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Validated stability indicating RP-HPLC method for the determination of Cobicistat in bulk and pharmaceutical formulations

K.Sureshbabu[1], B.Koteswararao[2], C.Rambabu[2]*

[1]Satavahana Degree College, Vijayawada, A.P, (INDIA)

[2]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P, (INDIA)

E-mail: rbchintala@gmail.com

ABSTRACT

A new validated RP-HPLC method was developed for the determination of Cobicistat in dosage form. The developed method produced high sensitivity, precision and accuracy. An isocratic C18 (Hypersil BDS, 150 mm x 4.6 mm, 5µ) column was used with mobile phase of composition acetonitrile : phosphate buffer (10 : 90 at pH 6.5) at a flow rate of 1.0 mL/min with UV detection at wavelength of 240 nm for Cobicistat. The retention time of the drug was 4.078 minutes. The developed method was validated for specificity, linearity, precision, accuracy and robustness as per ICH guidelines. Linearity was found in the range of 7.5 – 45.0 µg/ml. The percentage recoveries of the drug ranged from 99.23 – 99.97 %. Forced Degradation study was carried out by treating the sample under the acidic, alkaline, thermal and photo conditions. The results indicated that the any other impurity is not merging with the main peak. The proposed method could be used for routine analysis of Cobicistat in their dosage forms.

KEYWORDS

Liquid chromatography;
Cobicistat;
Dosage forms;
Determinaation;
Validation;
Forced degradation.

INTRODUCTION

Cobicistat,[1,3-thiazol-5-ylmethyl N-[(2R,5R)-5-[[(2S)-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]-4-morpholin-4-ylbutanoyl]amino]-1,6-diphenylhexan-2-yl] carbamate] is used in the treatment of human immunodeficiency virus (HIV). Cobicistat has no anti-HIV activity of its own[1]. In combination with three other drugs elvitegravir, emtricitabine, and tenofovir, Cobicistat is used in the treatment of HIV. The combination of the four drugs is popularly known as Quad – pill[2,3]. A thorough review of literature revealed that there are very few spectrophotometric and HPLC methods reported for the analysis of Cobicistat[4-6]. Hence, it was felt that there is a need of new analytical method development for the determination of Cobicistat in pharmaceutical dosage form. Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible stability indicating RP-HPLC method for the analysis of Cobicistat. The developed method was validated according to ICH guidelines[7].

MATERIALS AND METHODS

Materials

HPLC grade Merck make potassium dihydrogen

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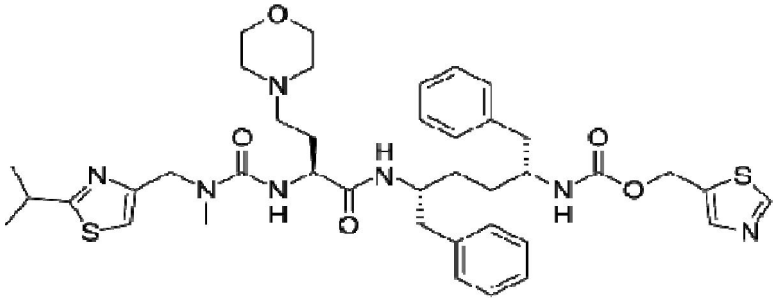

Figure 1 : Structure of Cobicistat

TABLE 1 : System suitability parameters for Cobicistat by proposed method

Name of the compound	Retention Time	Area	Height	USP Tailing	USP Plate count
Cobicistat	4.078	3767990	531698	1.10	8400

orthophosphate (KH_2PO_4), Orthophosphoric acid,, acetonitrile were used. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Tybost® tablets having 150 mg Cobicistat were procured from the local market. HPLC grade Milli-Q water was used throughout the analysis.

Instrumentation

Waters model 2695 LC chromatographic system, with UV-Visible detector with PDA of Waters (2996) make and a fixed injector equipped with 10µL loop was used for the chromatographic separation. The chromatogram was recorded at ambient temperature and peaks quantified by means of Empower software. Chromatographic separation was carried out on a C18 column [Hypersil BDS, 150mm x4.6mm 5µ]. Sartorious electronic balance was used for weighing the samples. Ultrasonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

Chromatographic separation of Cobicistat was carried on a C18 column. The mobile phase was composed of acetonitrile and phosphate buffer (pH 6.5) in the ratio of 10 : 90 v/v. [The buffer solution was prepared by dissolving 1.6 g of potassium dihydrogen orthophosphate in 1000 mLwater and the pH of the was adjusted to 6.5 using dilute orthophosphoric acid]. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1.0 ml/min. Detection was carried out at 240 nm at ambient temperature.

Method development

Preparation of standard stock solutions

Standard stock solution of Cobicistat was prepared by dissolving 15.0 mg in 50 mL volumetric flasks using 15mL of mobile phase. Later, the volumes were made upto the mark with mobile phase to obtain a final concentration of 300 µg/mL. From the above stock solution, 10 mL aliquot was pipetted in to a 100 mL volumetric flask and dissolved in 15mL of the mobile phase and made up to the mark with the diluent (mobile phase) to obtain a final concentration of Cobicistat of 30 µg/mL.

