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Research Article

STABILITY INDICATING UPLC METHOD FOR SIMULTANEOUS ESTIMATION OF COBICISTAT AND DARUNAVIR FROM ITS COMBINED TABLET DOSAGE FORM

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ABSTRACT

A reverse phase ultra performance liquid chromatographic method (RP-UPLC) was developed for the determination of Darunavir and Cobicistat in pure and tablet dosage forms. Separation was carried out by using Waters- Alliance UPLC system equipped with auto sampler, PDA detector, Aquity C18 (2.1 x 100mm, 1.7 µm, Make: BEH) column, 0.1% ortho phosphoric acid buffer and acetonitrile in the ratio 30:70 v/v at a flow rate of 0.27ml/min as mobile phase and detection at 242nm at ambient temperature. The active pharmaceutical ingredient was extracted from tablet dosage form using a mixture of acetonitrile and water (50:50) as diluent. The calibration graphs were linear and the method showed excellent recoveries for bulk and tablet dosage form. The developed UPLC method was validated and meets the requirements delineated by the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, Intermediate precision and robustness. The intra-day and inter-day variation was found be less than 1%. The method was reproducible and selective for the estimation of Darunavir and Cobicistat. Because the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one.

Keywords: Darunavir, Cobicistat and Method validation.

INTRODUCTION

Darunavir[1] is an Antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS. Nomenclature :-[(1S,2R)-3-[[(4-Aminophenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ester. Molecular formula is $C_{27}H_{37}N_3O_7S$. Molecular weight is 547.66. Melting point of drug is74^0C.[2] It is a amorphous white, solid, freely soluble in methanol, acetonitrile and soluble in ethanol. Darunavir contains a bis-tetrahydro-furnanyl (bis-THF) moiety and sulfonamide isostere; the drug is administered as its ethanolate salt. Its structural formula of Darunavir is shown in figure 1.

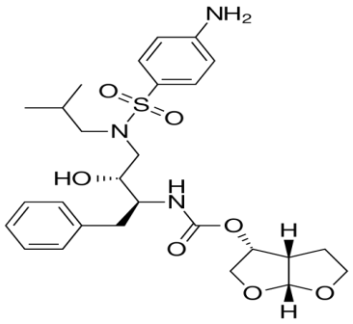

Fig. 1: Chemical structure of Darunavir

Cobicistat[3] Thiazol-5-ylmethyl N-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl) methyl-methyl-carbamoyl] amino]-4-morpholino-butanoyl] amino]-5-phenyl-pentyl] carbamate is a cytochrome P450 3A (CYP3A) inhibitor having molecular formula $C_{40}H_53N_7O_5S$, molecular weight 775g.mol-1. It is used for the treatment of human immunodeficiency virus (HIV) infection. Cobicistat is of interest for its ability to inhibit liver enzymes that metabolize other medications used to treat HIV, Elvitegravir an HIV integrase inhibitor[4,5,]. Cobicistat is a novel pharmocokinetic boosting agent without activity on HIV[5]. Its structural formula of Cobicistat is shown in figure 2.

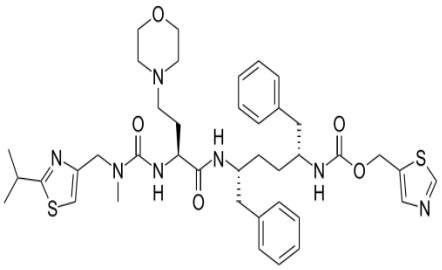

Fig. 2: Chemical structure of Cobicistat

The literature survey revealed that there are a very few HPLC and spectroscopic methods available for the determination of Darunavir and Cobicistat in pure and combined dosage forms. The present study was aimed to develop a new UPLC method for simultaneous estimation of Darunavir and Cobicistat in combined pharmaceutical dosage form.

Ultra Performance Liquid Chromatography, or UPLC for short, have been investigated and demonstrated high relevance and efficiency in the areas of speed and sensitivity of the analysis, as well as in the area of chromatographic resolution. One of its main characteristics lies in the fact that it utilizes very small particles and decreases the quantity of solvent required for processing. UPLC is a revolutionary tool in the industry that finds its origin in High Performance Liquid Chromatography (HPLC).[6] highlights the fact that the main modification made in the development of UPLC lies in the size of the materials or particles used during the process of separation.[7]

EXPERIMENTAL

Chemicals and reagents

Darunavir and Cobicistat bulk drugs were made available from Pharmatrain, Kukatpally, Hyderabad. Ortho phosphoric acid, methanol and Acetonitrile were obtained from Merck. All chemicals and reagent used were of HPLC grade, Milli-Q-water was used throughout the experiment.

