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Advances in Bioresearch

ORIGINAL ARTICLE

Method Development and Validation for the Quantification of Cobicistat and Atazanavir in Pure and Pharmaceutical Formulations by RP-HPLC

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ABSTRACT
A simple, precise, accurate, robust & cost-effective method was developed for the routine analysis. The method was successfully validated in terms of linearity, precision, accuracy as per ICH guidelines. The sample preparation was very simple and done with easily available solvent like acetonitrile. The linearity range of the present method is 25-160 µg/ml when compared to other methods with linearity range 45-135 µg/ml and 25- 67.5 µg/ml for Atazanavir and Cobicistat respectively. The LOD and LOQ of the present method was found to be 0.035 µg/ml and 0.11 µg/ml for Atazanavir and 0.025 µg/ml and 0.078 µg/ml for Cobcistat. Previous reported methods have higher LOD and LOQ values when compared to the present method. Hence it is a very sensitive method. The retention time of Atazanavir and Cobicistat was found to be 5.48 mins and 7.02 mins respectively which is almost equal to the other reported methods. Hence it can be concluded that the proposed method is a good approach for obtaining reliable results & is also found to be suitable for the routine analysis and quality control and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination.

***KEYWORDS**: Cobicistat, Atazanavir, RP-HPLC, ICH, Accuracy*.

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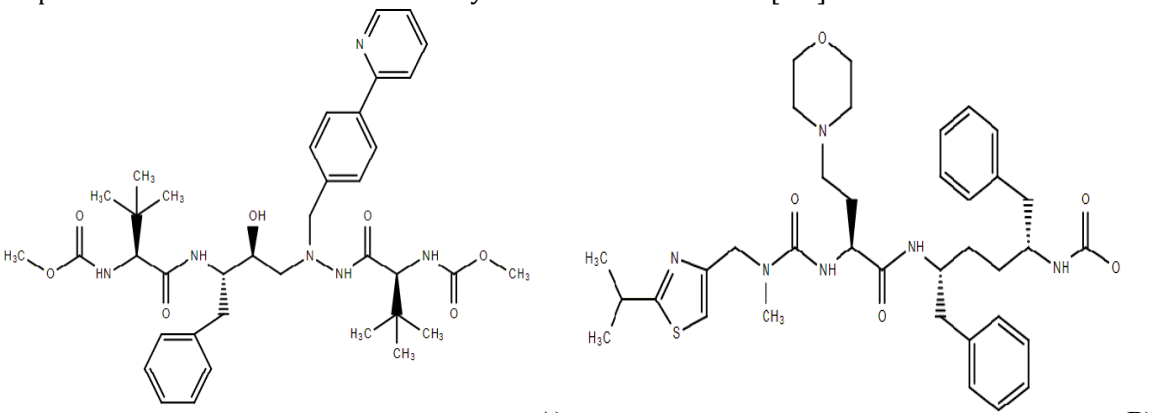
INTRODUCTION

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 atazanavir and darunavir and therefore enables increased anti-viral activity at a lower dosage Cobicistat does not have any anti-HIV activity on its own (Fig. 1). Cobicistat, marketed under the name Tybost (formerly GS-9350), indicated for treating 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 atazanavir or darunavir (once daily dosing regimen) in combination with other antiretroviral agents in the treatment of HIV-1 infection. increasing systemic exposure of anti-retrovirals (ARVs) without increasing dosage allows for better treatment Atazanavir selectively inhibits the virus specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing the formation of mature virions. Atazanavir is not active against HIV-2 [1-3].
Atazanavir (ATV) is an azapeptide HIV-1 protease inhibitor (PI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). HIV-1 protease is an enzyme required for the proteolytic

cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Atazanavir binds to the protease active site and inhibits the activity
of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs. Atazanivir is pharmacologically related but structurally different from other protease inhibitors and other currently available antiretrovirals [4-7].



A) B)

Fig. 1: Structure of A) Atazanavir and B) Cobicistat.

MATERIAL AND METHODS

Chemicals and reagents

HPLC grade methanol, acetonitrile and analytical grade sodium acetate was purchased from Merck (Mumbai, India). Cobicistat working standard was obtained as a gift sample from Mylan Laboratories, (Hyderabad, India) and Atazanavir working standard from Hetero drugs Ltd, Hyderabad, India. The Evotaz tablets (150 mg of Cobicistat and 300mg Atazanavir sulphate, Bristol-Myers Squibb Company, UK) were procured from the local pharmacy.

Instrumentation

Shimadzu gradient HPLC (JAPAN), HPLC column Phenomenex (250 x 4.6mm, 5µm), Mobile phase filtration unit (Pall Life sciences, Mumbai, India), LAB-INDIA U.V with Empower software, Sonicator, P^H meter (LAB-INDIA), digital balance (Denver).

Preparation of sodium acetate buffer

About 0.82 gm of sodium acetate (Mol. Wt. 82.04) was dissolved in water and the final volume was made up to 1000.0 ml. pH of the solution was adjusted to 4.2 with dilute ortho phosphoric acid.