Preparation of sample solutions

Transferred grinded sample of 20 tablets quantitatively equivalent to 138 mg Cobicistat into 50 mL volumetric flask, added 30 mL of diluent, sonicated to dissolve for 10 minutes, filtered and diluted to mark with diluents. 10 mL of this solution was diluted to 100 mL in volumetric flask.

Method validation

The developed HPLC method for the determination of Cobicistat was validated as per the ICH guidelines.

System suitability and system precision

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method

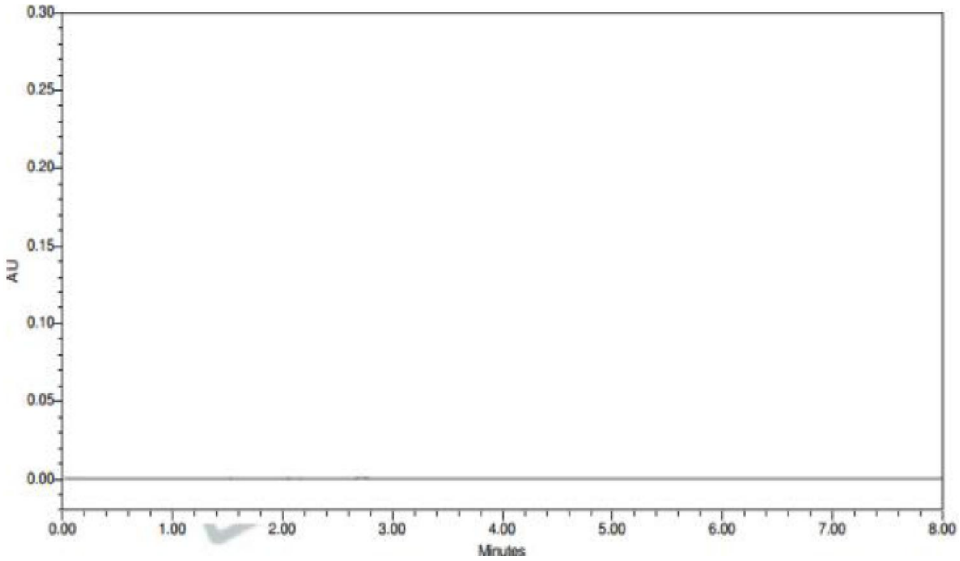

Figure 2 : Chromatogram of Cobicistat blank

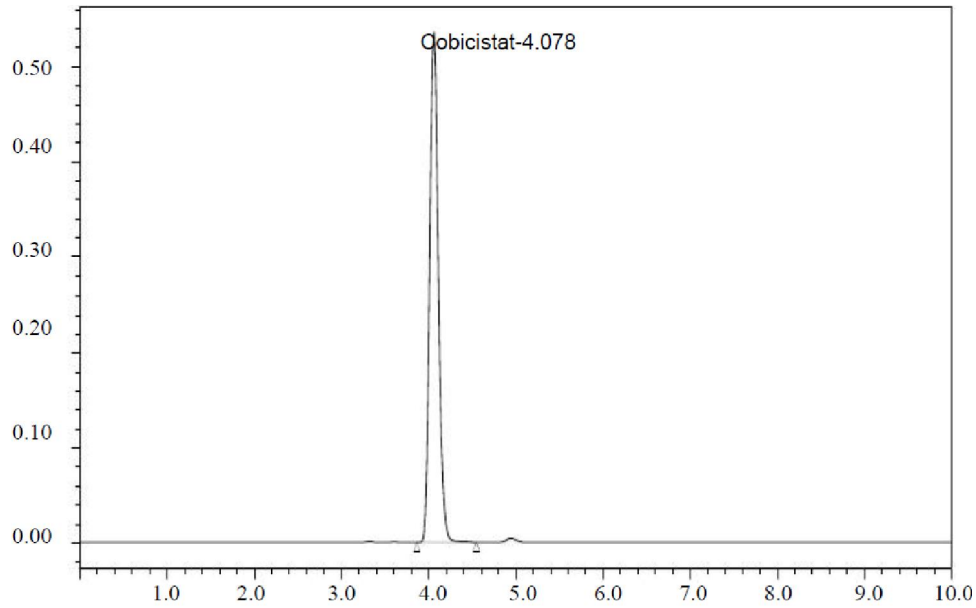

Figure 3 : Standard chromatogram of Cobicistat

include number of theoretical plates (efficiency), tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 30 µg/mL Cobicistat. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the TABLE 1.

Specificity

Blank interference

Specificity studies include application of the proposed method for blank, placebo solution, sample solution (control sample), standard solution. A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the above defined chromatographic conditions and the blank chromatogram was recorded. Chromatogram of Blank solution (Figure no.-2) showed no peaks at the retention time of Cobicistat peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Cobicistat in Tybost tablets. Similarly typical representative chromatogram of standard was also shown Figure-3

Forced degradation

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TABLE 2 : Forced degradation data for Cobicistat

Condition	Time (hours)	Retention time (min)	Retention time of additional degradation peak (min)	% Degradation	% of Active drug Present after Degradation
Acid Degradation	12	4.091	2.976 3.101 3.612 5.401	0.264 7.121 20.012 1.042	71.561
Alkaline Degradation	12	4.097	2.976 3.595 5.383	0.265 5.434 0.403	93.898
Thermal Degradation	08	4.090	3.330 3.613	0.604 0.403	98.993
Photo Degradation	08	4.098	3.611 5.382	2.454 0.432	97.114

