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

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ABSTRACT
A simple, Accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Darunavir in Tablet dosage form. Chromatogram was run through BDS (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrie in the ratio of 40:50A:10M was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Cobicistat and Darunavir was 210nm. Retention time of Cobicistat and Darunavir were found to be 3.170 min and 3.984 min. % RSD of the Cobicistat and Darunavir were and found to be 1.06 and 1.3 respectively. % Recover was Obtained as 100.71% and 100.2% for Cobicistat and Darunavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Darunavir were 0.107 ppm, 0.326 ppm and 0.333ppm, 1.009 ppm respectively. Regression equation of Cobicistat is y = 10306x + 1346 and of Darunavir is y = 8883.x + 2152.

KEYWORDS: Cobicistat, Darunavir, RP-HPLC.

INTRODUCTION
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 co administered agents that are metabolized by CYP3A enzymes. More specifically, cobicistat is indicated to increase systemic exposure of Darunavir or darunavir (once daily dosing regimen) in combination with other antiretroviral agents in the treatment of HIV-1 infection. Increasing systemic exposure of anti-retroviral (ARVs) without increasing dosage allows for better treatment outcomes and a decreased side effect profile. Darunavir belongs to the class of organic compounds known as amino benzene sulfonamides. These are organic compounds containing a benzene sulfonamide moiety with an amine group attached to the benzene ring.

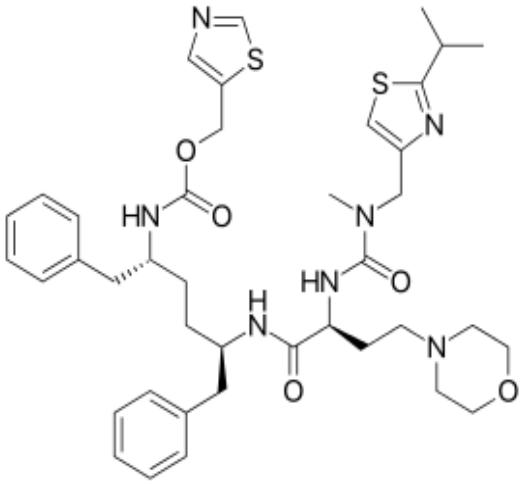
STRUCTURE


Fig-1: structure of Cobicistat.

Molecular Weight (MW) : 776.02.
Formula: $C_{40}H_{53}N_7O_5S_2$.
CAS No.: 1004316-88-4.
Storage: 3 years -20℃ powder 6 months-80℃ in solvent.
Solubility (25°C)*: DMSO 100 mg/mL (128.86 mM), Ethanol 100 mg/mL (128.86 mM) and Water <1 mg/mL (<1 mM).

IUPAC Name: (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.

Mechanism of action
Cobicistat is a mechanism-based inhibitor of cytochrome P450 3A (CYP3A) isoforms. Inhibition of CYP3A-mediated metabolism by cobicistat increases the systemic exposure of CYP3A substrates Darunavir and therefore enables increased anti-viral activity at a lower dosage. Cobicistat does not have any anti-HIV activity on its own.

Absorption
Median peak plasma concentrations were observed at 3.5 hours post-dose.

Protein binding: 97-98% bound to human plasma proteins.

Metabolism
Cobicistat is metabolized by CYP3A and to a minor extent by CYP2D6 enzymes and does not undergo glucuronidation.

Half-life
The terminal plasma half-life of cobicistat is approximately 3 to 4 hours.

Toxicity
The most common adverse reactions reported during clinical trials were jaundice (13%), ocular icterus (15%), and nausea (12%).

pKa value
Strongest Acidic is 14.18 and Strongest Basic is 6.69.

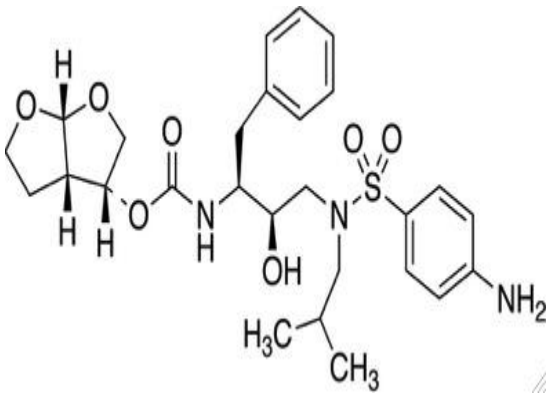


Fig-2: structure of darunavir.

Molecular Weight (MW) : 547.66358.
Formula: $C_{27}H_{37}N_3O_7S$.

IUPAC NAME: (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.

MECHANISM
Darunavir is a HIV protease inhibitor which prevents HIV replication by binding to the enzyme's active site, thereby preventing the dimerization and the catalytic activity of the HIV-1 protease. Darunavir selectively inhibits the cleavage of HIV encoded Gag-polyproteins in virus-infected cells, which prevents the formation of mature infectious virus particles.

