IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 5 Ver. VI (Sep. – Oct. 2017), PP 61-68
www.iosrjournals.org

Stability Indicating Rp-Hplc Method For The Simultaneous Estimation Of Atazanavir And Cobicistat In Bulk And Tablet Dosage Form

S. Naazneen[1*], A. Sridevi[2]
[1]*Assistant Professor, St Mary's College of Pharmacy, Secunderabad, Telangana, India.*
[2]*Professor, IPT, Sri Padmawathi Mahila VishwaVidyalayam, Tirupathi, Andhra Pradesh, India,*
Email: nazneenshaikphd@gmail.com

Abstract: *The combination of Atazanavir and Cobicistat combined dosage form is used for the treatment of HIV-1 infection. Review of Literature reveals that there is no stability indicating HPLC methods for simultaneous estimation of atazanavir and cobicistat from tablet dosage form. Hence a High Performance Liquid Chromatographic (HPLC) method was developed and validated for the estimation of Atazanavir and Cobicistat simultaneously. Chromatographic separation was optimized by gradient HPLC on a C18 column [Phenomenex, 250 x 4.6 mm, 5µ] utilizing a mobile phase consisting a mixture of 0.01M sodium acetate buffer of pH 4.2 , methanol and acetontrile in the ratio of 25:15:60 v/v at a flow rate of 1ml/min with UV detection at 235nm. The retention time of Atazanavir and Cobicistat was 5.48 min and 7.02 min respectively. Good Linearity obtained over the range of 25µg/ml to 150µg/ml for atazanavir and cobicistat. Correlation coefficient was found to be 0.999&0.998 for atazanavir & cobicistat respectively. The % RSD of precision for atazanavir and cobicistat was found to be 0.10 and 0.08 respectively. The % mean recovery was found to be 99.19-101.68% for atazanavir and 99.03-99.72.% for cobicistat. The validated economical method was applied for forced degradation study of Atazanavir and Cobicistat tablet.*

***Keywords*:** *Atazanavir and Cobicistat, stress study, HPLC method*

Date of Submission: 28-08-2017 Date of acceptance: 02-10-2017

I. Introduction

Atazanavir is chemically known as methyl N-[(1S)-1-{[(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-N'-{[4-(pyridin-2yl)phenyl]methyl}butanehydrazido]-1 phenylbutan-2-yl]carbamoyl}-2,2-dimethyl propyl]carbamate and Cobicistat is chemically known as thiazol-5-ylmethyl N-[1-benzyl-4-[[2-[[(2-isopropylthiazol-4-yl) methyl-methyl-carbamoyl] amino]-4-morpholino-butanoyl] amino]-5-phenyl-pentyl] carbamate. The combination of atazanavir sulphate and cobicistat combined dosage form is used for the treatment of HIV-1 infection [1-2]. After profound search from data and literature available, it was revealed that many analytical methods have been reported for the estimation of atazanavir sulphate and cobicistat individually and with other combinations including high performance liquid chromatography[3-9] , LC-MS[10-12], Ultraviolet Spectrophotometry[13-18], High performance thin layer chromatography (HPTLC)[19-20] methods. One stability indicating RP-UPLC method was reported for the simultaneous determination of Atazanavir and Cobicistat, components were separated by introducing mixture of 0.1% orthophosphoric acid buffer of pH 5.5, methanol and acetonitrile in the ratio 27:18:55 v/v as mobile phase at a flow rate of 0.27 ml/min. The components were detected at a wavelength of 245 nm [21]. Whereas there is no stability indicating HPLC methods were reported for simultaneous estimation of atazanavir and cobicistat. Hence a simple, rapid, sensitive and accurate stability indicating HPLC method was developed for the simultaneous estimation of atazanavir and cobicistat from bulk and pharmaceutical dosage form.

II. Materials And Methods

Chemicals and reagents: HPLC grade methanol, acetonitrile and analytical grade sodium acetate were 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.

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 UV Win software, Sonicator, P^H meter (LAB-INDIA), digital balance (Denver).

Preparation of 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 were prepared by diluting several aliquots of standard solutions of atazanavir and cobicistat.

