ISSN: 2348-8948
Vol: 3; Issue: 4

RESEARCH ARTICLE

A STABILITY-INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF COBICISTAT

K.KALYANI*,V.ANURADHA

*Department of Chemistry R.V.R & J.C College of Engineering, Guntur, Andhra Pradesh, India,

Department of BS & H, Vignan's Nirula Institute of Technology and Science, Guntur, A.P., India.

Email: kk.chem445@gmail.com

Date Received:	Date of Accepted:	Date Published:
3-Apr-2015	21-Apr-2015	24-Apr-2015

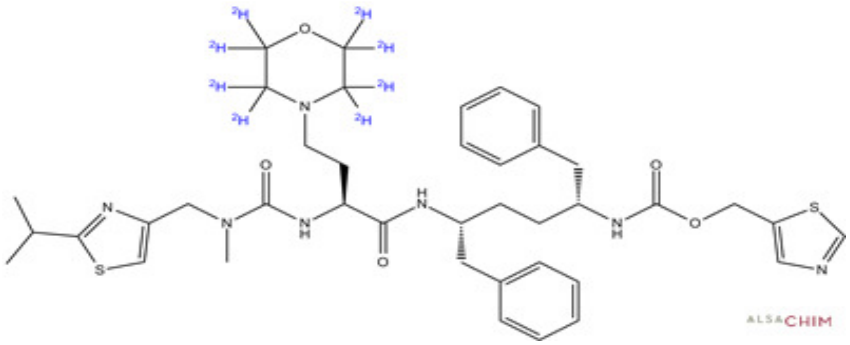
Abstract:
A new reverse phase – high performance liquid chromatography (RP-HPLC) have been developed and validated for the estimation of Cobicistat in bulk drug and pharmaceutical dosage form. The developed method is rapid, accurate, precise, simple and economical. The separation was carried out using column hypersil BDS C-18 (150mm x 4.6mm 5µ particle size) in isocratic mode, with mobile phase containing water: Acetonitrile (90:10). The flow rate 1.0 ml/min effluents are monitored at 240 nm. Chromatogram showed peak at a retention time of 4.09 min for Cobicistat. The method is validated according to ICH guidelines system suitability, linearity, precision, accuracy, specificity, ruggedness, robustness, LOD and LOQ. The calibration plot showed good linear relationship with $r^2 = 0.999$ in the concentration range of 7.5 to 45 µg/ml for Cobicistat. The LOD and LOQ were found to be 0.1472µg/ml and 0.4461 µg/ml. accuracy was found to be 99.61 %. Cobicistat also undergoes degradation in acids, alkalies, thermal and photolytic conditions. The method is specific for the estimation of Cobicistat in Cobicistat tablets.

Keywords: Cobicistat, RP-HPLC, Forced degradation, Method of validation

Introduction

Cobicistat 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 $C_{40}H_{53}N_7O_5S$, molecular weight 775 $g.mol^{-1}$. 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[1,2,3]. Cobicistat is a novel pharmocokinetic boosting agent without activity on HIV[3].

Literature review reveals some of the analytical methods reported for Cobicistat by RP-HPLC method and most of the work was done on biological fluids. RP-HPLC method in pharmaceutical dosage form developed for a specific, precise, accurate, rapid sensitive and faster elution[4]. A few RP-HPLC methods were reported with other drugs[5,6]. Simple and economic UV spectrophoto metric method has been developed for Coboicistat[7].However there was no stability indicating method reported for the drug Cobicistat. Hence the present study was aimed to develop a simple fast economical specific, accurate, precise, linear and sensitive stability indicating RP-HPLC method for the estimation of Cobicistat in bulk and its Pharmaceutical dosage form. The method was validated according to the ICH guidelines.



Structure of Cobicistat

Solubility: Soluble in Dimethyl sulfoxide,Ethyl acetate, Chloroform.
Category: Treatment of infection with the human immunodeficiency virus (HIV)[1,2]
Storage: Store at room temperature, 68°F to 77°F (20°C to 25°C).
Brand Name: Tybost (Gilead Sciences, Inc.)