ABSORPITION
The absolute oral bioavailability of a single 600 mg dose of darunavir alone and after co-administration with 100 mg ritonavir twice daily was 37% and 82%, respectively.

METABOLISM
Hepatic. Darunavir is extensively metabolized by CYP enzymes, primarily by CYP3A.

HALF LIFE
The terminal elimination half-life of darunavir was approximately 15 hours when combined with ritonavir.

pKa value: Strongest Acidic is 11.43 and Strongest Basic is 1.76.

MATERIALS AND METHODS
Cobicistat and Darunavir, Combination of Cobicistat and Darunavir tablet dosage forms, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc. Instrument**:** HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Cobicistat and Darunavir solutions.

Buffer: (0.1%OPA)
1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water.

Standard Preparation
Accurately Weighed and transferred working Standards of 7.5 mg of Cobicistat and 40 mg of Darunavir into a 25ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solution was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation
1tablet was weighed, powdered and then the weight was transferred into a 500mL volumetric flask, 100mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Method development

Chromatographic parameters were preliminary optimized to develop HPLC method for simultaneous cobicistat and darunavir with short analyses time (10min), and acceptable resolution (> 2). The isoabsoprtive point of cobicistat and darunavir selected was 210nm. In order to identify a suitable organic modifier, various compositions of acetonitrile and methanol were tested along with different buffers. Different columns like X-terra, Inertsil, Inspire columns were tried. Resolution was the major problem while we are developing method. Resolution was less very less when we are using one organic phase, to increase resolution acetonitrile were used in isocratic mode. Finally separation for simultaneous determination of cobicistat and darunavir was carried out by gradient elution with a flow rate of **1** ml/min inertsil (BDS 250 x 4.6 mm, 5µ.) The standard chromatogram was shown in Fig-3.

Optimized Method

Drugs were eluted with good resolution, retention time all the parameters like Plate count and Tailing factor were within the limits.

Mobile phase

Buffer and Acetonitrile and methanol taken in the ratio 40:50A:10M.

Chromatographic conditions

Flow rate: 1 ml/min
Column: BDS 250 x 4.6 mm, 5µ.
Detector wave length: 210nm
Column temperature: 30°C
Injection volume: 10µL
Run time: 7 min
Diluents: first dissolved in water: Acetonitrile 50:50

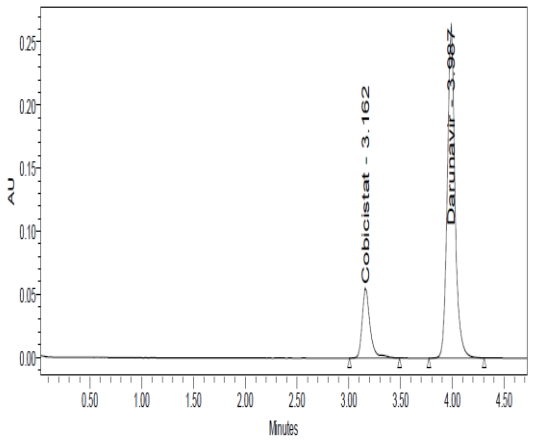


Fig-3: Optimized chromatogram of Cobicistat and Darunavir.

Method Validation

The above method was validated according to ICH guidelines to establish the performance characteristics of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method.

1. System suitability

All the system suitability parameters are within range and satisfactory as per ICH guidelines for Table: 2.1.

Table: 2.1 System suitability studies of Cobicistat and Darunavir method.

Property	Cobicistat	Darunavir
Retention time (t_R)	3.170± 0.3 min	3.984±0.3min
Theoretical plates (N)	9443 ± 163.48	15131± 163.48
Tailing factor (T)	1.29 ± 0.117	1.14± 0.117

2. Linearity

Six Linear concentrations of Cobicistat (7.5-45ppm) and Darunavir (40- 240ppm) are prepared and injected. Regression equation of the Cobicistat and Darunavir are found to be, y = 10306x + 1346, and y = 8883.x + 2152 and the regression co-efficient was 0.999. As shown Table: 2.2 and Fig: 4, Fig: 5.

Table: 2.2 Calibration data of Cobicistat and Darunavir method.

S.No	Concentration Cobicistat (µg/ml)	Response	Concentration Darunavir (µg/ml)	Response
1	0	0	0	0
2	7.5	83972	40	359687
3	15	155266	80	742168
4	22.5	230648	120	1056971
5	30	305803	160	1384921
6	37.5	389008	200	1774658
7	45	467853	240	2159056

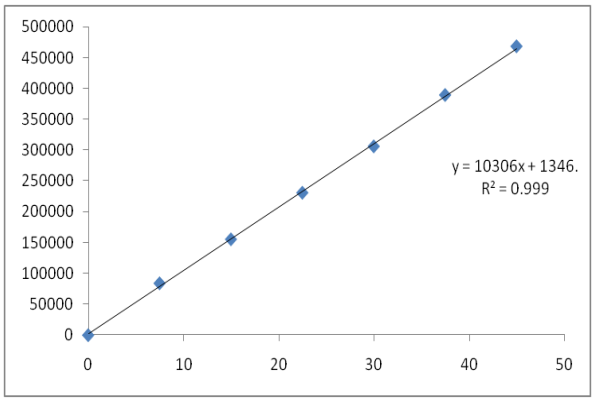


Fig: 4 Calibration curve of Cobicistat.