Preparation of sample solution: Twenty tablets 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. Transfer 1ml of solution into a 10 ml volumetric flask and diluted up to the mark with 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.

III. Results And Discussions

Chromatographic Conditions: Chromatographic Conditions the HPLC system consisted of Shimadzu gradient HPLC (JAPAN) with dual λ Absorbance UV detector. The wavelength of detection as set at 235nm. Separation was carried out in isocratic mode on Phenomenex C18 column (4.6x250mmx5µm) and the retention time of atazanavir and cobicistat was found to be 5.48 min and 7.02 min respectively (Fig1), using mobile phase consisting a mixture of 0.01M sodium acetate buffer of pH 4.2, methanol and acetontrile in the ratio of 25:15:60 v/v at a flow rate of 1ml/min. The mobile phase filtered through nylon milli pore (0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed with Ultra sonicator prior to use. Chromatography was carried out at room temperature 25^{o}C and maintains the column temperature at 32^{o}C.

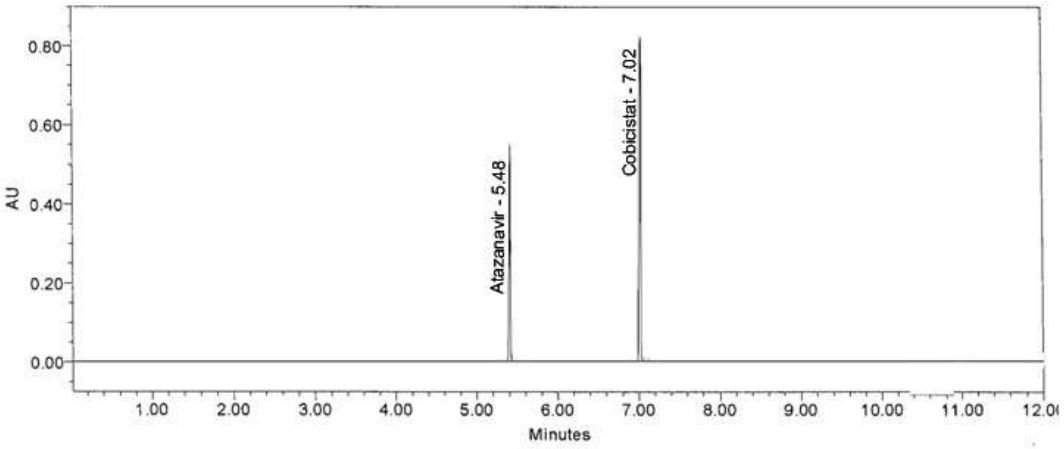

Figure 1: Chromatogram of Atazanavir and Cobicistat

The developed Method was validated for linearity, precision, accuracy, ruggedness and is applied for forced degradation studies as per the ICH guidelines [22-23].

IV. Method Validation

Linearity: Linear concentrations of both drugs were prepared and the best fit line was calculated. Wide range calibration was determined by solutions containing 25µg/ml to 150µg/ml (Table 1) for atazanavir and cobicistat. Correlation coefficient was found to be 0.999&0.998 for atazanavir & cobicistat respectively (Fig 2&3)

Table 1: Linearity Data for Atazanavir & Cobicistat

Atazanavir				
Conc (µg/ml)	**Area 1**	**Area 2**	**Area 3**	**Avg Area**
25	92826	92754.5	92822.5	92801
50	196479	196407.5	196475.5	196454
75	286626	286554.5	286622.5	286601
100	373493	373421.5	373489.5	373468
125	471928	471856.5	471924.5	471903
150	565386	565314.5	565382.5	565361
Intercept	3521	3450	3518	3399.733333
slope	3744	3744	3744	3744
Intercept Standard Deviation				40.15387071

LOD (µg/ml)				0.035392033
LOQ(µg/ml)				0.107248586
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				**66.50563886**
LOD (µg/ml)				**0.025892946**
LOQ(µg/ml)				**0.078463472**