2. Experimental details of cobicistat

2.1 Instruments and columns

Waters HPLC model 2695 Series quaternary Pump, auto sampler equipped with UV Visible detector synchronized against a waters alliance empower2 software was used for the present study. The column is maintained in a constant temperature column oven that can maintain 5ºC to 60ºC column temperature. Other equipment used are Power Sonicator, model no: 405, Hwashin Technology, Korea, The column used in the development for determination is Hypersil BDS C_{18} (150 mm× 4.6mm, 5µ).

2.2 Chemicals used

HPLC grade acetonitrile and water were purchased from Merck, Mumbai, India, Potassium dihydrogen orthophosphate and orthophosphoric acid AR grade purchased from SD Fine Chem, Mumbai, India.

The reference sample and branded formulation was supplied by Bio Leo Analytical labs, Hyderabad, Andhra Pradesh, India.

2.3 Selection of chromatographic method

Selection of chromatographic method in general is done taking into consideration of several parameters like the nature of the drugs, molecular weight and solubility. Since the drugs selected are polar in nature, RP-HPLC was selected for initial chromatographic condition because of its simplicity and suitability.

2.4 Selection of wave length (λ_{max})

An ideal wavelength is one that uses good response for the drugs to be detected Cobicistat in diluent the spectra was scanned on UV- visible spectrophotometer in the range of 200 nm to 400 nm against diluents as blank. The maximum absorbance of Cobicistat was found to be 240 nm.

2.5 Preparation of mobile phase

1.6gms of potassium dihydrogen orthophosphate was weighed and dissolved in 1000 mL of water, pH adjusted to 6.5 with dilute orthophosphoric acid. The solution was filtered through 0.4µ membrane filter and was degassed. Preparation of Buffer solution: Acetonitrile in a ratio of (90:10 V/V) was filtered through 0.05µ membrane filter and sonicated by using Power Sonicator (model no: 405, Hwashin Technology, Korea) before use. The flow rate of the mobile phase was maintained at 1mL/min. The column temperature was maintained at 30ºC and the detection of the drug was carried out at 240 nm.

2.6 Preparation of stock solution

About 15 mg of Cobicistat was weighed and transferred into 50 mL volumetric flask, 30 mL of diluent was added and the solution was sonicated to dissolve and dilute to the volume with mobile phase.

Standard preparation:

Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

2.7 Preparation of sample solution

Twenty tablets of Cobicistat was weighed and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 138 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. 10 µL of blank solution was injected as diluents of Standard solution, Disregard peaks due to blank and diluents if any.

2.8 Chromatographic conditions
Table : 1

3. Validated RP-HPLC method for cobicistat

3.1 Accuracy

For accuracy determination, three different concentrations were prepared separately i.e.50%, 100%, and 150% of analyte and the chromatograms were recorded for the same. The results obtained for recovery were found to be within the limits. Hence the proposed method was found to be accurate and precise. (table -3)

3.2 Precision

The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed in terms of variance, standard deviation, coefficient of variation of a series of measurement.

System precision

System precision was determined by injecting six homogenous preparation solutions into HPLC System concentration 30µg/mL.The mean, standard deviation and % RSD for peak areas of Cobicistat from standard solutions were calculated. The % RSD Cobicistat was found to be below 1.Hence the method is said to be Precise (table-2)

3.3 Method precision

Method precision was determined by injecting six sample solutions of Single batch were analysed as per test method Concentration 30µg/ml. The mean, standard deviation and % RSD for peak areas of Cobicistat from sample solutions were calculated. The % RSD Cobicistat was found to be below 1.Hence the method is said to be Precise.

3.4 Linearity

Linearity for Cobicistat was determined in the range of 7.5µg/ml– 45µg/ml. A graph was plotted with concentration on X-axis and peak area on Y- axis and correlation coefficient was determined (fig:1). The method was linear from the concentration of 7.5µg/ml–45µg/ml for the estimation of Cobicistat (table-5),

3.5 Ruggedness (intermediate precision)

The Ruggedness of the method has been verified by analysing the six samples of the same batch for method precision as per test method by different analyst using different instrument, different days. The analyst's prepared six samples of the same batch by two different analysts. Calculated %RSD for two different days in six samples for ruggedness results with the method precision (table-2).

