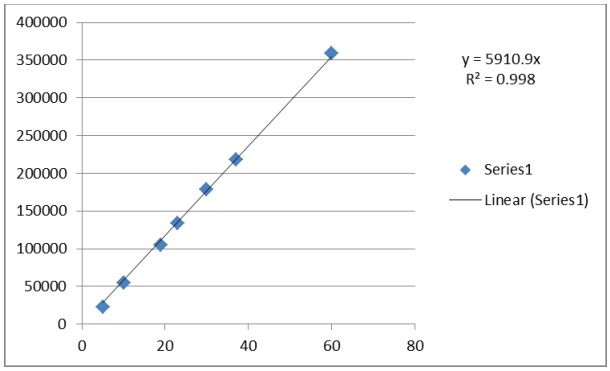


Fig.7: Linearity Plot of COBI

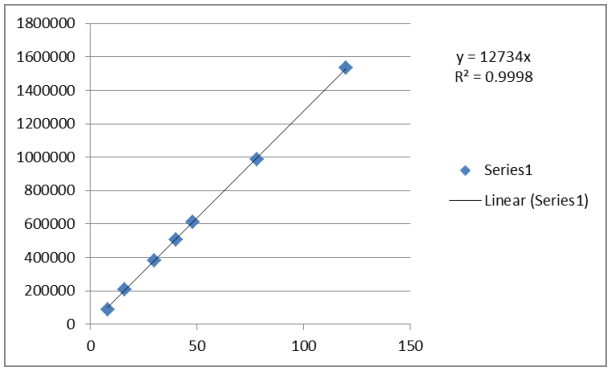


Fig.8: Linearity Plot of DRV

3. Accuracy

Table No. 4: Accuracy data (Triplicate values at 50, 100 and 150 percent levels) of DRV.

Concentration of spiked level	Amount added (µg)	Amount found (µg)	Percent Recovery	Mean (%) Recovery	% RSD
50%	15.4	15.41	100.06	100.28	0.32
	15.07	15.09	100.13		
	15.3	15.4	100.65		
100%	30.5	30.54	100.13	100.16	0.15
	30.14	30.15	100.03		
	30.2	30.3	100.33		
150%	45.4	45.42	100.04	100.04	0.01
	45.16	45.18	100.04		
	45.04	45.07	100.06		

Table No. 5: Accuracy data (Triplicate values at 50, 100 and 150 percent levels) of COBI

Concentration of spiked level	Amount added (µg)	Amount found (µg)	Percent Recovery	Mean (%) Recovery	%RSD
50%	7.51	7.53	100.26	99.73	0.53
	7.56	7.50	99.20		
	7.59	7.57	99.73		
100%	15.40	15.11	99.11	99.01	0.95
	15.80	15.49	98.03		
	15.60	15.43	99.91		
150%	22.52	22.56	100.17	99.95	
	22.57	22.52	99.77		
	22.53	22.51	99.91		

CONCLUSIONS

From the above discussion it can be concluded that the proposed method is precise, accurate and stability indicating. Therefore the proposed method can be used for routine quality control and analysis of the drug during stability studies in bulk samples and in tablet dosage forms.

Author Contribution

All authors Contributed equally

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