Preparation of Calibration standard solutions

Stock solutions (1mg/ml) of Atazanavir and Cobicistat were prepared in acetonitrile. Further dilutions were carried out using 60% Acetonitrile as diluent. Atazanavir and Cobicistat working standards of different concentrations ranging from 25-150µg/ml for Atazanavir and Cobicistat were prepared by diluting several aliquots of standard solutions of Atazanavir and Cobicistat.

Preparation of sample solution

Twenty tablets (Evotaz) each containing 150 mg of Cobicistat and 300 mg of Atazanavir were weighed and powdered equivalent to dose, transferred to a 100 mL volumetric flask, and extracted with 60% acetonitrile. The mixture was sonicated for 20 min in an ultrasonic bath. The volume was adjusted to 100 mL with the same solvent and then filtered. 1ml of the solution was transferred into a 10 ml volumetric flask and diluted up to the mark with the diluent. Further exactly 2 ml of the above dilute solution was introduced into a 10 ml volumetric flask and diluted up to the mark with diluent, and final concentration of Cobicitat and Atazanavir was found to be 30 and 60 µg/ml respectively.

Preparation of standard solution

Standard solution of concentration 30 µg/ml of Cobicistat and 60 µg/ml of Atazanavir were prepared by dissolving exactly 15 mg of Cobicistat and 30 mg of Atazanavir sulphate drug product in a 100 ml clean volumetric flask containing diluent (60% acetonitrile) and sonicated and made up to the mark and filtered through 4.5 µ filter under vacuum filtration. Further exactly 2 ml of the standard solution was diluted to 10 ml.

Assay procedure

The HPLC system was equilibrated and the sample and standard solutions were analyzed as per optimized chromatographic conditions in triplicate (n=3) and the percent of assay was calculated from the peak area of standard and sample.

%Assay= (AT/AS) x (WS/WT) x (DT/DS) x (P/100) x (Average weight/Label claim) x 100.

Where, AT = Average area counts of test, AS = Average area counts of standard**,** WS = Weight of standard taken in mg, WT = Weight of sample taken in mg, DT = Dilution of test**,** DS = Dilution of standard**,** P = Percentage purity of working standard, LC = Label claim of the drug in mg.

The developed method was validated for parameters like linearity, precision, accuracy, ruggedness, limit of detection and limit of quantification in analytical solution. The stability indicating nature of the method was checked by performing stability studies.

Stability studies

The objective of stability studies is to determine the percent of the sample found to be stable when it was subjected to different chemical and physical degradation conditions such as 0.1N HCl (acid hydrolysis), 0.1N NaOH (base hydrolysis), 3% H_2O_2 (oxidation), heat (thermal decomposition) and UV-light (radiation decomposition) for specified time [9].

Preparation of Degradation samples for Specificity Study

In order to establish whether the analytical method and the assay were stability-indicating, EVOTAZ tablets and pure active pharmaceutical ingredient (API) of both Atazanavir and Cobicistat were stressed under various conditions to conduct forced degradation studies. As these drugs are freely soluble in acetonitrile, it was used as a solvent and diluent in all the forced degradation studies.

RESULTS AND DISCUSSION

Method Development

The development of liquid chromatographic method was based on physico-chemical properties such as molecular weight, molecular formula, chemical structure, solubility, pKa value and UV absorption maxima of selected drugs. The selected drugs were completely soluble in water and methanol. Hence a reversed phase liquid chromatographic technique was adopted. The optimum chromatographic conditions were established by different trials by changing one of the chromatographic conditions such as column, mobile phase and its composition, flow rate of the mobile phase, injection volume, run time, column temperature and detection wavelength and keeping others constant.

Optimized Chromatographic Conditions

The HPLC system consisted of Shimadzu gradient HPLC (JAPAN) with dual λ absorbance UV detector. The wavelength of detection was set at 235nm. Separation was carried out in isocratic mode on Phenomenex C18 column (4.6 x 250mm x 5µm) and the retention time of Atazanavir and Cobicistat was found to be 5.48 min and 7.02 min respectively (Fig. 2), mobile phase used consisted of a mixture of 0.01M sodium acetate buffer of pH 4.2, methanol and acetonitrile in the ratio of 25:15:60 v/v at a flow rate of 1ml/min. The mobile phase was filtered through nylon milli pore (0.2µm) membrane filter and degassed with Ultra sonicator prior to use. Chromatography was carried out at room temperature at 25°C and the column temperature was maintained at 32°C.