3.6 Robustness

To evaluate the robustness, the following small deliberate variations are made in the method and analyzed the sample in triplicate.
Flow rate (±10%)
Column temperature (± 5^0C)
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 Column temperature (table 2, 4).

3.7 Specificity

Specificity shall be established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of Cobicistat with respect to mobile phase, Diluents and degradants.The specificity studies include deliberate degradation of the tablet sample by exposure to stress conditions. Specificity studies also include blank, sample solution (control sample), standard solution were injected into the HPLC system. There was no interference from the blank at the retention time of the peaks. Peak purity data reveals that Cobicistat were homogeneous and there was no interference at the retention time of Cobicistat peaks.

Forced degradation Studies:

Forced degradation or accelerated degradation is a process whereby the natural degradation rate of a product or material is increased by the application of additional stress. Forced degradation or stress test in is undertaken to demonstrate specificity when developing stability- indicating methods, particularly when little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development; manufacturing and packaging in which knowledge of chemical behavior can be used improve a drug product.

Forced Degradation study was carried out by treating the sample under the following 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. Inject 10 µL of blank solution, Standard solution, Disregard peaks due to blank.

Acid degradation: 10 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermediate shaking for 15 min. To this flask 5mL of 0.1N HCl was added and sonicated

for 30 minutes neutralized with 5 ml of 0.1N NaOH and diluted to volume with diluents and was analyzed as per the test method by injecting into HPLC system for 12hrs[8, 9].

Alkali degradation: 10 mL of the above stock solution was transferred into 100 mL volumetric flask, and added 50 mL of diluent with intermediate shaking for 15 min. To this flask 5 mL of 0.1N NaOH was added and sonicated for 30 minutes neutralized with 5 mL of 0.1N HCl and diluted to volume with diluents and was analyzed as per the test method for 12hrs[9].

Thermal degradation: The Drug substance was taken in Petri dish and exposed to a temperature of 105ºC for 8hrs. Then the sample was taken and diluted with the diluent for further analysis. Treated sample was analyzed as per the test method[10].

Photolytic degradation: Sample was exposed to UV light for 8hrs. Treated sample was analyzed as per the test method[11].

4. Analysis of marketed formulations

The fixed chromatographic conditions were applied for the estimation of Cobicistat (TYBOST 150 mg) formulation by RP-HPLC method. Weighed twenty tablets of Cobicistat and powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 138 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. Inject 10 µL of blank solution, Standard solution, Disregard peaks due to blank.

Recording of chromatograms

The standard solutions stabilize the system until stable baseline is obtained. Initially inject the blank solution and diluents. The standard chromatograms were recorded by injected standard solutions and the peak areas of standard chromatograms were noted. A calibration graph was plotted using peak area Vs concentration. Then the sample solution was injected and the amount of Cobicistat present in the formulation was calculated from the calibration curve. The amount of Cobicistat present in per tablet was found to be 101.4 ± 0.159 mg. Total label claim for TYBOST formulation was 150 mg.

4.1 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the developed method were determined by analyzing progressively low concentration of the standard solutions using the developed methods. The LOD is the concentration of the analyte that gives a measurable response (Signal to noise ratio 3.3). The LOD of Cobicistat was found to be 0.1472 µg/mL.LOQ is the lowest concentration of the analyze which gives repose that can be accurately quantified (Signal to noise ratio 10). The LOQ of the Cobicistat was found to be 0.4461 µg/ml.

