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A stability indicating RP-HPLC method for simultaneous estimation of darunavir and cobicistat in bulk and tablet dosage form

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ABSTRACT

The purpose of the present investigation was to develop a new RP-HPLC method for simultaneous estimation of Darunavir and cobicistat as per ICH guidelines. The HPLC separation was carried out by reverse phase Chromatography was carried out on an BDSC 18column (4.6x150mm, 5µ particle size) with a isocratic mobile phase composed of ortho phosphoric acid buffer, Acetonitrile, (50:50v/v) at a flow rate of 1mL/min. The column temperature was maintained at30°C and the detection was carried out using a PDA detector at 210nm. The retention times for Darunavir and cobicistat and were 2.018 min and 2.721 min respectively. The percentage recoveries of Darunavir and cobicistat were 99.97 % and 99.95 % respectively. The relative standard deviation for assay of tablets was found to be less than2%. The % RSD for method precision was found to be 0.9 for both Darunavir and cobicistat. The correlation coefficient for Darunavir and cobicistat was found to be 0.9994 and 0.9996 respectively. The detection limits were found to be 0.039µg/mL and 0.210µg/mL for Darunavir and cobicistat respectively. The quantitation limits were found to be 0.117µg/mL and 0.638µg/mL for Darunavir and cobicistat respectively. The proposed method was fast, accurate, precise and sensitive hence it can be employed for the simultaneous quantification of Darunavir and cobicistat in the dosage form, bulk drugs as well as for routine analysis in quality control.

Key words: Darunavir, cobicistat, simultaneous quantification, stability-indicating method, RP-HPLC method.

INTRODUCTION

Darunavir is chemically named as (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl N-[(2S,3R)-3-hydroxy-4-[N-(2-methylpropyl)4-aminobenzenesulfonamido]-1-phenylbutan-2-yl]carbamate Fig.(1). It is used as a protease inhibitor used to treat HIV. It acts on the HIV aspartyl protease which the virus needs to cleave the HIV polyprotein into its functional fragments. It was developed to increase interactions with HIV Protease and to be more resistant against HIV- 1 protease mutations[1-3]. 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 is chemically named as(1,3-thiazol-5-yl)methylN-[(2R,5R)-5-[(2S)-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}-4-(morpholin-4-yl)butanamido]-1,6-diphenylhexan-2-yl]carbamate Fig. (2). Cobicistat, trade name Tybost (formerly GS-9350), is a licensed drug for use 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 coadministered agents that are metabolized by CYP3A enzymes [4-6]. More specifically, cobicistat is indicated to increase systemic

exposure of atazanavir 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.

Literature survey reveals that various UV-spectroscopy [7] & HPLC assay methods are reported for the estimation of Darunavir and cobicistat individually[8-9] and in-combination[10-12]with other drugs. To the best of our knowledge there is one official method for the simultaneous estimation of Darunavir and cobicistat by RP-HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop sensitive and economically method for simultaneous estimation and validation of Darunavir and cobicist at in tablet formulation in accordance with the ICH guidelines [13].

MATERIALS AND METHODS

Instrumentation

RP-HPLC waters 2695 separation module equipped with 2996Photodiode Array Detector (PDA) was employed in this method. The Empower 2 software was used for LC peak integration along with data acquisition and data processing. The column used for separation of analytes is Hypersil BDSC18, (150 x 4.6 mm, 5µ).

Chemicals and Reagents

The reference samples of Darunavirand cobicistat were provided as gift samples from Spectrum pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merckchemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets(PREZCOBIX; Dosage: Darunavir-800mg &cobicistat-150mg)were purchased from the local pharmacy.

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a Hypersil BDSC18, (150 x 4.6 mm, 5µ) column using mobile phase consisting of ortho phosphoric acid Buffer: Acetonitrile in the ration of 50:50%v/v at a flow rate of 1.0 ml/min. The mobile phase was filtered through 0.45µm nylon filter and sonicated for 5 min in ultrasonic bath. Samples were analysed at 210nm at an injection volume of 10 µL.

Preparation of ortho phosphoric acid Buffer:

Buffer was prepared by mixing 1 ml of ortho phosphoric acid and 900 ml of milli-Q water in a 1000ml of Volumetric flask and degas to sonicate and finally make up the volume with water.

Preparation of Diluent

A mixture of water and methanol in the ratio 50:50 [%v/v] was used as a Diluent.

Preparation of Blank

Diluent was used as a blank.