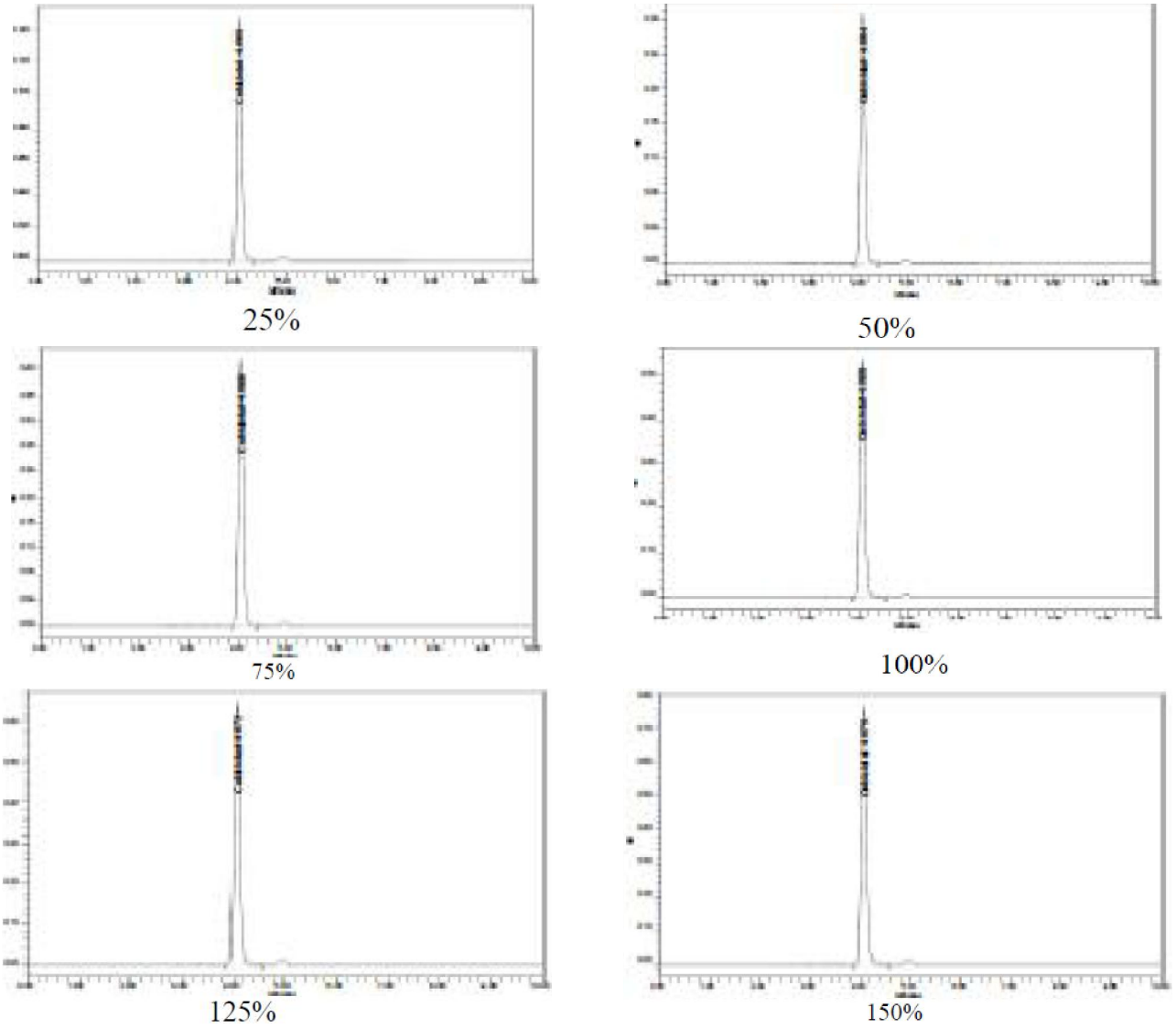

Figure 4 : Linearity chromatograms of Cobicistat

The specificity studies also include deliberate degradation of the tablet sample by exposure to stress conditions. Forced Degradation study was carried out by treating the sample under the acidic, alkaline,

TABLE 3 : Linearity studies for Cobicistat by proposed method

Concentration(µg/ml)	Area (µv²sec)
7.5	903546
15.0	1822435
22.5	2732656
30.0	3692323
37.5	4633259
45.0	5565046