5. Results and Discussion of Cobicistat

In order to achieve optimum separation of the component peaks, mixtures of Acetonitrile buffer in different combinations were tested as mobile phase on a Hypersil BDS C18 (150 mm× 4.6mm, 5µ) stationary phase. The mobile phase composition of potassium dihydrogen orthophosphate: Acetonitrile in the ratio of 90:10 (v/v) and pH adjusted to 6.5 with dilute orthophosphoric acid were selected as the chromatographic peaks were well defined and resolved with no tailing. A good linear relationship (r2 = 0.9999) was observed in the range of 7.5 - 45µg/ml for Cobicistat (fig-1). The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The system precision was established by six replicate injections of the standard solutions containing analytes of interest. The value of relative standard deviation was found to be 0.11 indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found to be 0.34 indicating the injection repeatability of the method. Recovery values obtained from the proposed method indicates the method is accurate. Six samples of the same batch were prepared by two different days. The %RSD was calculated for two different day's analyst in six samples for ruggedness (intermediate precision) results with the method precision. The method is rugged within the limit. 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 column temperature (table-4). The specificity studies include deliberate degradation of the tablet sample by exposure to stress conditions. Specificity studies also include blank, diluents, sample solution (control sample), standard solution were injected into the HPLC system. There was no interference from the blank and diluents at the retention time of the peaks. Peak purity data reveals that Cobicistat were homogeneous and there was no interference at the retention time of Cobicistat peaks. The specificity of the HPLC method was determined by forced degradation studies as per ICH guidelines [8, 9, 10, 11]. Cobicistat was found to degrade 29% under acidic, where Cobicistat undergoes negligible degradation by alkaline, thermal, photolytic (table-6). Two new peaks were observed in the chromatogram of acidic degrade sample of the drug (fig- 6) compared to standard. No peaks were observed in the chromatograms of the alkaline, thermal, photolytic degrade samples of the drug (fig-7, 8,9). The results obtained indicate that the drug

Table : 1

Method parameters	Optimisation conditions
Column	Hypersil BDS C18, 150 X 4.6, 5µ.
Flow Rate	1.0 ml/min
Wave length	240 nm
Column température	30°C
Injection volume	10 µL
Diluent	Mobile Phase
Elution type	Isocratic
Needle Wash solution	Water: Acetonitrile (90:10)

Table 2: Summary of validation parameters:

No.	Validation Parameters	Results
1	**Precision**	The method is precise
	RT (min)	% RSD 0.043
2	**Method precision**	
	RT (min)	% RSD 0.086
3	**Ruggedness**(For Day-1 and Day-2)	
	RT (min)	% RSD 0.11
4	**Linearity**	Correlation coefficient (r^2) = 0.9999 Concentration range = 7.5 - 45(µg/mL)
5	**Robustness** Change in Column temperature at ± 5 1. Column temperature at 25^0C 2. Column temperature at 35^0C Change in flow rate at ±0.2mL/min 1.flow rate at 0.8mL/min 2.flow rate at 1.2mL/min	The method is robust for change in flow rate and Column temperature. % RSD 0.76
6	**Specificity** Interference from diluents and interference from force degradation	No interference at the retention time of Cobicistat peaks
7	LOD(µg/ml)	0.1472 (µg/ml)
8	LOQ (µg/ml)	0.4461 (µg/ml)

Table:3 Determination of accuracy for Cobicistat

Accuracy	Amount added (mg/ml)	Amount recovered (mg/ml)	% recovered
50%	69	49.62	99.23
100%	138	99.65	99.65
150%	207	149.62	99.97
Overall mean of 3 levels % recovery			99.61

Table no: 4 Robustness data for Cobicistat

Variations	Chromatographic parameters	
	Retention time (min)	Area (μV^2 Sec)
Change in Column temperature at ± 5 1. Column temperature at 25^0C 2. Column temperature at 35^0C	 4.083 4.087	 3592189 3896576
Change in flow rate at ±0.2mL/min 1.flow rate at 0.8mL/min 2.flow rate at 1.2mL/min	 3.613 4.697	 3314360 4356729

Table no: 5 Linearity data for Cobicistat (n=6)

Concentration of Cobicistat (µg/ml)	peak area (μV^2 Sec)
7.5	903546
15	1822435
22.5	2732656
30	3692323
37.5	4633259
45	5565046

Concentration range (µg/mL) = 7.5 - 45
Correlation coefficient (r^2) = 0.9999
Slope (m) = 124570
Intercept (b) = 45086