Equipments

The Waters UPLC system with a PDA detector was used for method development and validation. The output signal was monitored and processed by using Empower software.

Chromatographic condition

The mobile phase used 0.1% Ortho phosphoric acid buffer and Acetonitrile in the isocratic mode employing at a flow rate of 0.27 ml/min. The analytical column used Aquity C18 (2.1 x 100mm, 1.7

µm, Make: BEH). The detection was carried out at a wavelength of 242nm for a run time of 3min. Diluent used as water and Acetonitrile in the ratio of 50:50 v/v.

Preparation of solutions

Preparation of 0.1% ortho phosphoric acid buffer

Pipetted 1ml of OPA into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water.

Preparation of mobile phase

A mixture of the above buffer and acetonitrile in (30:70) ratio was prepared, degassed in ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard solution

Accurately weigh and transfer about 800 mg of Darunavir and 150mg of Cobicistat working standard into a 100 ml clean dry volumetric flasks add Diluent and sonicate to dissolve it completely and make volume up to the mark with the Diluent (Stock solution). Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Assay of Pharmaceutical Dosage form: (Sample Preparation)

Twenty tablets of Prezcobix were weighed to get the average weight and then grind. An amount of powder equivalent to about 800 mg of Darunavir and 150mg of Cobicistat into a 100 ml clean dry volumetric flasks add Diluent and sonicate to dissolve it completely and make volume up to the mark with the Diluent (Stock solution). Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Further pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

System suitability parameters

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.27ml/min for 2 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 4 µL of standard into Aquity C18 (2.1 x 100mm, 1.7 µm, Make: BEH) column, the mobile phase of composition 0.1% orthophosphoric acid buffer and acetonitrile in the ratio 30:70 v/v was allowed to flow through the column at a flow rate of 0.27ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

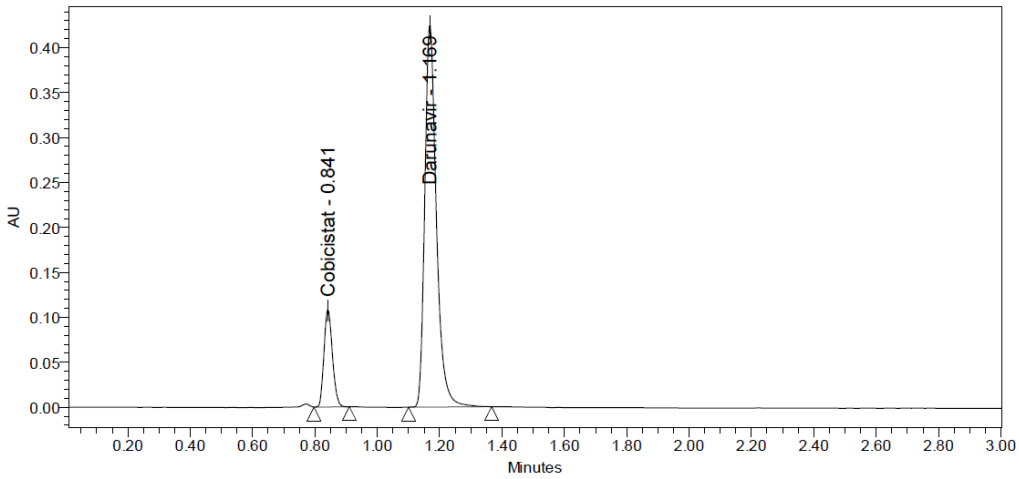

Fig. 3: Standard chromatogram

Table 1: System suitability parameters

Parameters	Darunavir	Cobicistat
Retention time	0.841	1.169
USP Plate count	4352.49	4929.44
USP Tailing	1.17	1.33

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine Darunavir and Cobicistat in their tablet dosage form. The result obtained for Darunavir and Cobicistat was comparable with the corresponding labeled amounts and they were shown in Table-2.