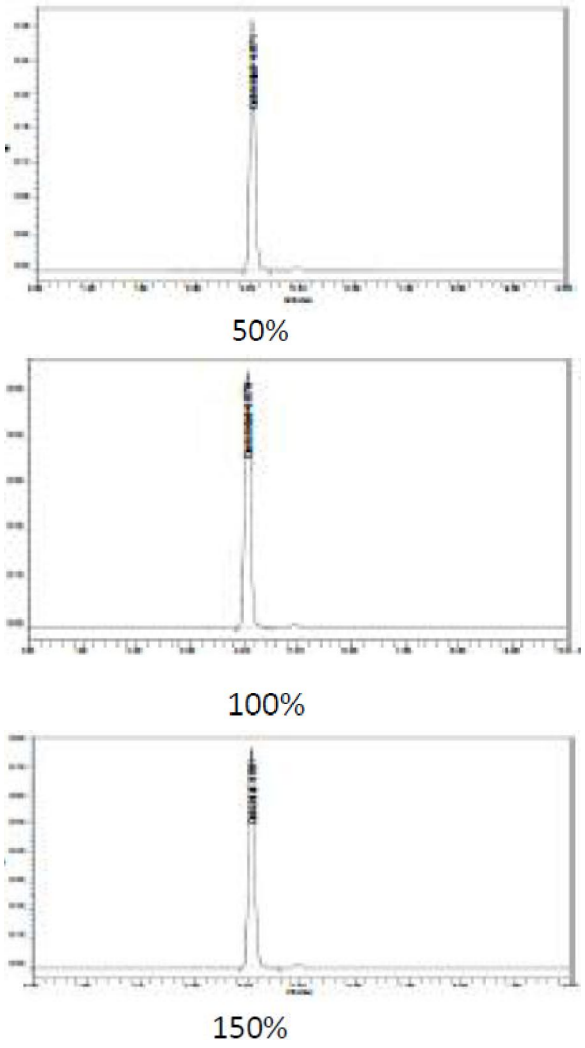

Figure 5 : Accuracy chromatograms of Cobicistat

thermal and photo conditions. Weighed twenty tablets of Cobicistat and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 15 mg was transferred into 50 mL volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 50 mL with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. The results pertaining to these degradation conditions were given in TABLE - 2

Linearity and range

Standard curve was obtained in the concentration range of 7.5 – 45.0 µg/ml for Cobicistat. Linear regression analysis was used to evaluate the linearity of the curve. To assess the linearity of the proposed method, slope, intercept and correlation coefficient [r^2] of standard curve were calculated and the calibration plot was shown in Figure-10. The results were given in the TABLE- 3. From the data obtained, the method was found to be linear within the proposed range. The linearity chromatograms were given in Figure - 4

Accuracy

Accuracy is expressed in terms of percentage recovery. Recovery % is determined by the standard addition method. In the present study recovery studies were carried out at 50%, 100% and 150% spiked levels. The results of Recovery % were given in TABLE 4 and Chromatograms of accuracy were presented in Figure - 5.

Precision

The precision of the method was assessed by six replicate injections of 100% test concentration. The precision was expressed in terms of standard deviation and %RSD. The results were given in TABLE 5 and chromatograms in Figure -6. The system precision was also analysed and the results were given TABLE 6 and the corresponding chromatograms were represented in Figure -7.

Ruggedness

Degree of reproducibility of test results was obtained by analyzing the same sample under variety of normal test conditions such as different ana-

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TABLE 4 : Accuracy data for Cobicistat

S. No	Cobicistat		
	Area(μv^2sec)		
	50%	100%	150%
Injection 1	1829089	3699154	5566905
Injection 2	1839904	3699095	5564389
Injection 3	1854092	3697650	5561290
Average	1841028	3698633	5564195
Amount recovered (µg)	49.62	99.65	149.96
*% Recovery	99.23	99.65	99.37

TABLE 5 : Data for method precision of Cobicistat

S. NO	Cobicistat	
	Retention time	Area
Injection 1	4.074	3801545
Injection 2	4.077	3814329
Injection 3	4.079	3830890
Injection 4	4.083	3835530
Injection 5	4.081	3833254
Injection 6	4.075	3820984
Average	4.078	3822755
Standard deviation	0.003	13142.3
%RSD	0.086	0.34

TABLE 6 : Data for system precision of Cobicistat

S. NO	Cobicistat	
	Retention time	Area
Injection 1	4.071	3724354
Injection 2	4.073	3729085
Injection 3	4.075	3733145
Injection 4	4.072	3726546
Injection 5	4.070	3720985
Injection 6	4.073	3725466
Average	4.072	3726597
Standarddeviation0	0.002	4168.3
% RSD	0.043	0.11

lysts, instruments, days, reagents, column etc. The Ruggedness of the method was verified by analyzing the six samples of same batch for method precision as per test method on two different days. The analyst's prepared six sample of the same batch on two different day's. Calculated %RSD on two different days in six samples for ruggedness results with the method precision. The results of ruggedness were given in TABLE 7 and the chromatograms were given in Figure - 8

LOD and LOQ

The formulae 3.3 σ/S and 10 σ/S were used to calculate LOD and LOQ respectively. σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves.