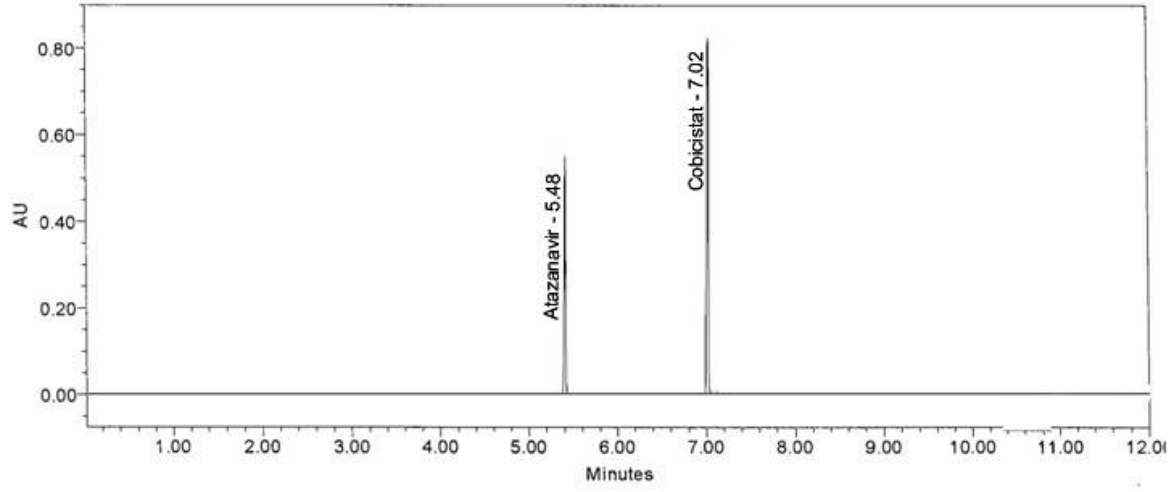


Figure. 2: Chromatogram of Atazanavir and Cobicistat

The developed method was validated for linearity, precision, accuracy, ruggedness and was applied for forced degradation studies as per the ICH guidelines. (Q1A(R2) ICH, Geneva., 2005).

Method validation

Linearity

Linear concentrations of both drugs were prepared and the best fit line was calculated. Wide range calibration was determined [9-12] by solutions containing 25µg/ml to 150µg/ml for Atazanavir and Cobicistat each (Table 1). Correlation coefficient was found to be 0.999 & 0.998 for Atazanavir and Cobicistat respectively (Fig 3 and 4).

Table 1: Linearity Data for Atazanavir & Cobicistat

Atazanavir				
Conc (µg/ml)	**Area 1**	**Area 2**	**Area 3**	**Avg Area**
25	92826	92754	92822	92801
50	196479	196407	196475	196454
75	286626	286554	286622	286601
100	373493	373421	373489	373468
125	471928	471856	471924	471903
150	565386	565314	565382	565361
Intercept	3521	3450	3518	3399
Slope	3744	3744	3744	3744
Intercept Standard Deviation				40.15
LOD (µg/ml)				0.035
LOQ(µg/ml)				0.11
Cobicistat				
Conc (µg/ml)	**Area 1**	**Area 2**	**Area 3**	**Avg Area**
25	206754	206621	206689	206688
50	433082	432949	433017	433016
75	682282	682149	682217	682216
100	839203	839070	839138	839137
125	1078082	1077949	1078017	1078016
150	1271682	1271549	1271617	1271616
Intercept	10191	10058	10126	10125
Slope	8476	8476	8476	**8476**
Intercept Standard Deviation				**66.50**
LOD (µg/ml)				**0.025**
LOQ(µg/ml)				**0.078**