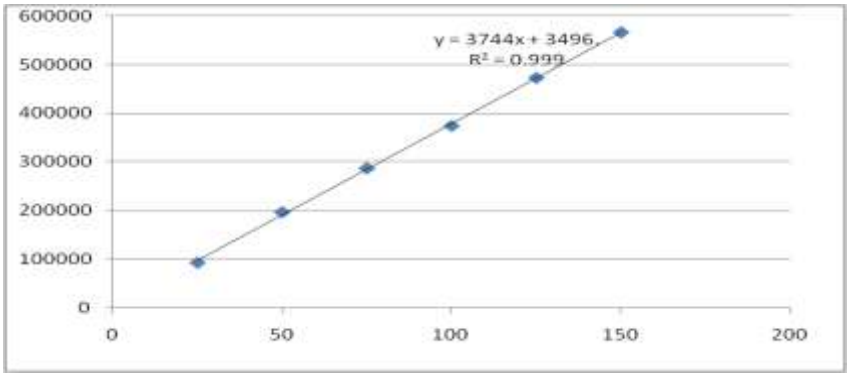

Figure 2: Calibration Curve of Atazanavir

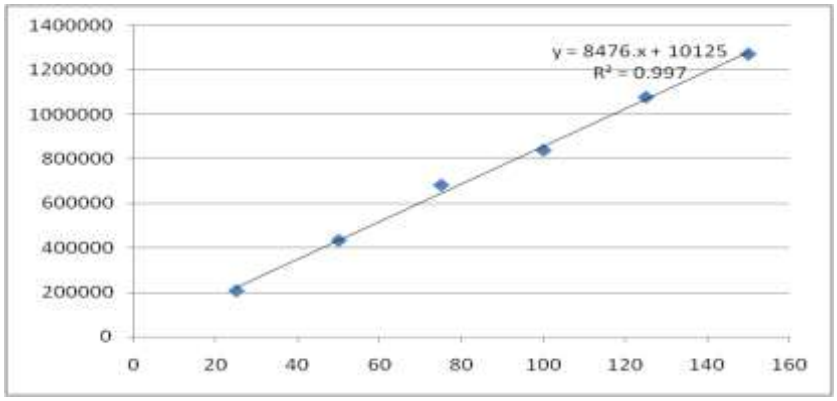

Figure 3: Calibration Curve of Cobicistat

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD is 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) & recording the chromatograms of six test solutions. The % RSD of atazanavir and cobicistat was found to be 0.10 and 0.08 respectively.

Table 2: Method Precision Data for Atazanavir

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 in 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 & 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. 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%) & 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 % CV 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 Stress Degradation Studies:

Stress degradation studies were performed as per the ICH guidelinesQ1A (R2) Stability Testing of New Drug Substances and Products, using the proposed validated analytical method. (Table 6&7)

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 solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. (Fig 4)

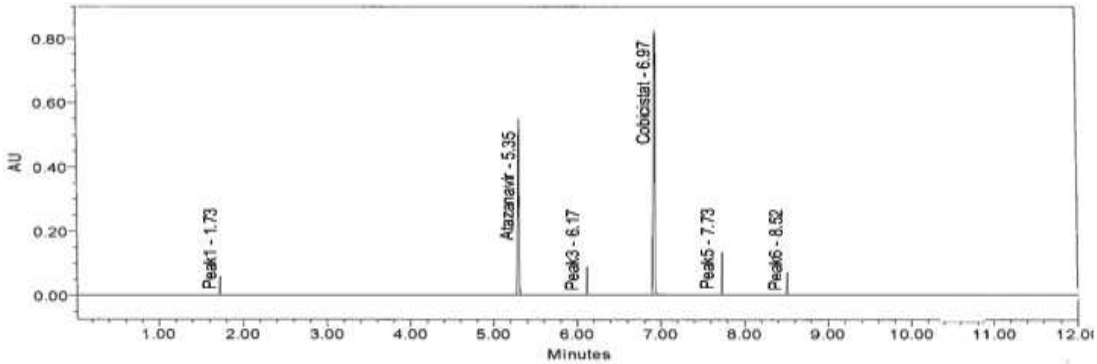

Figure 4: Chromatogram of Acid Degradation

Alkali Degradation Studies: To 1ml of stock solution of 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 5).