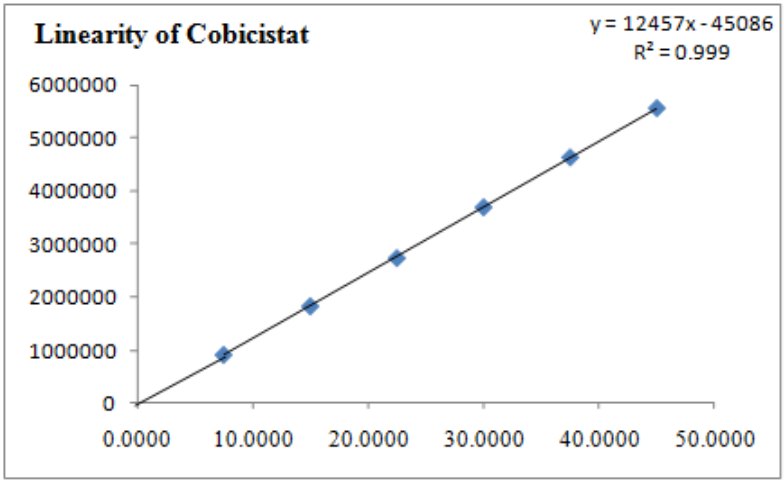
Table no: 6 Forced Degradation data for Cobicistat

Condition	Time (hours)	Retention time (min)	% of Active drug Present after Degradation
Acid Degradation	12	4.091	71.561
Alkaline Degradation	12	4.097	93.898
Thermal Degradation	08	4.090	98.993
Photo Degradation	08	4.098	97.114

Table no: 7 Analysis of marketed formulation (Assay) data for Cobicistat (n=3)

Drug	Quantity claim (mg/tablet)	*Quantity found (mg/tablet) ± SD	* % Assay found ± SD
Cobicistat	150	148.48 ± 2	98.99 ± 1.4

Fig. 1: Calibration curve for Cobicistat



concentration (µg/ml)