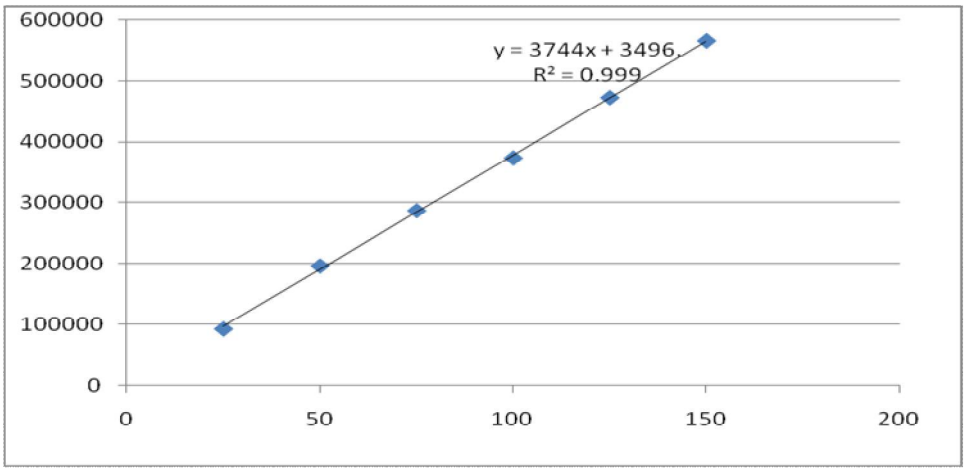


Fig. 3: Calibration Curve of Atazanavir

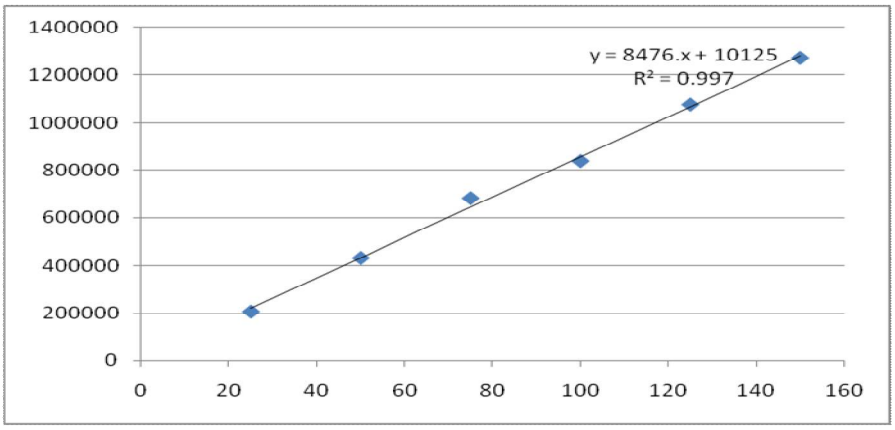


Fig. 4: Calibration Curve of Cobicistat

Limit of Detection (LOD) and Limit of Quantification (LOQ);

The LOD was calculated using the formula 3.3 times σ/s where "σ" is standard deviation of the intercept obtained for calibration curve and "s" is the slope of the calibration curve. Similarly LOQ is calculated using the formula 10 times σ/s. The calculated LOD and LOQ are shown in Table 1.

Precision

The intraday precision was demonstrated by injecting six test solutions of Atazanavir and Cobicistat with 10 µg/ml and 25µg/ml respectively as per the test procedure (Table 2) and recorded the chromatograms of six test solutions. The % RSD of Atazanavir and Cobicistat was found to be 0.10 and 0.08 respectively [13-16].

Table 2: Method Precision Data for Atazanavir and Cobicistat.

Atazanavir (50µg/ml)		**Cobicistat (125µg/ml)**
S.No	**Area**	**Area**
1	196479	1078082
2	196107	1076854
3	196175	1077127
4	196559	1078192
5	196487	1079283
6	196568	1078517
Mean	196396	1078009
SD	201.83	897.98
%RSD	0.10	0.083

Intermediate Precision;

Intermediate precision of the analytical method was determined by performing method precision on three successive days by different analysts under same experimental condition. Assay of all six replicate sample preparations was determined and the mean % RSD of Atazanavir and Cobicistat was found to be 0.57 and 0.82 respectively (Table 3).

Table 3: Intermediate Precision Data for Atazanavir and Cobicistat

Atazanavir Area for 50µg/ml					**Cobicistat Area for 125µg/ml**			
S.No	**Day-1**	**Day-2**	**Day-3**	**Avg**	**Day-1**	**Day-2**	**Day-3**	**Avg**
1	193086	192590	192693	192790	1035926	1032098	1031622	1033215
2	195715	195519	195723	195652	1044700	1041076	1031401	1039059
3	193783	195586	194390	194586	1034973	1031747	1042673	1036464
4	196166	195769	195773	195903	1046036	1042107	1041731	1043291
5	196094	193898	195201	195064	1057124	1053593	1052816	1054511
6	193175	195978	195682	194945	1056360	1054130	1045055	1051848
Mean	194670	194890	194910	194823	1045853	1042459	1040883	1043065
SD	1475.73	1350.18	1205.8	1106.1	9547.06	9841.87	8238.72	8542.15
%RSD	**0.75**	**0.69**	**0.62**	0.57	**0.91**	**0.94**	**0.79**	0.82

Accuracy

Accuracy of the method was established by performing recovery studies according to the ICH guidelines [9, 17]. Spiked samples were prepared by spiking pre-analyzed sample solutions with pure drug at three different concentration levels each in triplicate. Mean percentage recovery values at three different concentrations of the two drugs was calculated. The % mean recovery of Atazanavir (99.19-101.68%) and Cobicistat (99.03-99.72%) at each level was within the limits of 98% and 102% (Table 4)

Table 4: Accuracy data of Atazanavir and Cobicistat

Accuracy of Atazanavir						
S.N0.	**Conc.**	**Calculated Concn.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1	50	50.48	100.95			
2	50	51.28	102.56	101.68	0.81	0.80
3	50	50.76	101.52			
1	100	99.03	99.032			
2	100	99.41	99.409	99.19	0.19	0.19
3	100	99.16	99.156			
1	150	149.3	99.517			
2	150	150.3	100.21	99.75	0.40	0.40
3	150	149.3	99.522			
Accuracy of Cobicistat						
S.N0.	**Conc.**	**Calculated concn.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1	50	49.31	98.62			
2	50	49.91	99.79	99.66	0.97	0.98
3	50	50.28	100.55			
1	100	99.47	99.48			
2	100	98.99	98.98	99.73	0.88	0.89
3	100	100.7	100.71			
1	150	148.23	98.83			
2	150	148.91	99.23	99.038	0.20	0.21
3	150	148.63	99.05			

Ruggedness

The ruggedness of method was calculated with six injections of 75µg/ml in two batches using two different columns. The % covariance of ruggedness for Atazanavir was 0.40 with column-1 and 0.36 with column-2 and the % CV of ruggedness for Cobicistat was 0.43 with column-1 and 0.31 with column-2 (Table-5), which is within acceptance limits.