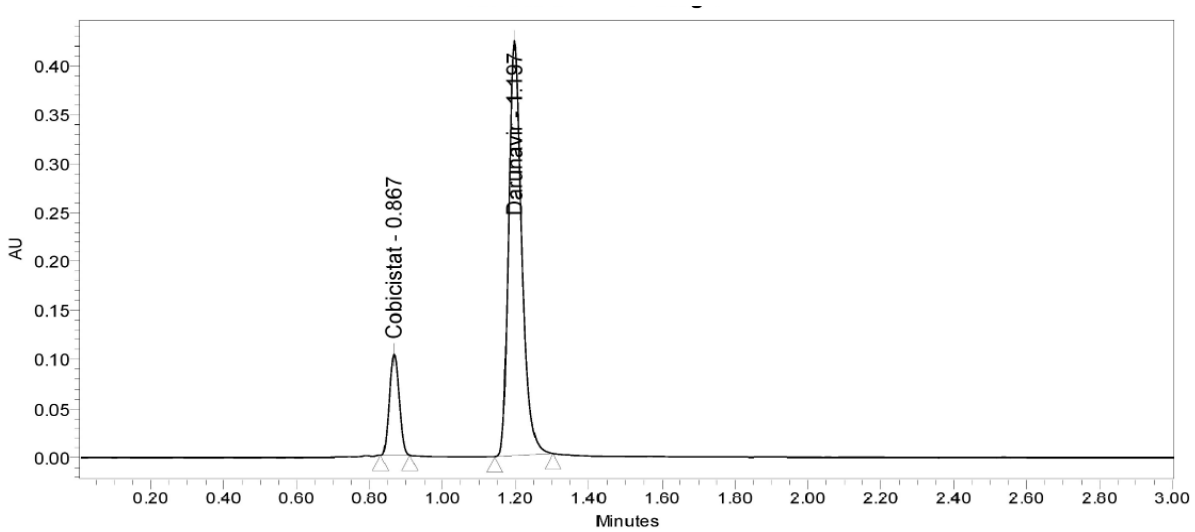

Fig. 4: Sample chromatogram

Table 2: Assay results for Darunavir and Cobicistat

Name of the drug	Label Claim (mg)	% Assay
Darunavir	800	98.24
Cobicistat	150	101.5

Method validation

The method was validated in accordance with ICH guidelines.[8]

Linearity

Linearity of the method was studied by injecting five concentrations of the drugs intriplicate prepared in the range of 40-200 µg/ml and for Darunavir, 7.5-37.5 µg/ml for Cobicistat into the UPLC system. Linear graphs were plotted by using the peak are as against concentration in µg/ml from which the correlation coefficients, slopes and *Y*-intercepts of the calibration curves were determined. The results were shown in table 3.

Table 3: Linearity results for Darunavir and Cobicistat

Parameters	Darunavir	Cobicistat
Concentration range(µg/ml)	40-200	7.5-37.5
Correlation coefficient	0.999	0.999
Intercept	54185	50932
Slope	14336	24312

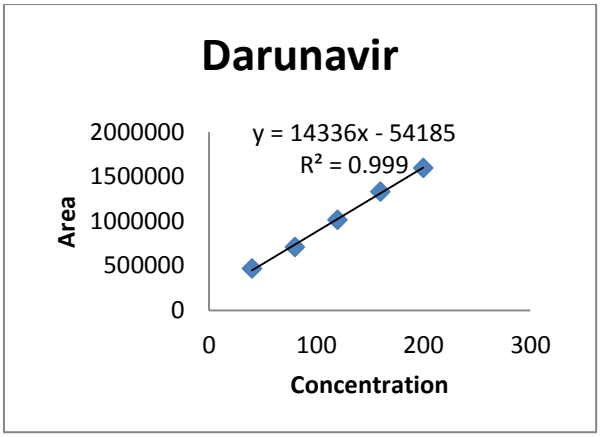

Fig. 5: Linearity graph for Darunavir

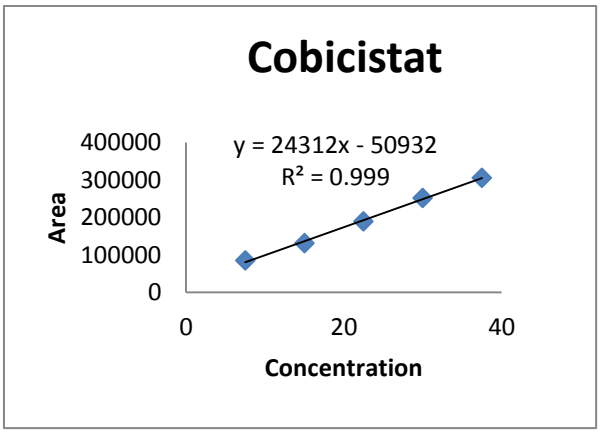

Fig. 6: Linearity graph for Cobicistat

Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet sample. Percent recovery was calculated by comparing the area with preanalysed sample. For all the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated by subtracting the total area from preanalysed sample area. The results were shown in Table-4.