Fig.2: Standard Chromatogram of Cobicistat

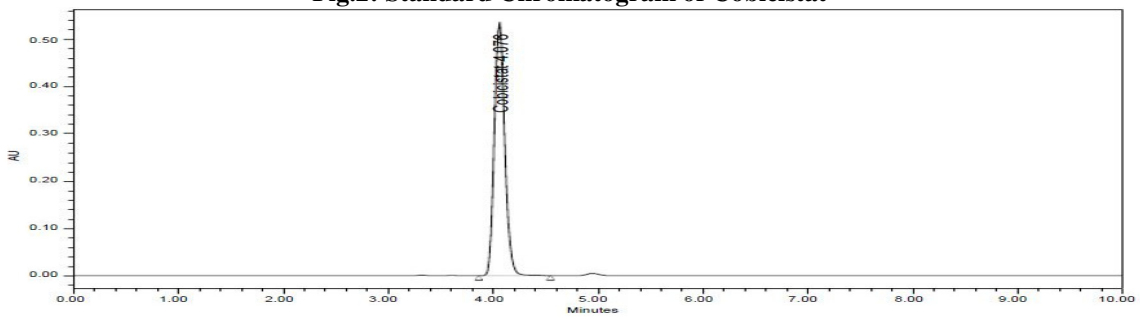


Fig.3: Accuracy chromatogram of Cobicistat

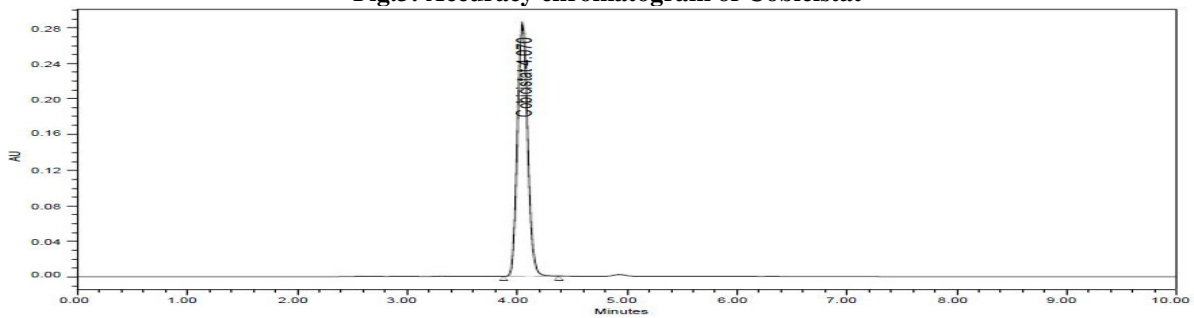


Fig.4: Precision chromatogram of Cobicistat

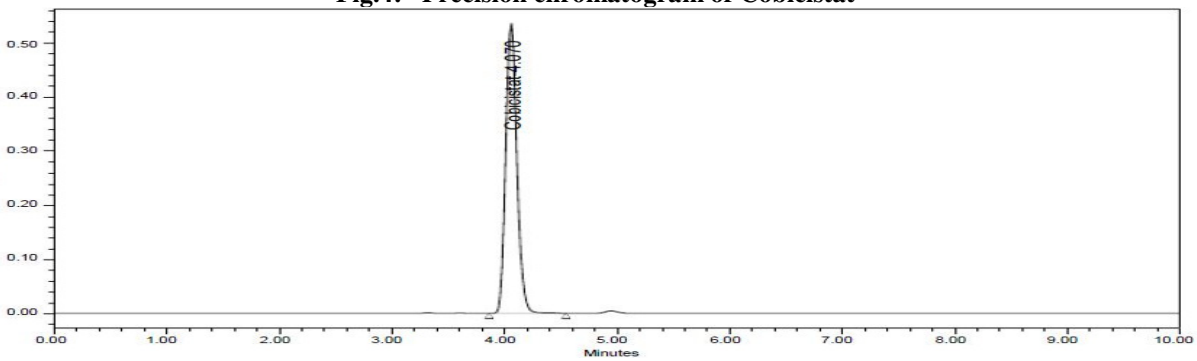


Fig.5: Linearity chromatogram of Cobicistat