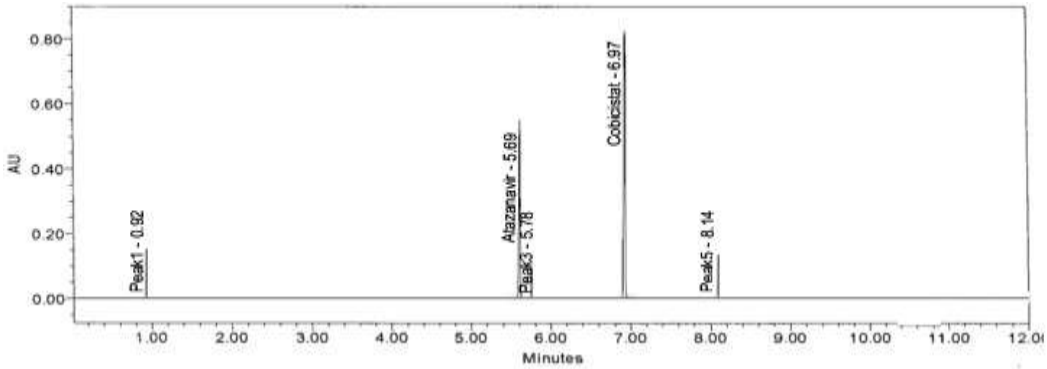

Figure 5: Chromatogram of Base Degradation

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 6).

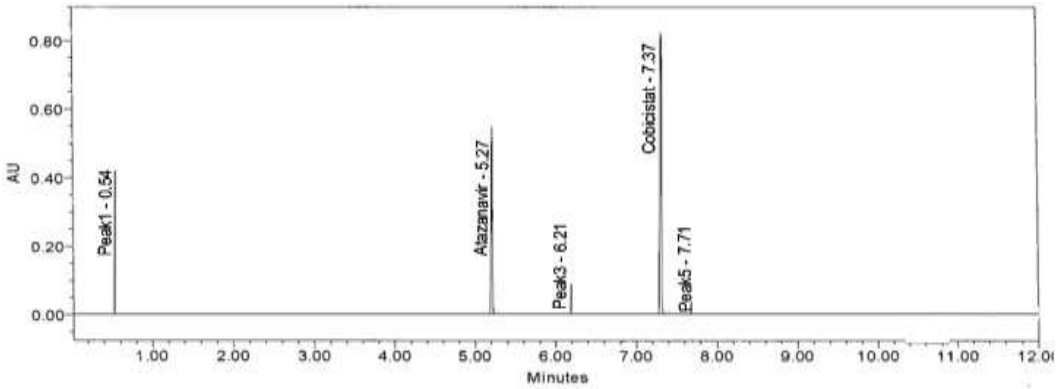

Figure 6: Chromatogram of Oxidative Degradation

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 200 Watt hours/m^2 in photo stability chamber. 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 7).

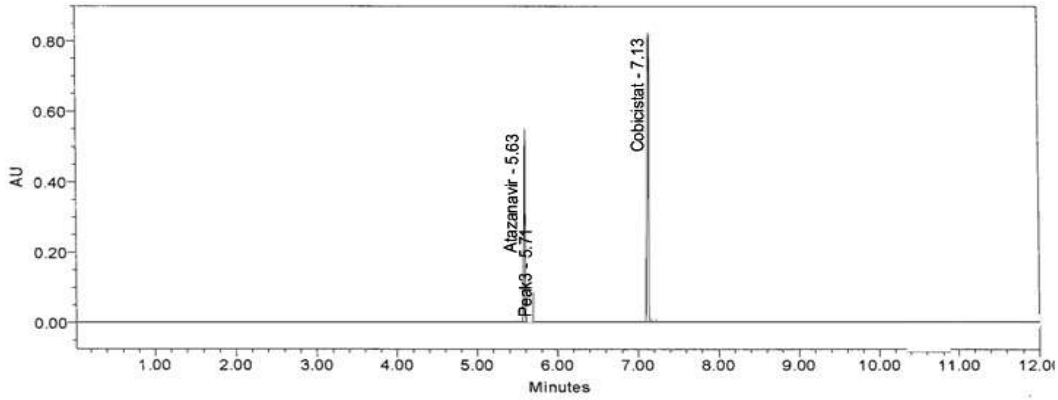

Figure 7: Chromatogram of UV Degradation

Thermal degradation studies:

The 1ml of stock solution of standard drug and sample of atazanavir and cobicistat was exposed to temperature 105^{o}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 8)

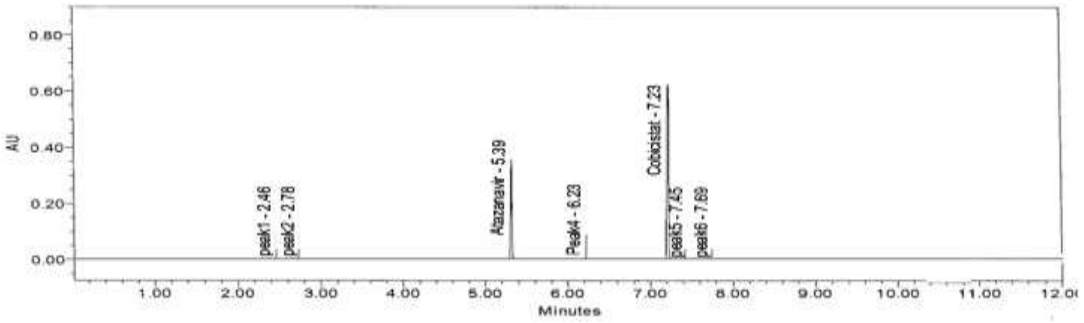

Figure 8: Chromatogram of Thermal Degradation Study

Table 6: Results of Stress Degradation Studies of Cobicistat

Sno	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 7: Results of Stress Degradation Studies of Atazanavir

Sno	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 undergoes significant degradation in acidic, oxidation, alkaline, and UV. 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 shows 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.

V. Conclusion

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.

References

[1] Croom KF, Dhillon S, Keam SJ. Atazanavir-A Review of its Use in the Management of HIV-1 Infection. Drugs.69, 2009, 1107-1140.

[2] Lepist EI, Phan TK, Roy A, Tong L, MacLennan K, Murray B, Ray AS. Cobicistat Boosts the Intestinal Absorption of Transport Substrates, Including HIV Protease Inhibitors and GS-7340, In vitro, Antimicrobial Agents and Chemotherapy. 56, 2012; 5409–5413.

[3] Chitturi SR, Somannavar YS, Peruri BG, Nallapati S, Sharma HK, Budidet SR, Handa VK, Vurimindi HB. Gradient RP-HPLC method for the determination of potential impurities in atazanavir sulfate, J Pharm Biomed Anal. 55, 2011, 31-47.

[4] Dario C, Franco M, Diego R, Norberto P. Determination of Atazanavir in Human Plasma by High-Performance Liquid Chromatography with UV Detection, J. Chromatogr. Sci. 46, 2008, 485-488.

[5] Sathish KK, Sujana K, Prameela RA. New validated RP-HPLC method for the determination of Atazanavir Sulphate in bulk and dosage form, Der. Pharma. Chemica. 4, 2012,1305-1310.

[6] Srinivasu K, Venkateswara Rao J, Appala Raju N, Mukkanti K. A Validated RP-HPLC Method for the Determination of Atazanavir in Pharmaceutical Dosage Form, E-Journal of Chemistry. 8, 2011, 453-456.

[7] Shiny G, Satyavati D. Development and validation of RPHPLC method for the analysis of Cobicistat and related impurities in bulk and pharmaceutical dosage forms, Asian J. Pharm. Ana. 5, 2015, 1-8.

[8] Urooj F, Mamatha T, Rajesh GG. A novel RP-HPLC method development and validation of Cobicistat in bulk drug and tablet dosage form, Der Pharmacia Sinica. 5, 2014, 99-105.

[9] Anusha T, Ashwini G, Annapurna RC, Aravind S, Abbarajuprasana L, Avinash K. Method development and validation for the simultaneous estimation of Atazanavir and Ritonavir in pharmaceutical dosage form by RP-HPLC, Int. J. Pharm. Chem. Bio. Sci. 3, 2012, 44-54

[10] Xu XS, Rose A, Demers R, Eley T, Ryan J, Stouffer B, Cojocaru L, Arnold M. Quantitative determination of free/bound atazanavir via high-throughput equilibrium dialysis and LC-MS/MS, and the application in ex vivo samples, Bioanalysis. 6, 2014, 3169-3182.