Preparation of Standard Solution

Accurately weighed and transferred 40mg mg of Darunavir (1600µg/ml**)** in to 25ml of clean dry volumetric flask, add 17ml of diluent then sonicated for 10min and make up the volume with diluent. From this solution 1ml was pipetted out into a 10ml of clean volumetric flask and make up the final volume with diluent.

Accurately weighed 7.5mg of cobicistat (300µg/ml) and transferred into 25ml of clean dry volumetric flask, add 17ml of diluent then sonicated for 10 min and make up the final volume with diluent. From this solution 1ml was pipetted out into a 10ml of clean volumetric flask and make up the final volume with diluent.

Preparation of sample solution

10 tablets was weighed, powdered and then weighed equivalent to 150mg of Cobicistat (or) 800mg of Darunavir was transferred into a 500mL volumetric flask, 300mL of diluent added and sonicated for 25 min, further the volume made up with diluents and mixed (Stock solution). From this solution 1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluents and mixed and filtered.

Label Claim: 800 mg DARUNAVIR, 150 mg COBICSTAT

Method Validation
The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, Robustness, LOQ & LOD.

Specificity
To assess the method's specificity, working blank, placebo, standard and sample solutions into HPLC system to check the co-elution, if any, at the retention time of Darunavir and cobicistat peak.

Specificity of the method was also confirmed by forced degradation study, it was carried out to determine whether there are any interference of any impurities may be unexpected to present in retention time of analytical peaks. Forced degradation studies are carried out by using 2N HCl, 2N NaOH, thermal degradation Hydrogen peroxide and Photo degradation.

Accuracy
The accuracy was determined by calculating % recoveries of Darunavir and cobicistat. It was carried out by adding known amounts of each analyte corresponding to three concentration levels (50, 100, and 150%) of the labelled claim to the excipients. At each level, six determinations were performed and the accuracy results were expressed as percent analyte recovered by the proposed method.

Precision
Precision of an analytical method is usually expressed as the standard deviation. The repeatability studies were carried out by estimating response of Darunavir and cobicistat six times. The intra-day and inter-day precision studies (intermediate precision) were carried out by estimating the corresponding responses three times on the same day and on three different days for three different concentrations and the results are reported in terms of relative standard deviation.

Linearity
The purpose of the test for linearity is to demonstrate that the entire analytical system (including detector and data acquisition) exhibits a linear response and is directly proportional over the relevant concentration range for the target concentration of the analyte. The linear regression data for the calibration plot is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance.

Robustness
Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase, flow rate and temperature. This deliberate change in the method has no affect on the peak tailing, peak area and theoretical plates and finally the method was found to be robust.

Limit of Detection & Limit of Quantitation
The LOD can be defined as the smallest level of analyte that gives a measurable response and LOQ was determined as the lowest amount of analyte that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations,

$LOD=3.3 \times s/s$ and
$LOQ=10 \times s/s$,

Where s = standard deviation, S= slope of the calibration curve.

Assay of Darunavir and cobicistatin Tablet
Assay of marketed product was carried out by using the developed method. Sample solutions were prepared and injected into RP-HPLC system. The sample solution was scanned at 210nm. The drug estimated was found to be 98.7% for Darunavir & 99.3% for cobicistat. The chromatogram showed two single peaks of Darunavir and cobicistat was observed with retention times of 2.021 and 2.725 min (Fig.4).

RESULTS AND DISCUSSION

Optimized Chromatographic conditions

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Darunavir and cobicistat. The method developed was proceeding with wavelength selection and the optimized wavelength was 210 nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used to get better resolution. Finally the analysis was performed by using ortho phosphoric acid Buffer: Acetonitrile in the ration of 50:50 % v/v at a flow rate 1.0 ml/min at an injection volume of 10 µL and separation was carried by using Hypersil BDSC18, (150 x 4.6 mm, 5µ)column. The proposed method was optimized to give a sharp peak with minimum tailing for Darunavir and cobicistat. (Fig.3). The optimized conditions were given in Table 1.

Specificity of the method was assessed by comparing the chromatograms of blank, placebo, drug standards and mixed standard solutions to those obtained for tablet solutions. Retention times of the drugs in standard solutions, in the mixed standard solutions and in the sample solutions were the same. This result indicated specificity of the method. Furthermore, there was no interference from the excipients present in the tablets; thus, the method was considered specific.

Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Degradation studies were carried out under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis and the drug substances were degraded in all conditions. Acid and base hydrolysis was performed by exposing the drug substances with 2N HCl and 2N NaOH at 60 ^{o}C for 30min and it was showed degradation of Darunavir and cobicistat with degraded products peak at retention time 2.019 min& 2.730 min respectively .

Degradation studies under oxidative conditions were performed by heating the drug sample with 20% H_2O_2 at 60 ^{o}C and degraded product peaks were observed. Both Darunavir and cobicistat are sensitive to acid and alkali and there was no degradation occurs under UV light and thermal conditions. The results of forced degradation studies were given in Table 2.

Precision was evaluated by a known concentration of Darunavir and cobicistat was injected six times and corresponding peaks were recorded and % RSD was calculated and found within the limits. The low % RSD value was indicated that the method was precise and reproducible and the results were shown in the table Table 3.

Accuracy of the method was proved by performing recovery studies on the commercial formulation at 50, 100 and 150% level. % Recoveries of Darunavir and cobicistat ranges from 99.97%and 99.95%in simultaneous equation method and the results were shown in the Table 4. Linearity was established by analyzing different concentrations of Darunavir and cobicistat respectively. The calibration curve was plotted with the area obtained versus concentration of both Darunavir and cobicistat (Fig 5&6). In the present study six concentrations were chosen ranging between 40-240 µg/mL of Darunavir and 7.5-45 µg/mL of cobicistat. The regression equation and correlation coefficient for Darunavir and cobicistat was found to be

y = 7294.x + 18800 and R^2=0.9994 and
y = 16728x + 2781and R^2=0.9996 respectively and results were given in Table 5.

Robustness of the method is the ability of the method to remain unaffected by small deliberate changes in parameters like flow rate, mobile phase composition and column temperature. To study the effect of flow rate of the mobile phase it was changed to 0.1 units from 1.0 mL to 0.9 Ml and 1.1 mL. The effect of column temperature also checked by changing temperature to ± 5 ^{o}C. This deliberate change in the above parameters has no significant effect on chromatographic behavior of the samples and results were given in Table 6.