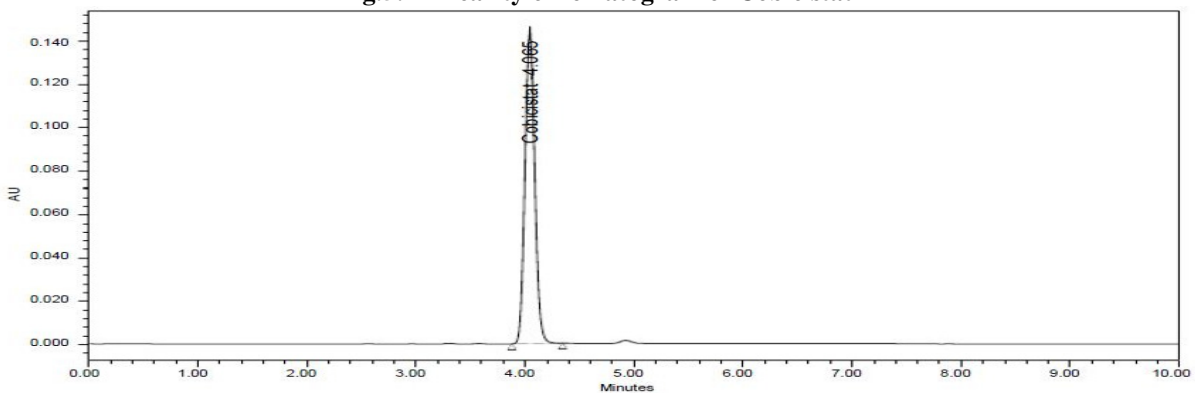


Fig.6: Chromatogram of Acid degradation

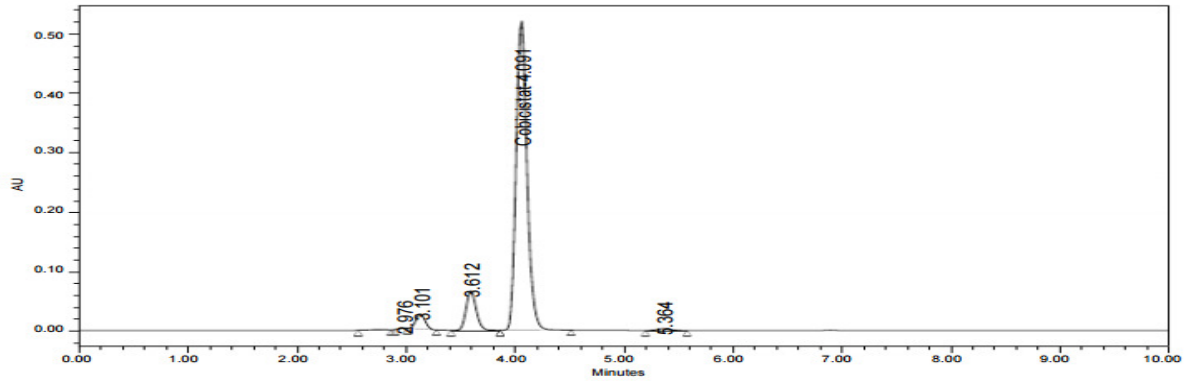


Fig.7: Chromatogram of Alkali degradation

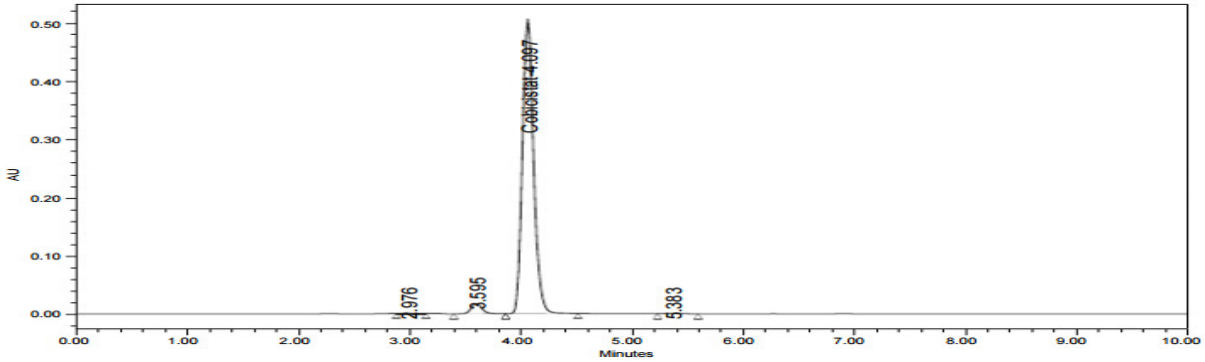


Fig.8: Chromatogram of Thermal degradation

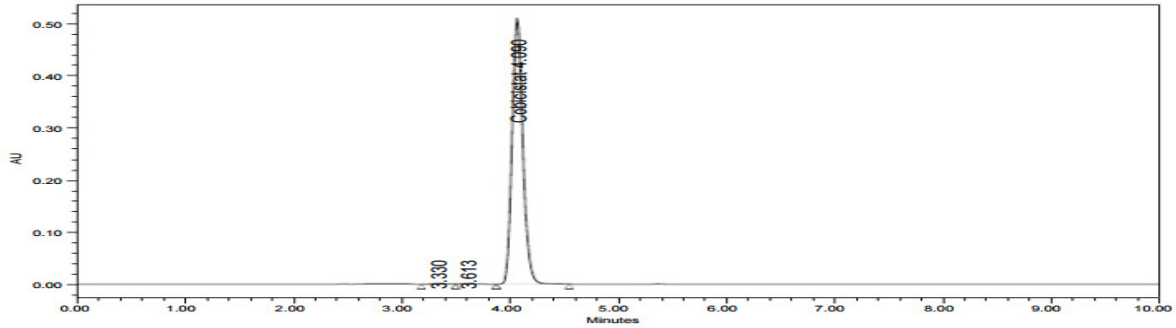
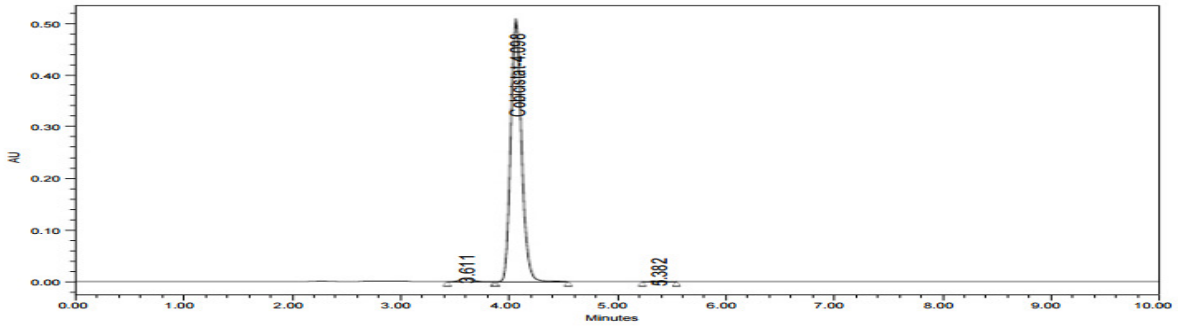