Table 5: Results of Ruggedness

	Atazanavir 75µg/ml		**Cobicistat 75µg/ml**	
S.NO	**Column 1**	**Column 2**	**Column 1**	**Column 2**
1	75.02	75.08	75.04	75.08
2	74.56	75.02	75.02	74.88
3	74.34	74.79	74.34	74.62
4	74.54	74.82	74.72	74.96
5	75.09	74.61	74.55	74.59
6	74.55	74.34	74.29	74.51
Mean	74.68	74.78	74.66	74.77
± SD	0.30	0.273	0.32	0.23
% CV	0.40	0.364	0.43	0.31
% Accuracy	99.58	99.70	99.54	99.70

Results of Assay Studies:

The mean percent of assay of Evotaz tablet (containing 150 mg of Cobicistat and 300 mg of Atazanavir) was determined by comparing the peak area of standard and formulation and found to be 98.5 for Atazanavir and 99.6 for Cobicistat, and the results are given in Table 6.

Table 6: Results of assay studies of Atazanavir and Cobicistat

S. No.	Standard (Evotz)		Sample		% Assay	
	Atazanavir 60 µg/ml	Cobicistat 30 µg/ml	Atazanavir 60 µg/ml	Cobicistat 30 µg/ml	Atazanavir	Cobicistat
1	229300	272912	224714	276187	98.4	101.2
2	235774	259849	226343	258549	96.3	99.5
3	222782	248104	224714	245623	100.8	99.2
Mean	229286	260288	225257	260120	98.5	99.96

Results of Stress Degradation Studies

Stress degradation studies were performed as per the ICH guidelines Q1A (R2) Stability Testing of New Drug Substances and Products, using the proposed validated analytical method(Table 7 and 8).

Acid Degradation studies

To 1ml of stock solution Atazanavir and Cobicistat, 1ml of 2N HCl was added and refluxed for 30min at 60^0c. From the above solution 10µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Fig 5)

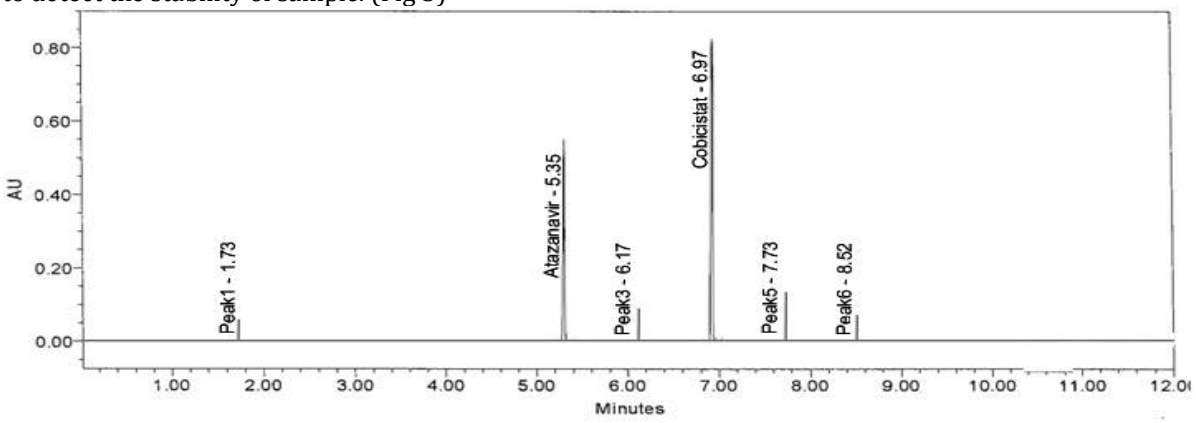


Fig. 5: Chromatogram of acid degradation

Alkali Degradation Studies

To 1ml of stock solution of standard drug and sample Atazanavir and Cobicistat, 1ml of 2N NaOH was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample (Fig 6).