Fig 1: Chemical Structure of Darunavir

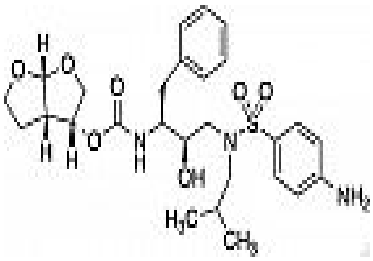

Fig 2: Chemical Structure of cobicistat

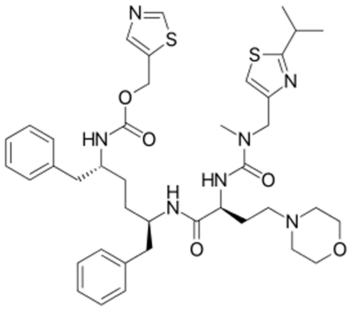

Fig 3: Standard Chromatogram of Darunavir andcobicistat

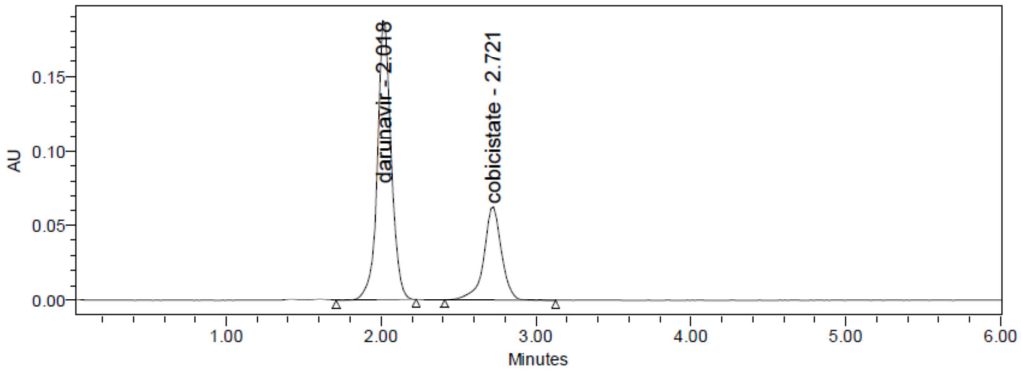

Fig 4: A typical chromatogram of Darunavir andcobicistat in tablet dosage form

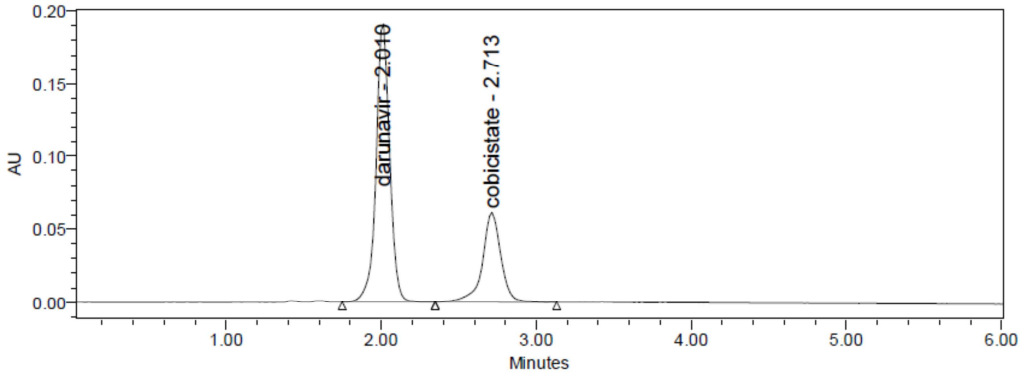

Fig 5: Linearity curve of Darunavir

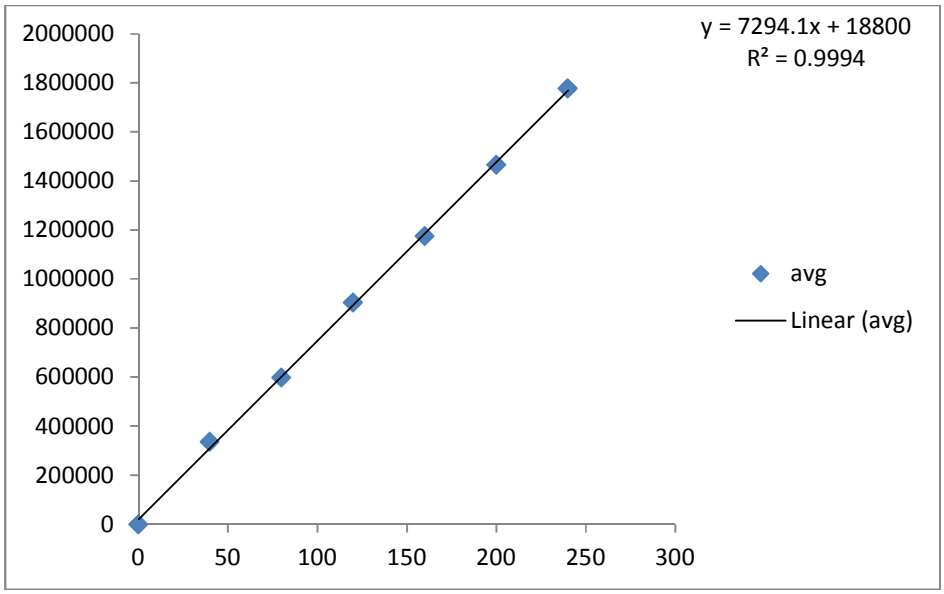

Fig 6: Linearity curve of cobicistat

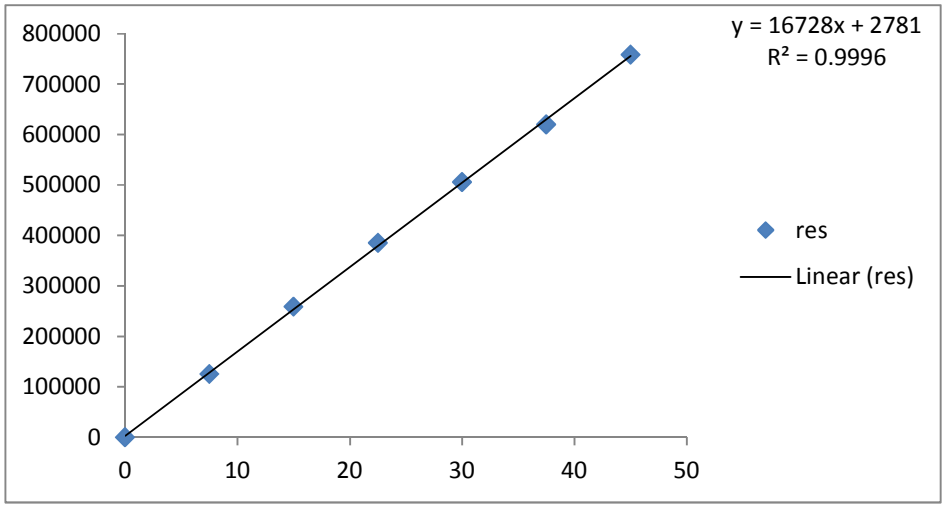

Table 1: Optimized Chromatographic conditions for Darunavir and cobicistat

Parameter	Condition
Mobile Phase	ortho phosphoric acid Buffer: Acetonitrile: (50:50%V/V)
Column	BDSC18, 150 x 4.6 mm,5µ.
Wave length	210nm
Flow rate	1.0 mL/min
Injection volume	10µL
Run time	6 min
Diluent	Water: Methanol (50:50)

Table 2: Results of Forced Degradation Studies Darunavir and cobicistat

S.No	Injection	DARUNAVIR		COBICISTAT	
		% Assay	% Degradation	% Assay	%Degradation
1	Acid Degradation	95.24	3.47	95.80	3.50
2.	Base Degradation	96.81	1.90	96.92	2.38
3.	Peroxide	97.24	1.47	97.64	1.66
4.	Thermal Degradation	98.07	0.64	98.16	1.44
5.	UV Degradation	99.04	0.38	99.09	0.26
6.	Neutral degradation	99.18	0.46	99.12	0.18

Table 3: Precision method of proposed RP-HPLC method for Darunavir and cobicistat

Drug	Mean Area	% RSD
DARUNAVIR	1151631	0.9
COBICISTAT	504155	0.9

Table 4: % Recovery Data for Darunavir andcobicistat

Drug	Spiked Level %	% Recovery	% RSD
DARUNAVIR	50	99.25	1.12
	100	100.80	0.56
	150	99.86	1.58
COBICISTAT	50	99.34	1.35
	100	100.27	1.19
	150	100.23	1.05

Table 5: Results of Linearity for Darunavir and cobicistat

S.No	DARUNAVIR		COBICISTAT	
	Conc. (µg/ml)	Peak Area	Conc. (µg/ml)	Peak Area
1	40	336534.5	7.5	125096
2	80	598635	15	258706.5
3	120	904302	22.5	385690.5
4	160	1174831	30	505974.5
5	200	1466758.5	37.5	620293.5
6	240	1777584	45	758437.5