Fig.9: Chromatogram of Photolytic degradation



undergoes degradation under acidic condition, where as it relatively stable under alkaline, thermal, photolytic conditions. Hence the developed RP-HPLC method was found to be simple, rapid, sensitive, accurate, precise and specific for the estimation of Cobicistat in Cobicistat tablets. The sample solution was injected and the amount of Cobicistat present in the formulation was calculated from the calibration curve. The amount of Cobicistat present in per tablet was found to be 148.48 ± 2 mg (table-7). Total label claim for TYBOST formulation was 150 mg.

6. Conclusion

The HPLC method developed and validated allows a simple and fast quantitative determination of Cobicistat from its formulation. All the validation parameters were found to be within the limits according to the ICH guidelines[12,13]. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged, and robust and can be involved in the routine analysis of the marketed formulation.

7. Acknowledgements

The author acknowledges the management and principal of R.V.R&J.C College of engineering and CHIPS Pharmaceutical Sciences for their kind support.

REFERENCES OF COBICISTAT

1. Website http:// en.wikipedia.org/ wiki/ Cobicistat.
2. www.hivclinic.ca December 2014. Page no-1 International Conference on Harmonisation, Geneva, October, 1993.
3. Christian Manzardo and Jose M.Gatell A new pardigm for HIV-1 treatment, Aids Rev 2014, 16, 35-42.
4. Urooj Fatimal, T.Mamatha and RjeshGoudGajula, A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form, Der Pharmacia Sinica, 2014, 5(5), 99-105.
5. Kavitha, K. Y.; Geetha, G.; Hariprasad, R.; Venkatnarayana, R.; Subramanian, G. development and validation of RP-HPLC analytical method for simultaneous estimation of Emtricitabine, Rilpivirine, Tenofovir Disoproxilfumarate and its pharmaceutical dosage forms, 2013, 4 (1), 150-155
6. V.V. Raveendra Babu 1,*, Pankaj K.Sharma 2 and I. Singhvi , A New Gradient Liquid Chromatographic Method for simultaneous Estimation of Tenofovir DisoproxilFumarate, Cobicistat, Emtricitabine and Elvitegravir in bulk drug and tablet dosage form, Asian Journal of Chemistry; (2014), 26(18) , 6233-6237.
7. Chandni Saha, Md. Nazeeruddin Ahmed, Development and validation of a simple uv spectrophotometric method for the determination of cobicistat in its bulk form, indo American Journal of Pharmaceutical Research, 2014, 14(12) ,5792 - 5796.
8. ICH, Stability testing of new drug substances and products. Geneva: International Conference on Harmonization. IFPMA, 2003.
9. ICH, Q1A, Stability Testing of New Drug Substances and Products. In: Proceedings of the International Conference on Harmonization, Geneva, March, 1994. 23.
10. ICH, Q2A, Hamonised Tripartite Guideline, Test On Validation of Analytical Procedures, IFPMA. In: Proceedings of the International Conference on Harmonization, Geneva, March, 1994. 23.
11. ICH, Q2B, Hamonised Tripartite Guideline, Validation of Analytical Procedure: Methodology, IFPMA, In: Proceedings of the International Conference on Harmonization, Geneva, March, 1996.
12. ICH, Q1A (R2): Stability Testing of New Drug Substances and Products, ICH Harmonized Tripartite Guideline, Geneva Switzerland, 2003.
13. ICH Q2 (R1): Validation of Analytical Procedures: Text and Methodology, ICH Harmonized Tripartite Guideline, Geneva Switzerland, 2003.