[11] Schuster A, Burzawa S, Jemal M, Loizillon E, Couerbe P, Whigan D. Quantitative determination of the HIV protease inhibitor atazanavir (BMS-232632) in human plasma by liquid chromatography-tandem mass spectrometry following automated solid-phase extraction, J Chromatogr B Analyt Technol Biomed Life Sci. 788, 2003, 377-386.

[12] Laxminarayana B, Nageswara RP, Ajitha M, Srinivas LD, Rajnarayana K. Simultaneous Determination of Ritonavir and Atazanavir in Human Plasma by LC-MS/MS and Its Pharmacokinetic Application, Am. J. Pharm Tech Res. 2, 2012, 558-571.

[13] Dey S, Reddy YV, Reddy Thirupathi, Sahoo SK, Murthy P N, Mohapatra S, Patro SS. Method development and validation for the estimation of atazanavir in bulk and pharmaceutical dosage forms and its stress degradation studies using UV-Vis spectrophotometric method, International Journal of Pharma & Bio Sciences. 1, 2010, 1-3.

[14] Nilesh AB, Shailendra PK, Shailesh NS, Saroj VS, Vishnu PC. Spectrophotometric simultaneous determination of Atazanavir and Ritonavir in combined tablet dosage form by ratio derivative and area under curve method, Der. Pharma. Chemica. 4, 2012, 208-213.

[15] Konidala SK, Sujana K. Development and validation of UV spectroscopic method for determination of atazanavir sulphate in bulk and formulation, International Journal of Pharmacy & Pharmaceutical Sciences. 4, 2012, 614-620.

[16] Nanda RK, Kulkarni AA, Yadav PB. Simultaneous spectrophotometric estimation of Atazanavir sulfate and Ritonavir in tablet, Der. Pharma. Chemica. 3, 2011, 84-88.

[17] Bari NAK, Shailendra P, Sharma SN, Shirse SV, Choudhari VP. Spectrophotometric simultaneous determination of atazanavir and ritonavir in combined tablet dosage form by ratio derivative and area under curve method, Der Pharma Chemica. 4, 2012, 208-212.

[18] Khan PS, Reddy PR, Reddy VK. Determination of atazanavir sulfate in pharmaceutical dosage forms by extraction spectrophotometry using acid dyes, Journal of Chemical & Pharmaceutical Research. 5, 2013, 104-110.

[19] Manoj G, Anil B, Bhanubhai S, Ishwarsinh R, Urvish D, Arpit P, Ketan V. Simultaneous Determination of Ritonavir and Atazanavir in combined tablet dosage form by HPTLC, Asian. J. Biomed. Pharm. Sci. 2, 2012, 15-19.

[20] Kuppusamy S, Karunakaran K, Thambichetty A, Manasa D, Bhargavi K, Kagitala V, Kiran S. Development and Validation of HPTLC Method for the Estimation of Atazanavir Sulphate in Bulk Drugs and Combined Dosage Form, International Journal of Pharmaceutical Sciences Review & Research. 25, 2014, 217-222.

[21] Valli Purnima B, Santha Kumari M, Ramu G, Vijaya Bhaskara Reddy T, Srinivas Rao Y, Ramachandran D. Assay and stability studies of cobicistat and atazanavir sulphate in combined dosage form by RP-UPLC method. Der Pharmacia Lettre. 8 (2), 2016, 435-446.

[22] ICH guidelines for the stability of new drug substances and products. Q1A(R2) ICH, Geneva; 2005.1-13.

[23] ICH guidelines for validation of analytical procedures: text and methodology. Q2(R1) ICH, Geneva; 2005, 1-14.

S. Naazneen. " Stability Indicating Rp-Hplc Method For The Simultaneous Estimation Of Atazanavir And Cobicistat In Bulk And Tablet Dosage Form." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 5, 2017, pp. 61–68.