Robustness

Robustness was assessed by varying the parameters such as percent organic content, pH of the mo-

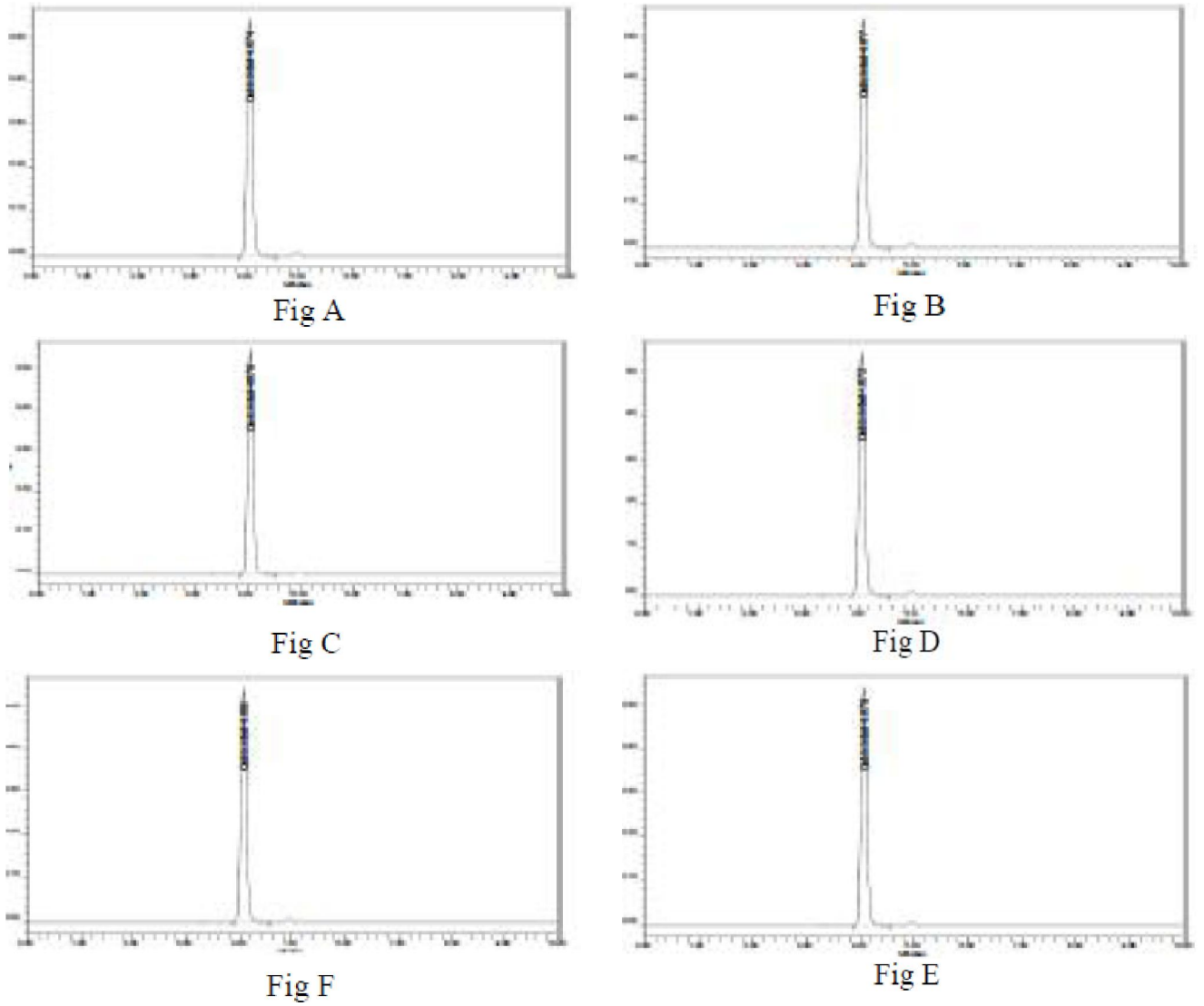

Figure 6 : Method precision chromatograms of Cobicistat

TABLE 7 : Data for ruggedness of Cobicistat

		Day 1		Day 2	
S. No		**Retention Time**	**Area**	**Retention Time**	**Area**
1	Injection 1	4.074	3801545	4.083	3799035
2	Injection 2	4.077	3814329	4.085	3812903
3	Injection 3	4.079	3830890	4.081	3810122
4	Injection 4	4.083	3835530	4.087	3832239
5	Injection 5	4.081	3833254	4.089	3838099
6	Injection 6	4.075	3820984	4.079	3815048
Average		4.078	3822755	4.084	3817908
Std Dev		0.003	13142.3	0.004	14581.5
% RSD		0.086	0.34	0.092	0.382

bile phase, buffer concentration, temperature, injection volume and flow rate. In the present investigation, a variation of ± 0.1 mL/min in the flow rate, change in temperature were adopted to study Robustness. The results were tabulated in TABLE 8 and chromatograms of robustness were given in Figure -9.

