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## Development of an RP-HPLC method for multicomponent tablet formulation containing Cobicistat and Darunavir

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### ABSTRACT

A reverse phase-high performance liquid chromatographic method for simultaneous analysis of Cobicistat (COB) and Darunavir (DAR) in a tablet dosage form has been developed and validated. The method was performed with Durashell C 18 column (250 X 4.6 mm) 5-micron particle column with 60:10:30 of buffer (1 ml of Orthophosphoric acid in 1000 ml of water), methanol and acetonitrile respectively as mobile phase, at a flow rate of 1 ml per minute. UV detection at 210 nm. By the proposed method, COB and DAR were eluted with retention times of 4.88 and 4.35 minutes respectively. The method was validated as per the ICH guidelines. The method was simple, rapid, specific, accurate, precise, reliable and reproducible. Calibration curve plots were linear over the concentration ranges of 19.55 to 28.60 mcg/ml for COB and 103 to 149 mcg/ml for DAR. Limit of detection was 1.98, and 2.46 mcg/ml and Limit of quantitation were 6.01 and 7.38 mcg/ml for COB and DAR respectively. Robustness was studied by determining the effect of small deliberate variations in the chromatographic conditions. The conditions studied were flow rate was altered by  $\pm 0.5$  ml/minute and the detection wavelength was altered by  $\pm 2$  nm.



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### INTRODUCTION

Darunavir is an antiretroviral drug of the protease inhibitor class. Chemically it is [(1R,5S,6R)-2,8-dioxabicyclo[3.3.0]oct-6-yl] N-[(2S,3R)-4-[(4-aminophenyl)sulfonyl]- (2-methylpropyl)amino]-3-hydroxy-1-phenyl- butan-2-yl] carbamate.

Cobicistat is a licensed drug for use in the treatment of human immunodeficiency virus infection (HIV/AIDS).

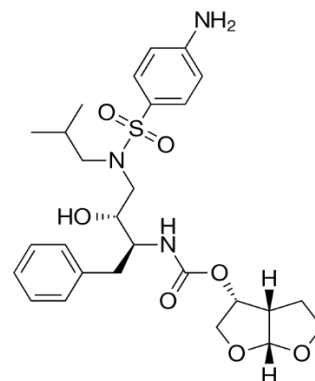


Figure 1: Structure of Darunavir

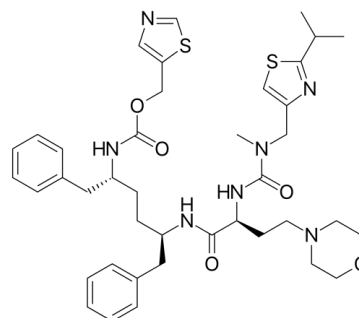


Figure 2: Structure of Cobicistat

Chemically it 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.

Literature survey shows that many UV spectroscopy (Chandni Saha *et al.*, 2014) and HPLC assay methods are reported for estimation of COB and DAR individually (Patel *et al.*, 2012; Urooj Fatima *et al.*, 2014) and in combination of both COB and DAR (Mallikarjunarao N *et al.*, 2016; Paul Richards *et al.*, 2016; Rizwan *et al.*, 2016). HPLC determined plasma concentration of darunavir and reported (Takahashi *et al.*, 2007). The RP-HPLC method was reported for the simultaneous estimation of COB and DAR in tablet dosage forms (Sigamala S *et al.*, 2016). Stability is indicating HPLC-DAD method was reported for the simultaneous estimation of Emtricitabine, Elvitegravir, Cobicistat and Tenofovir in the tablet dosage forms (Mallikarjunarao N *et al.*, 2016). RP-HPLC Method was reported for the assay and degradation study of cobicistat and atazanavir sulphate in bulk and combined dosage form (Leela Madhuri P *et al.*, 2016). A bioanalytical method was developed and validated for the simultaneous estimation of atazanavir and cobicistat by RP-HPLC in human plasma (Mathews *et al.*, 2017). The method was reported for the stability indicating an RP-HPLC method for the simultaneous estimation of COB and DAR in bulk and pharmaceutical combined dosage forms (Sathish Kumar Reddy *et al.*, 2016).

## EXPERIMENTAL

### Materials and methods

Pharmaceutical grade working standards Cobicistat and Darunavir were obtained from Bright Labs, Hyderabad, India. All chemicals and reagents were HPLC grade and were purchased from SD Fine Chemicals Limited, Mumbai. HPLC grade water was collected from the (Milli Q academic) Millipore.

### Instrumentation

The analysis was carried out on a THERMO SCIENTIFIC HPLC system equipped with the ACCELA-1250 pump with autosampler and a photodiode array detector. CHROM/QUEST software performed data acquisition, data handling and instrumentation control. Column C 18 (250 X 4.6 mm) 5-micron particle column was used to optimise the method.