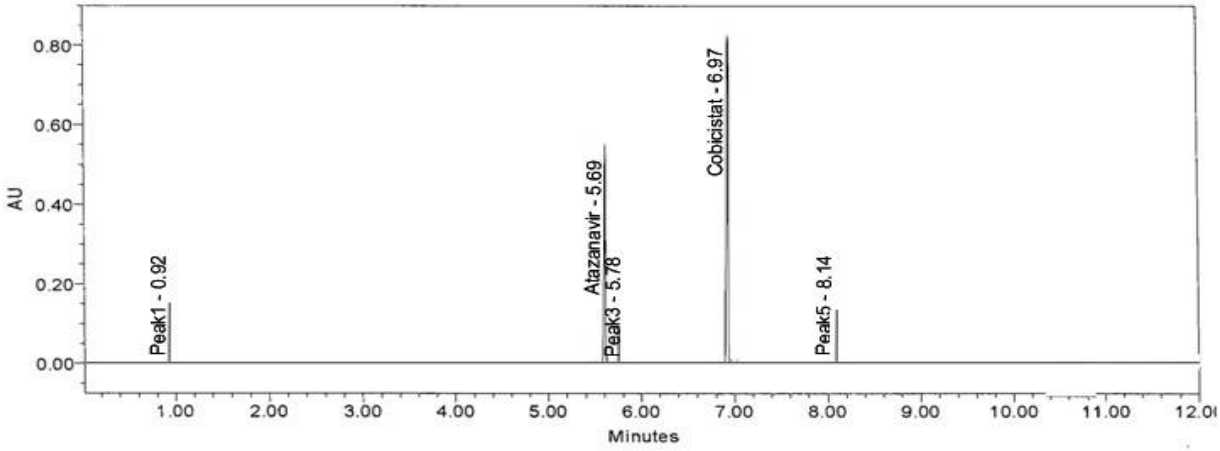


Fig. 6: Chromatogram of Base Degradation

Oxidative Degradation

To 1ml of stock solution of standard drug and sample of Atazanavir and Cobicistat, 1ml of 20% H_2O_2 was added and refluxed for 30min at 60^0c. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample (Fig 7).

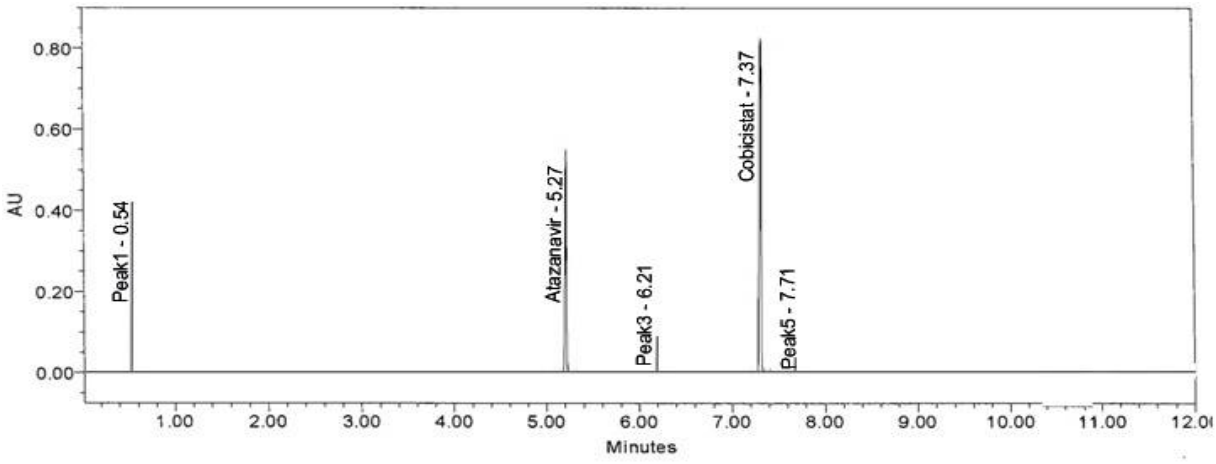


Fig. 7: Chromatogram of Oxidative Degradation

Photo Stability Studies

The photochemical stability of the drug was also studied by exposing the 25 µg/ml solution to UV light by keeping the beaker in UV Chamber for 7days or in 200 Watt hours/m^2 in photo stability chamber. For HPLC study, from the above solution 10µl was injected into the system and the chromatograms were recorded to detect the stability of sample (Fig 8).