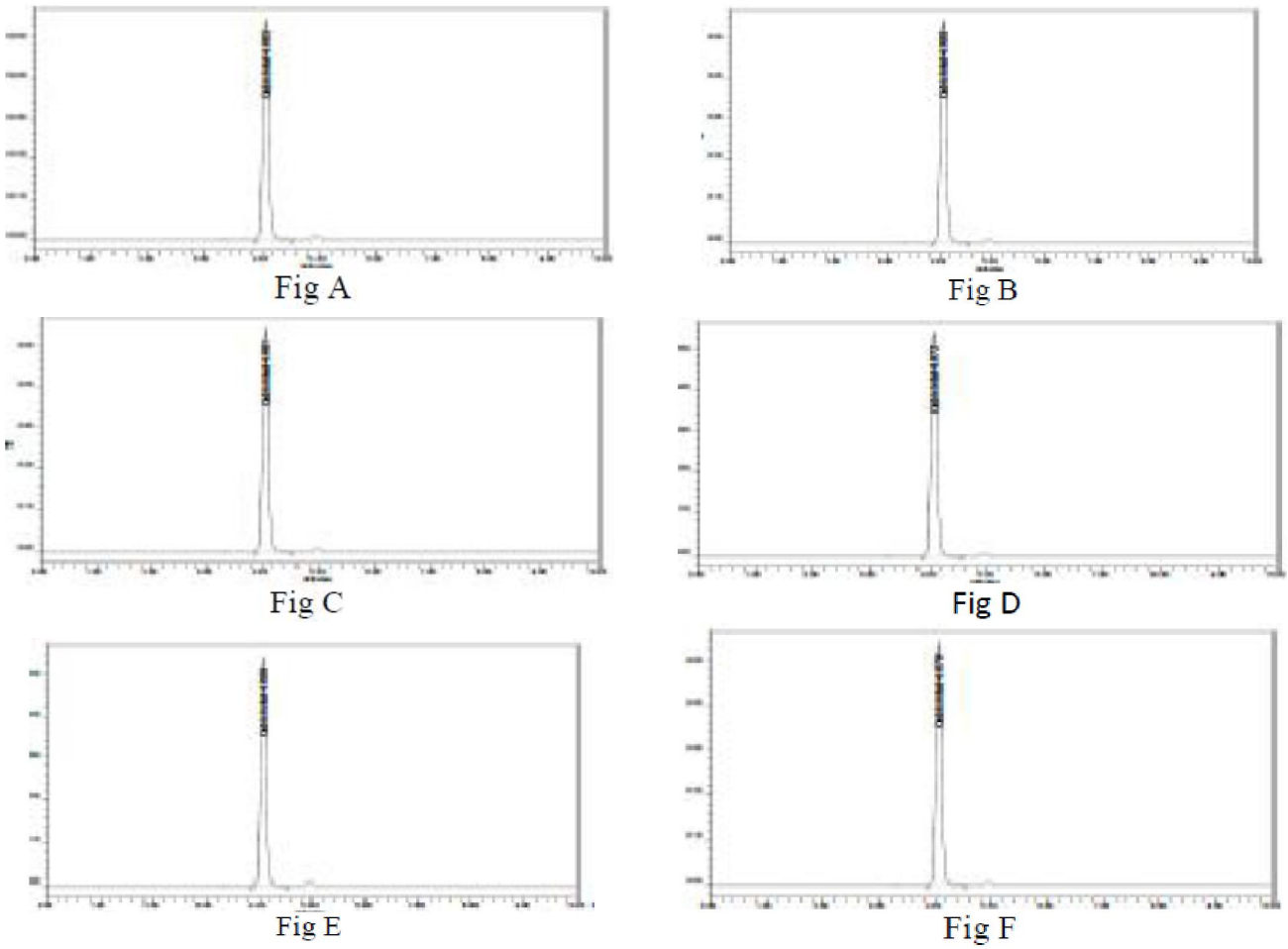

Figure 7 : System precision chromatograms of Cobicistat

RESULTS AND DISCUSSION

In present study a new analytical method reversed phase HPLC method for the determination of Cobicistat tablets in combined dosage form. The column used in this method is Hypersil BDS C18, 150 mm X 4.6 mm, 5µ with a flow rate of 1.0ml/min at a wavelength 240 nm and Coloumn temperature is 30°C. The mobile phase preparation was done by using buffer potassium di hydrogen ortho phosphate (pH 6.5). The mobile phase combination was Buffer: ACN (90:10). The run time was set for 10 minutes. The retention time of Cobicistat was 4.078 minutes. The developed method is specific for the determination of Cobicistat and the same was known from the blank, placebo and forced degradation studies, as no other peak was found at the retention time of Cobicistat during these studies. The new HPLC method developed and validated for determination of Cobicistat in pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of drug in its solid combined dosage form by RP-HPLC method. The linearity range for Cobicistat is 7.5-45.0 µg/ml the co-relation co-efficient was found to be 0.999. The percentage RSD obtained for system precision of Cobicistat was 0.11. The percentage RSD obtained for method precision of Cobicistat was 0.34. The Limit of detection and Limit of Quantification values for Cobicistat are 0.1472 and 0.4461 µg/mL respectively.

The Ruggedness of the method has been verified by analyzing the six samples of same batch for method precision as per test method by different analysts using different instrument, different days. The analyst's prepared six sample of the same batch on two different day's. calculated %RSD for two dif-