### Optimization of RP-HPLC Method

Durashell C 18 column (250 X 4.6 mm) 5-micron particle column with 60:10:30 of Buffer (1 ml of orthophosphoric acid is diluted to 1000 ml with

water), methanol and acetonitrile respectively as mobile phase, at a flow rate of 1 ml per minute. UV detection at 210 nm

### Mobile phase preparation and diluent

Mixing of buffer (1 ml of orthophosphoric acid in 1000 ml of water), HPLC grade methanol and acetonitrile in the ratio of 60:30:10 respectively, and pH was adjusted to 5.5 with triethylamine. The dilution of the sample also made with the above mobile phase.

### Preparation of standard solution of Cobicistat

Weighed accurately about 30 mg of cobicistat and transferred into a 50 ml volumetric flask, and added about 15 ml of diluent (mobile phase), sonicated for 5 minutes to dissolve and diluted to 50 ml with diluent. Pipetted out 1 ml of the above solution into a 25 ml volumetric flask and diluted up to the mark with diluent. (Figure 1).

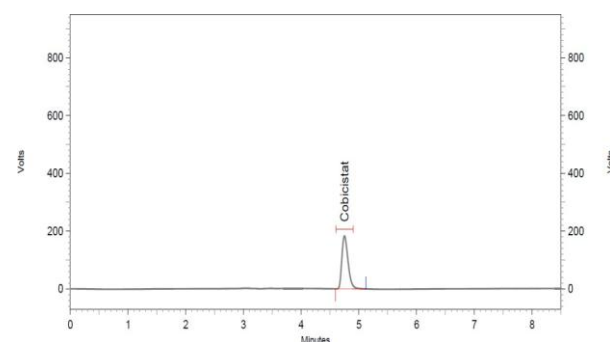


Figure 3: Chromatogram of standard Cobicistat

### Preparation of standard solution of Darunavir

Weighed accurately about 160 mg of darunavir and transferred into a 50 ml volumetric flask, and added about 15 ml of diluent (mobile phase), sonicated for 5 minutes to dissolve and diluted to 50 ml with diluent. Pipetted out 1 ml of the above solution into a 25 ml volumetric flask and diluted up to the mark with diluent. (Figure 2).

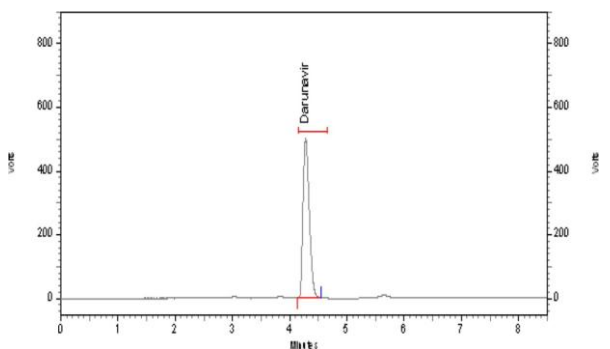
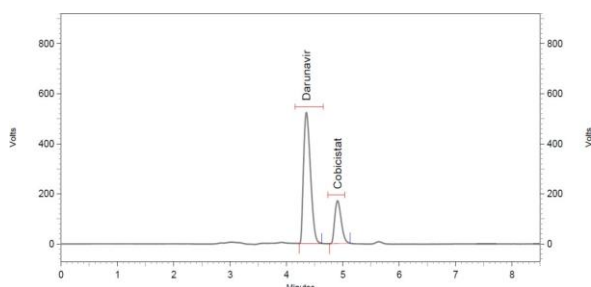


Figure 4: Chromatogram of a Standard solution of Darunavir

### Sample Preparation

Analysis of tablet dosage form was performed by determining average weight of the tablets using 20 tablets. Then the tablets were crushed into a fine

powder. The powder equivalent to 30 mg of COB and 160 mg of DAR was transferred into a 50 ml volumetric flask and added about 15 ml of diluent (mobile phase), sonicated for 5 minutes to dissolve and diluted to 50 ml with diluent. Pipetted out 1 ml of the above solution into a 25 ml volumetric flask and diluted up to the mark with diluent. The final concentration was 15 mcg/ml of COB and 80mcg/ml of DAR. Then injected the above solution into the chromatographic system and analysed quantitatively. The analysis was repeated six times, and the possibility of interference of excipients from the tablet dosage form was examined. (Figure 3).



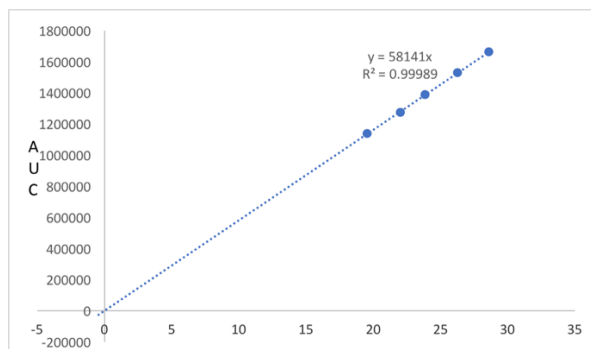
**Figure 5: Chromatogram of COB and DAR at 210 nm from Pharmaceutical dosage form**

**Method validation**

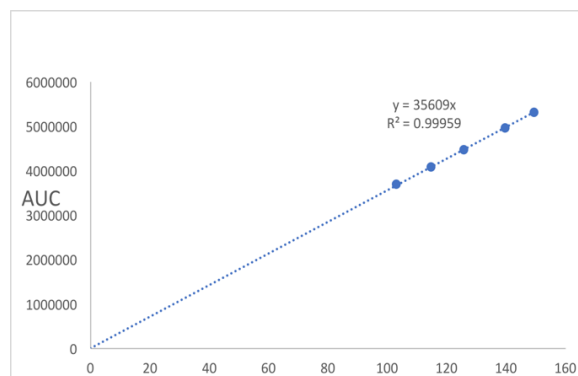
The proposed analytical method was validated as per the ICH guidelines. The following validation parameters were performed: Linearity, accuracy, precision, system suitability, limit of detection, limit of quantitation and robustness.