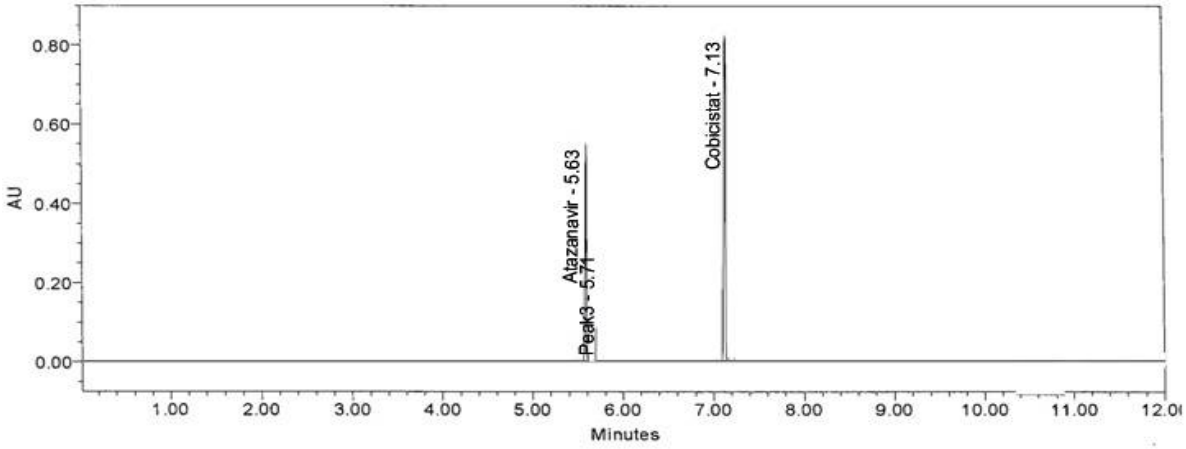


Fig. 8: Chromatogram of UV Degradation

Thermal degradation studies;

The 1ml of stock solution of standard drug and sample of Atazanavir and Cobicistat was exposed to temperature 105°C for 24hrs. For HPLC study, from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Fig 9)

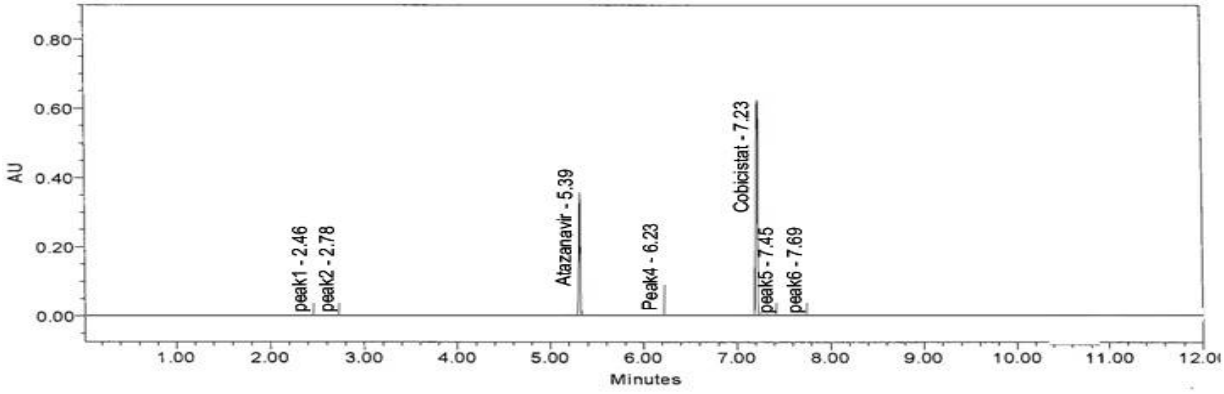


Fig. 9: Chromatogram of Thermal Degradation Study

Table 7: Results of Stress Degradation Studies of Cobicistat

S.No	Stress conditions	Time	% Assay	% Degradation	Purity angle	Purity threshold
1	Acid Degradation	30 min	91.8	8.2	0.14	0.18
2	Base Degradation	30 min	92.4	7.6	0.21	0.24
3	Peroxide Degradation	30 min	88.5	11.5	0.21	0.26
4	UV Degradation	7 days	98.6	1.4	0.20	0.22
5	Thermal Degradation	24hrs	96.3	3.7	0.18	0.21

Table 8: Results of Stress Degradation Studies of Atazanavir

S. No	Stress conditions	Time	% Assay	% Degradation	Purity angle	Purity threshold
1	Acid Degradation	30 min	92.2	7.8	0.15	0.18
2	Base Degradation	30 min	91.6	8.4	0.17	0.23
3	Peroxide Degradation	30 min	90.1	9.9	0.21	0.24
4	UV Degradation	7 days	92.2	7.8	0.15	0.21
5	Thermal degradation	24hrs	95.7	4.3	0.17	0.23

Atazanavir and Cobicistat exhibited significant degradation when exposed to acidic, oxidation, alkaline and UV conditions. Comparatively more degradation was found with base for Cobicistat and with peroxide for Atazanavir. As per ICH guidelines peak purity angle should be less than peak purity threshold. Hence, method of the analysis of Atazanavir and Cobicistat in tablet dosage form showed that the degradation product doesn't interfere with the analytical determination. Hence the proposed analytical method is also useful for the determination of Atazanavir and Cobicistat stability in sample of pharmaceutical dosage form.

CONCLUSION

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Atazanavir & Cobicistat in bulk and tablet dosage form. A simple, precise, accurate, robust & cost-effective method was developed for the routine analysis. The method was successfully validated in terms of linearity, precision, accuracy as per ICH guidelines. The sample preparation was very simple and done with easily available solvent like acetonitrile. The linearity range of the present method is 25-160 µg/ml when compared to other methods with linearity range 45-135 µg/ml and 25- 67.5 µg/ml for Atazanavir and Cobicistat respectively. The LOD and LOQ of the present method was found to be 0.035 µg/ml and 0.11 µg/ml for Atazanavir and 0.025 µg/ml and 0.078 µg/ml for Cobcistat. Previous reported methods have higher LOD and LOQ values when compared to the present method. Hence it is a very sensitive method. The retention time of Atazanavir and Cobicistat was found to be 5.2 mins and 6.9 mins respectively which is almost equal to the other reported methods. Hence it can be concluded that the proposed method is a good approach for obtaining reliable results & is also found to be suitable for the routine analysis and quality control and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination.