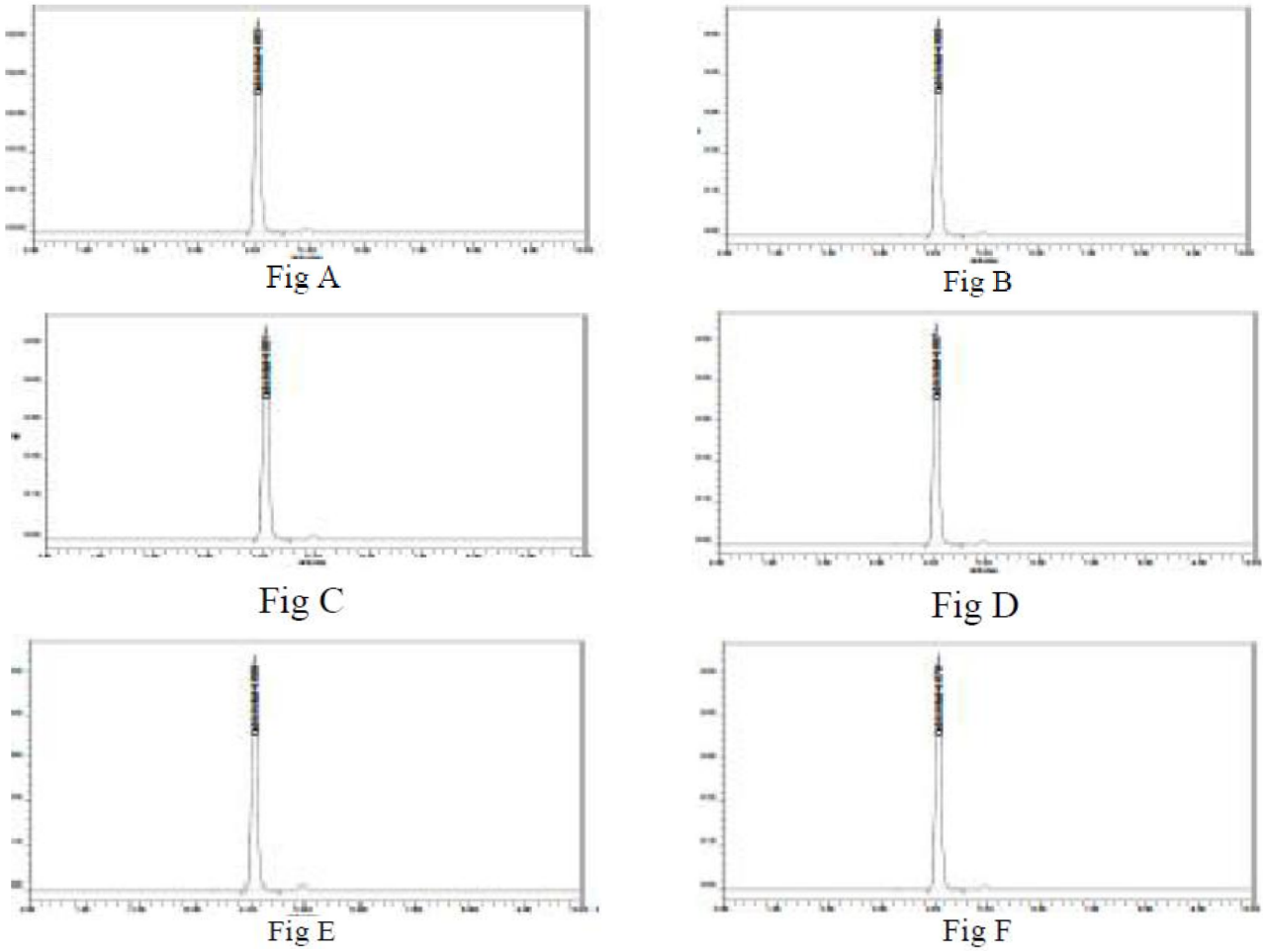

Figure 8 : Ruggedness chromatograms (Day 2) of Cobicistat

TABLE 8 : Data for robustness study of Cobicistat

S. No.	Cobicistat	
	RT	Ar Area (μv^2 sec)
	Standard	
1	2.429	949845
	Robust-1 Flow -1	
2	3.613	3314360
	Robust-2 Flow-2	
3	4.697	4356729
	Robust-3 Temp - 1	
4	4.083	3592189
	Robust-4 Temp -2	
5	4.087	3896576

ferent days in six samples for ruggedness results with the method precision. The system suitability was evaluated in each condition and compared the results with method precision results the method is robust for change in flow rate and temperature. No peak was observed at the retention time of Cobicistat and the developed method was found to be specific.

The sample solution was injected and the amount of Cobicistat present in the formulation was calculated from the calibration curve. The amount of