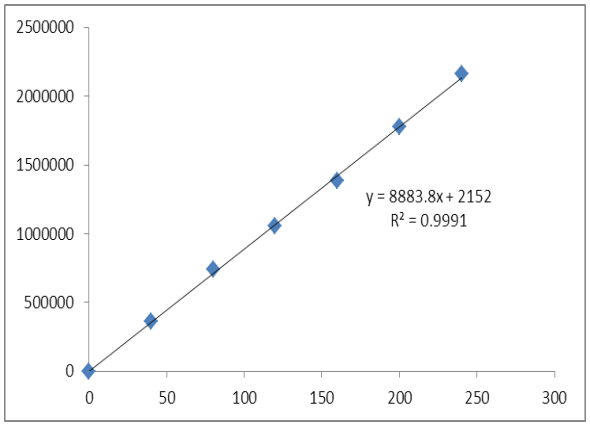


Fig: 5 Calibration curve of Darunavir.

3. Precision

Six different samples of both drugs were performed and % RSD for Cobicistat and Darunavir were found to be 0.44 % and 0.2% respectively for Table: 2.3.

Table: 2.3 Repeatability results for Cobicistat and Darunavir.

Sr. No.	Cobicistat	Darunavir
1	298971	1368391
2	298293	1367203
3	300896	1372673
4	301407	1374295
5	299571	1375155
6	301312	1370158
Mean	300075	1371313
Std. Dev.	1313.5288	3233
%RSD	0.44	0.2

4. Accuracy

Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 2.4.

Table: 2.4 Accuracy results of Cobicistat and Darunavir.

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Cobicistat	15	15.0195	100.13	1.06
	30	30.045	100.15	0.63
	45	44.9055	99.79	0.45
Darunavir	80	80.17	100.21	1.14
	160	160.53	100.33	0.71
	240	242.99	101.25	0.87

5. LOD

Limit of detection was calculated by into Cobicistat and Darunavir method and LOD for Cobicistat was found to be 0.107 and Darunavir was 0.333 respectively.

LOQ

Limit of Quantification was calculated by into Cobicistat and Darunavir method and LOQ for Cobicistat and Darunavir were found to be 0.326 and 1.009 respectively.

6. Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines as for shown Table 2.5.

Table 2.5 Robustness data of Cobicistat and Darunavir method.

S.NO	Robustness condition	Cobicistat %RSD	Darunavir %RSD
1	Flow minus	0.8	1.3
2	Flow Plus	0.2	0.4
3	Mobile phase minus	0.2	2.5
4	Mobile phase Plus	0.1	0.7
5	Temperature minus	0.3	2.5
6	Temperature Plus	0.6	0.4

7. Assay

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average % Assay was calculated and found to be 100.71% and 100.2% for Cobicistat and Darunavir respectively as Table 2.6.

Table 2.6 Assay of Tablet.

S. No.	Cobicistat %Assay	Darunavir %Assay
1	101.57	99.79
2	100.53	98.15
3	98.70	99.96
4	101.11	100.64
5	100.81	101.97
6	101.57	100.64
AVG	100.71	100.2
STDEV	1.0688	1.2609
%RSD	1.06	1.26

8. Degradation studies

Standards and degraded samples are injected and calculated the percentage of drug degraded in solution by applying different conditions like acid, alkali and oxidative, photolytic, thermal and neutral analysis shown as Table 2.7.

Table 2.7.degradation data.

Type of degradation	Cobicistat			Darunavir		
	AREA	% RECOVERED	% DEGRADED	AREA	% RECOVERED	% DEGRADED
Acid	291350	97.00	3.00	1323975	96.45	3.55
Base	292953	97.53	2.47	1341109	97.70	2.30
Peroxide	289814	96.48	3.52	1313083	95.66	4.34
Thermal	300012	99.88	0.12	1360729	99.13	0.87
Uv	300085	99.90	0.10	1368190	99.67	0.33
Water	300052	99.89	0.11	1363635	99.34	0.66

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Darunavir in Tablet dosage form. Retention time of Cobicistat and Darunavir were found to be 3.170 min and 3.984 min. % RSD of the Cobicistat and Darunavir were and found to be 1.06 and 1.3 respectively. % Recover was Obtained as 100.71% and 100.2% for Cobicistat and Darunavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Darunavir were 0.107 ppm, 0.326 ppm and 0.333ppm, 1.009 ppm respectively. Regression equation of Cobicistat is y = 10306x + 1346, and of Darunavir is y = 8883.x + 2152. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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