REFERENCES

1. Mathias AA, German P, Murray BP, Wei L, Jain A, West S, et al. (2010). "Pharmacokinetics and pharmacodynamics of GS-9350: a novel pharmacokinetic enhancer without anti-HIV activity". Clinical Pharmacology and Therapeutics. 87 (3): 322–9.
2. Lepist EI, Phan TK, Roy A, Tong L, Maclennan K, Murray B, Ray AS (2012). "Cobicistat boosts the intestinal absorption of transport substrates, including HIV protease inhibitors and GS-7340, in vitro". Antimicrobial Agents and Chemotherapy. 56 (10): 5409–13.
3. Sevrioukova IF, Poulos TL (2013). "Dissecting cytochrome P450 3A4-ligand interactions using ritonavir analogues". Biochemistry. 52 (26): 4474–81.
4. Croom KF, Dhillon S, Keam SJ (2009). "Atazanavir: a review of its use in the management of HIV-1 infection". Drugs. 69 (8): 1107–1140.
5. Gammal RS, Court MH, Haidar CE, Iwuchukwu OF, Gaur AH, Alvarellos M, et al. (2016). "Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing". Clinical Pharmacology and Therapeutics. 99 (4): 363–369.
6. Kohl NE, Emini EA, Schleif WA, Davis LJ, Heimbach JC, Dixon RA, et al. (1988). "Active human immunodeficiency virus protease is required for viral infectivity". Proceedings of the National Academy of Sciences of the United States of America. 85 (13): 4686–4690.
7. Bold G, Fässler A, Capraro HG, Cozens R, Klimkait T, Lazdins J, et al. (1998). "New aza-dipeptide analogues as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development". Journal of Medicinal Chemistry. 41 (18): 3387–3401.

8. International Conference on Harmonization, ICH Guidelines, Validation of Analytical Procedures Technical Requirements for Registration of Pharmaceuticals for Human Use: Text and Methodology Q 2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.
9. Kiranmaie GS, Nagaraju P, Mounika V and Priyadarshini GI: (2016). Development and validation of stability indicating RPHPLC method for simultaneous estimation of darunavir and cobicistat in the pharmaceutical dosage form. European Journal of Pharmaceutical and Medical Research ; 3(12): 405-10.
10. 13. Babu R, Sharma V and Singhvi PK: (2014). A new gradient liquid chromatographic method for simultaneous estimation of tenofovir disoproxil fumarate, cobicistat, emtricitabine and elvitegravir in bulk drug and tablet dosage form. Asian Journal of Chemistry; 26(18): 6233-37.
11. Olin JL, Spooner LM and Klibanov OM: (2012). lvitegravir/ Cobicistat/Emtricitabine/Tenofovir disoproxil fumarate single tablet for HIV-1 infection treatment. Annals of Pharmacotherapy; 46(12): 1671-77.
12. Runja C, Kumar PR and Avanapu SR: (2016). A validated stability-indicating RP-HPLC method for the determination of emtricitabine, tenofovir disoproxil fumarate, elvitegravir and cobicistat in the pharmaceutical dosage form. Journal of Chromatographic Science; 54(5): 759-64.
13. Sathish J, Prasad K and Babu KS: (2016). A stability-indicating RP-HPLC method for simultaneous estimation of darunavir and cobicistat in bulk and tablet dosage form. Der Pharmacia Letter; 8(12): 89-96
14. Mallikarjuna N and Sankar DS: (2016). Development and validation of stability-indicating HPLC-DAD method for simultaneous determination of emtricitabine, elvitegravir, cobicistat and tenofovir in their tablet dosage forms. Indian Journal of Pharmaceutical Education and Research; 50(1): 205-11.
15. Nagaraju P, Richards MP, Chandrasekhar KB and Kumar S: (2016). RP-HPLC method development and validation for simultaneous estimation of attazanavir and cobicistat in tablet dosage form. World journal of pharmacy and pharmaceutical Sciences; 5(8): 650-71.
16. Gummaluri R, Parthasarathi N and Madhulika GA: (2016). Simultaneous method for determination of emtricitabine, tenofovir disoproxil fumarate, elvitegravir and cobicistat in tablets by HPLC. Indian Journal of Pharmaceutical Sciences ; 78(4): 532-37.
17. Rao PP, Reddy DM and Ramachandran D: (2014). Stability indicating HPLC method for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage. World Journal of Pharmaceutical Sciences; 2(12): 1822-29.