**Linearity**

The linearity of the method was studied by injecting the mixed standard solutions with the concentration ranges from 19.55 to 28.60 mcg/ml and 103 to 149 mcg/ml of COB and DAR respectively. The above concentrations were arrived out of the ranges from 10 to 30 mcg/ml and 90 to 650 mcg/ml for COB and DAR respectively. The experiment was repeated 6 times by injecting constant injection volume, and the peak areas were plotted against the concentration to obtain the linearity graphs. (Figure 4 and Figure 5 & Table 1 and Table 2).



**Figure 6: Linearity for Cobicistat**



**Figure 7: Linearity for Darunavir**

**Table 1: Linearity for Cobicistat**

Concentration in mcg/ml	AUC
19.55	1137429
22.01	1275770
23.86	1387999
26.27	1528301
28.6	1663779

**Table 2: Linearity for Darunavir**

Concentration in mcg/ml	AUC
103	3692283
114.7	4080011
125.7	4470709
139.6	4966689
149.4	5314781

**Precision**

The precision of the optimised method was evaluated by performing six independent assays of the test sample. The % RSD of assay values was calculated.

**System suitability**

The system suitability parameters including tailing factor, theoretical plates, repeatability and resolution between COB and DAR peaks were defined.

**Limit of detection and Limit of quantitation**

The LOD and LOQ were determined from the linearity data by using the slope of the line and standard deviation of Y-intercept. LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analyte that can be detected and quantified with acceptable accuracy and precision.

**Accuracy**

Accuracy was carried out by spiking the pure drug to the test sample (tablet formulation) in four different levels, that is 100%, 110%, 120% and 130%. Each level was repeated three times, and the percentage recovery and %RSD was calculated, the results of accuracy are given in Table 3.

**Table 3: Accuracy study for Darunavir**

Percentage level	Standard Area	Sample Area*	Theoretical (mg)	Experimental mg/tab*	Percentage* (%)
100	4540691	4443500	800	798.05	99.76
110	4540691	4879163	880	876.29	109.54
120	4540691	5340095	960	959.07	119.88
130	4540691	5784718	1040	1038.93	129.87

\*Ave of 3 determinations

**Table 4: Accuracy study for Cobicistat**

Percentage level	Standard Area	Sample Area*	Theoretical (mg)	Experimental mg/tab*	Percentage* (%)
100	1396135.6	1390380	150	150.12	100.80
110	1396135.6	1529521	165	165.14	110.59
120	1396135.6	1661471	180	179.39	119.63
130	1396135.6	1801076	195	194.46	129.64

\*Ave of 3 determinations

**Table 5: Assay**

Standard area	Sample area	mg/tab*	Percentage* (%)
For Darunavir			
4540691	4432841	798.13	99.77
For Cobicistat			
1396135.6	1387401	150.18	100.12

\*Ave of 6 determinations

**Analysis of marketed dosage form**

An above description assayed the marketed tablet formulation. The peak areas were measured at 210nm, and the determination of sample concentrations was using multilevel calibration developed on the same system under the similar conditions using regression analysis, the assay results are given in Table 4.

**Robustness**

Robustness was studied by determining the effect of small deliberate variations in the chromatographic conditions. The conditions studied were flow rate was altered by  $\pm 0.5$  ml/min. and the detection wavelength was altered by  $\pm 2$  nm. These chromatographic variations are evaluated for resolution between COB and DAR.

**RESULTS AND DISCUSSIONS**

The simultaneous estimation of COB and DAR was done by the RP-HPLC method. The optimised method consists of 60:30:10 of buffer, methanol and acetonitrile respectively as the mobile phase, at a flow rate of 1 ml per minute. UV detection at 210 nm. The retention time of the COB and DAR is 4.3 and 4.8 minutes respectively. The linearity of the method was demonstrated over the concentration ranges of 19 to 29 mcg/ml for COB and 103 to 149 mcg/ml for DAR. Limit of detection 1.98 and 2.46 mcg/ml and Limit of quantitation were 6.01 and 7.38 mcg/ml for COB and DAR respectively. The accuracy was performed at four different levels, and all the levels the % RSD is

within the limit (<2%). The Robustness study includes deliberate deviation of flow rate and detection wavelength, shows that

**CONCLUSION**

The proposed HPLC method for the estimation of COB and DAR is simple, accurate and precise. The method can be utilised for routine analysis to determine DAR and COB in bulk drugs and formulations without prior separation.

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