Table 4: Accuracy results for Darunavir and Cobicistat

Drug Name	Level (%)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery (%)
Darunavir	50	542083	20.0	19.72	98.58	98.74
	100	1088547	40.0	39.59	98.98	
	150	1627611	60.0	59.20	98.66	
Cobicistat	50	104511	7.5	7.60	100.08	99.20
	100	203845	15.0	14.82	98.80	
	150	305566	22.5	22.21	98.73	

Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic conditions. For the intermediate precision study, repeatability study was carried out in different day under the same chromatographic conditions. The sample was injected in six replicate for intermediate precision and six replicate for precision. The peak area for injections was recorded. The mean and % relative standard deviation (%RSD) was calculated. From the data obtained the developed UPLC method was found to be precise. The Precision results were shown in Table-5 and ID Precision in Table-6.

Table 5: Precision results for Darunavir and Cobicistat

Injection	Darunavir	Cobicistat
Injection 1	1084739	200512
Injection 2	1080882	203186
Injection 3	1081781	201237
Injection 4	1085765	202277
Injection 5	1089123	202477
Injection 6	1083271	204097
Average	1084260	202297.7
Standard deviation	**2989.24**	**1294.06**
% RSD	**0.3**	**0.6**

Table 6: ID Precision results for Darunavir and Cobicistat

Injection	Darunavir	Cobicistat
Injection 1	1077595	205876
Injection 2	1084635	203869
Injection 3	1082058	201993
Injection 4	1089340	205153
Injection 5	1102113	207803
Injection 6	1099623	205981
Average	815370.3	1442223
Standard deviation	**2844.8**	**18375.8**
% RSD	**0.35**	**1.27**

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio (±10%), flow rate (0.27ml/min). It was observed that there were no marked changes in system suitability parameters, which demonstrated that the developed UPLC method is robust.

Limit of detection and Limit of quantification

The limit of detection (LOD) and limit of quantitation (LOQ) of the method were determined by standard deviation of response and slope method.

Degradation studies

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Exforge using the proposed method.

Preparation of Stock Solution
Accurately weigh and transfer about 800 mg of Darunavir and 150 mg of Cobicistat working standard into a 100 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent (Stock solution). Further pipette 1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Acid degradation condition
Pipette 1.5ml of the above stock solution into a 10ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at room temperature for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Alkali degradation condition
Pipette 1.5 ml of the above stock solution into a 10ml volumetric flask and add 3 ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at room temperature for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.45 microns syringe filters and place in vials.

Thermal induced degradation
The sample was taken in petridish and kept in Hot air oven at 105^0C for 48 hours. Then the sample was taken and diluted with diluents to prepare 120 ug/ml, 30 ug/ml of Darunavir, Cobicistat and injected into UPLC and analysed.

Oxidative degradation
Pipette 1.5 ml of the above stock solution into a 50ml volumetric flask 3 ml of 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 30 min. Filter the solution with 0.45 microns syringe filters and place in vials.

CONCLUSION
In the present work a new, accurate, precise and robust UPLC method was developed and validated for estimation of Darunavir and Cobicistat in pharmaceutical dosage form in accordance with the ICH parameters. The method gives good resolution between both the compounds with a short analysis time (3 min). Linearity is observed in the concentration range of 40-200 µg/ml for Darunavir and 7.5-37.5 µg/ml for Cobicistat drugs at 242 nm. The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be useful for the routine analysis of the Darunavir and Cobicistat in combined dosage form without any interference of excipients.

REFERENCES
1. Martindale: The complete drug reference. 36th edition, Pharmaceutical press, Lambeth High Street, London. 2009;869.
2. Goldwirt L, Chhuna S, Rey E, Launay O, Viard P, Pons P and Jullien V. Quantification of Darunavir (TMC114) in human plasma by high-performance liquid chromatography with ultra-violet detection, Journal of Chromatography B. 2007;857:327-331.
3. https://aidsinfo.nih.gov/drugs/536/cobicistat/.
4. www.hivclinic.ca December 2014. Page no-1 International Conference on Harmonisation, Geneva, 1993.
5. Christian Manzardo and Jose M. Gatell A new pardigm for HIV-1 treatment. Aids Rev. 2014; 16:35-42.
6. Swartz ME and Murphy B. Pharmaceutical Formulation Quality. 2004;6(5):40.
7. Van JJ, Zuiderweg EJ and Klinkenberg A. Longitudinal diffusion and resistance to mass transfer as causes of non ideality in chromatography. Chemical Engineering Science. 1956;5: 271–289.
8. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology.Q2 (R1), USA. 2005:1-13.