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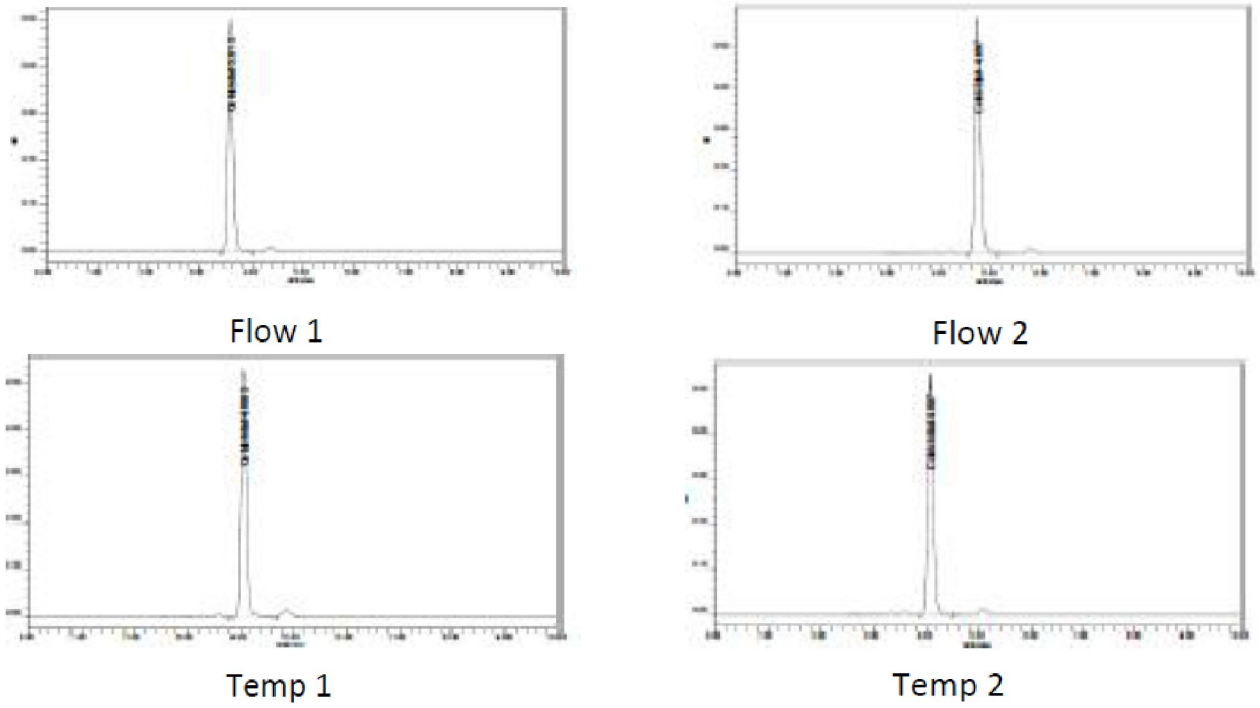

Figure 9 : Robustness chromatograms of Cobicistat

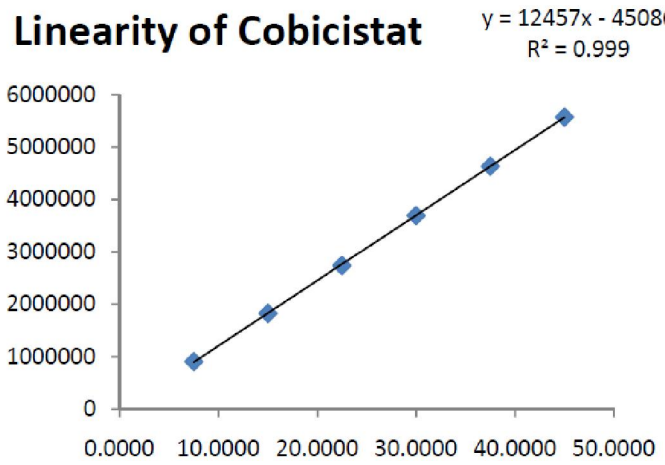

Figure 10 : Calibration curve for Cobicistat

Cobicistat found in the commercial sample as per the developed method was 148.48 mg against to the 150 mg present in the tablet and the assay of Cobicistat was found to be 98.99%.

CONCLUSION

The proposed method for Cobicistat was simple, selective, reproducible and specific with good precision and accuracy. The developed method was proved to be superior to most of the reported methods. The proposed method for estimation of selected drug was successfully applied either in pure form or tablet dosage form. More over the low solvent consumption along with small retention time of 4.078 for Cobicistat seems to be cost effective when compared to other developed methods shown in literature review. The proposed method can be used as alternative methods to the reported ones for the routine determination of selected drug under the study in tablet dosage form.

REFERENCES

[1] E.I.Lepist, T.K.Phan, A.Roy, L.Tong, K.MacLennan, B.Murray, A.S.Ray; Cobicistat

Boosts the Intestinal Absorption of Transport Substrates, Including HIV Protease Inhibitors and GS-7340, in Vitro.Antimicrobial Agents and Chemotherapy, **56(10)**, **(2012)**.

[2] L.Highleyman; Elvitegravir "Quad" Single-tablet Regimen Shows Continued HIV Suppression at 48 Weeks, HIV and Hepatitis.com.

[3] R.Elion, J.Gathe, B.Rashbaum, others; The single-tablet regimen of elvitegravir/cobicistat/emtricitabine/tenofovirdisoproxil fumarate (EVG/COBI/FTC/TDF; Quad) maintains a high rate of virologic suppression, and Cobicistat (COBI) is an Effective Pharmacoenhancer Through 48 Weeks, 50th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2010), Boston, September, 12–15 **(2010)**.

[4] Urooj Fatima, T.Mamatha1, Gajula Rajesh Goud; Der pharmacia sinica, Pelagia research library, **5(5)**, 99-105 **(2014)**.

[5] Raghu Ram Jampala, V.Kiran Kumar, Nemala Appala Raju; Pharmaceutical Methods, **5(1)**, 7-13 **(2014)**.

[6] K,Kavitha, Y.Geetha, G.Hariprasad, R.Venkatnarayana, R.Subramanian; Academic Journal Pharmacie Globale, **4(1)**,1-5 **(2013)**.

[7] International Conference on Harmonization (ICH) Harmonised Tripartite Validation of Analytical Procedures: Text and Methodology, **Q2(R1)**, **(2005)**.