Table 6: Robustness Data for Darunavir and cobicistat

Parameters	Changed Condition	Mean Peak Area		USP plate count	
		DAR	COB	DAR	COB
Flow rate (mL/min)	0.9ml	1303252	558299	2453	2805
	1.0ml	1165470	507202	2651	3094
	1.1ml	1133767	501041	2440	2765
Temperature (±5)	25^0C	1301077	565588	2456	2775
	30 ^{0}C	1165470	507202	2651	3094
	35^0C	1125373	500009	2457	2768
Mobile phase (±5%)	**70:**30 %v/v	1096183	449769	2524	2765
	75:25 %v/v	1165470	507202	2651	3094
	80:20 %v/v	1114292	480048	2498	2713

DAR*-DARUNAVIR****COB****-COBICISTAT*

Table 7: Results of LOD and LOQ for Darunavir and cobicistat

Drug	LOD (µg/ml)	LOQ (µg/ml)
DARUNAVIR	0.039	0.117
COBICISTAT	0.210	0.638

LOD and LOQ of Darunavir and cobicistat were evaluated based on relative standard deviation of the response and slope of the calibration curve. The detection limits were found to be 0.039µg/mL and 0.210µg/mL for Darunavir and cobicistat respectively. The quantitation limits were found to be 0.117µg/mL and 0.638µg/mL for Darunavir and cobicistat respectively. The results were given in the Table 7.

CONCLUSION

A new stability- indicating RP-HPLC method has been developed for estimation of Darunavir and cobicistat in bulk and pharmaceutical dosage form. The developed method was validated and it was found to be simple, sensitive, precise, and robust and it can be used for the routine analysis of Darunavir and cobicistat in both bulk and pharmaceutical dosage forms. The forced degradation studies were carried out in accordance with ICH guidelines and the results revealed suitability of the method to study stability of Darunavir and cobicistat under various degradation conditions like acid, base, oxidative, thermal, UV and photolytic degradations. Finally it was concluded that the method is simple, sensitive and has the ability to separate the drug from degradation products and excipients found in the dosage form.

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