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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DRUGS COBICISTAT AND ATAZANAVIR BY USING RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Atazanavir in Tablet dosage form. Chromatogram was run through ODS (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrie in the ratio of 45:55A was pumped through column at a flow rate of 1ml/min. Temperature was maintained at 30°C. Optimized wavelength for Cobicistat and Atazanavir was 270nm. Retention time of Cobicistat and Atazanavir were found to be 5.023 min and 3.579 min. %RSD of the Cobicistat and Atazanavir were and found to be 1.6 and 0.31 respectively. %Recover was Obtained as 100.3% and 100.21% for Cobicistat and Atazanavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Atazanavir were 0.87 ppm, 1.64 ppm and 1.33ppm, 4.17 ppm respectively. Regression equation of Cobicistat is y = 4985.x + 1820, and of Atazanavir is y = 33657x + 698.2.

Key words: Cobicistat, Atazanavir, RP-HPLC.

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INTRODUCTION

A core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and qualitative evaluation. In practice, quantifying an analyte in a complex sample becomes an exercise in problem resolving. To be efficient and effective, analytical chemist must know the tools that are available to tackle a wide variety of problems (1). Analytical chemistry is divided into two branches qualitative and quantitative. In this qualitative method provides information about the identity of atomic or molecular species or functional groups in the sample. A quantitative method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and bioanalytical samples. In pharma industry, spectrophotometric and chromatographic methods have gained the significance in recent studies. Spectrophotometric methods (2-6) are defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. There electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into

two main types, Qualitative and Quantitative-Qualitative Inorganic Analysis seeks to establish the presence of a inorganic compound in a sample or given element. Quantitative analysis seeks to establish the amount of a compound in a sample or given element. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample. There are various techniques used for analysis of mixture of compounds. Spectroscopy used to measure the interaction of the molecules with electromagnetic radiation. Then chromatography is the collective term for a family of laboratory techniques for the separation of mixtures and comprises passing a mixture of samples dissolved in a "mobile phase" along a stationary phase. The analyte which is separated, to be measured from other molecules in the mixture and allows it is to be isolated. Analytical Chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a compound. Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications in industrial prodcuctions, biomedical engineering, environmental monitoring, forensic sciences and so on. Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the coloured substances by filtration through column, followed by developments with pure solvents. High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. According to IUPAC, chromatography is a physical method of separation in which components will be separated or distributed between stationary and mobile phases. High performance liquid Chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is forced through the column at high speed as a result, the analysis time is reduced by 1-2 orders of the magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possibly increasing the column efficiency substantially. The importance of chromatography is increasing rapidly in pharmaceutical analysis for the exact differentiation, selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and the intermediates. The reasons for the popularity of the method is its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species or thermally fragile ones and its wide spread applicability to substances that are of prime interest to the industry. Sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation. The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high- pressure liquid chromatography, which is frequently called high-performance liquid chromatography. Both terms can be abbreviated as HPLC (7).

Cobicistat, trade name Tybost (formerly GS-9350), is a licensed drug for use in the treatment of infection with human immunodeficiency virus (HIV). Although it does not have any anti- HIV activity, cobicistat acts as a pharmacokinetic enhancer by inhibiting cytochrome P450 3A isoforms (CYP3A) and therefore increases the systemic exposure of coadministered agents that are metabolized by CYP3A enzymes. 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.

Atazanavir (formerly known as BMS-232632) is an antiretroviral drug of the protease inhibitor (PI) class. Like other antiretrovirals, it is used to treat infection of human immunodeficiency virus (HIV). Atazanavir is distinguished from other PIs in that it can be given once-daily (rather than requiring multiple doses per day) and has lesser effects on the patient's lipid profile (the amounts of cholesterol and other fatty substances in the blood). 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.Aim is to develop a new HPLC method for simultaneous estimation of Cobicistat and Atazanavir To develop a validated method according to ICH guidelines. To apply a validated technique for the estimation of Cobicistat and Atazanavir in pharmaceutical formulation.

MATERIALS AND METHODS (8-12)

Buffer: (0.1%OPA)

1ML of Ortho phosphoric acid solution in a 1000ml of Volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Standard Preparation

Accurately Weighed and transferred working Standards of 15 mg of Cobicistat and 30 mg of Atazanavir into a 10ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solution was taken into a 10ml volumetric flask and made up to 10ml.

Sample Preparation

1tablet was weighed, powdered and then the weight was transferred into a 100mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Cobicistat and Atazanavir are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 37.5ppm, 75ppm, 112.5ppm, 150ppm, 187.5ppm, 225ppm of Cobicistat and 75ppm, 150ppm, 225ppm 300ppm, 375ppm, 450ppm of Atazanavir. Accuracy preparations from the formulation solution take 0.5ml ,1ml , 1.5ml, was transferred to 10 ml volumetric flask and make up the volume to get 50% 100% and 150% solution concentrations.

RESULTS AND DISCUSSION

Systemsuitability- All the system suitability parameters are within range and satisfactory as per ICH guidelines (Table-1).

Table-1 System suitability studies of Cobicistat and Atazanavir method

Property	Cobicistat	Atazanavir
Retention time (t_R)	5.023± 0.3 min	3.579±0.3min
Theoretical plates (N)	8825 ± 163.48	11105± 163.48
Tailing factor (T)	1.6 ± 0.117	1.0± 0.117

Linearity- Six Linear concentrations of Cobicistat(37.5-225ppm) and Atazanavir (75ppm to 450ppm) are prepared and Injected. Regression equation of the the Cobicistat and Atazanavir are found to be, y = 4984.x + 1780, and y = 8340.x + 983.3 and the regression co-efficient was 0.999 (Table-2 and fig-1-6).

Table-2 Calibration data of Cobicistat and Atazanavir method.

S.no	Concentration Cobicistat (µg/ml)	Response	Concentration Atazanavir (µg/ml)	Response
1	0	0	0	0
2	37.5	191562	6.25	225114
3	75	390278	12.5	414984
4	112.5	546372	18.75	622694
5	150	738859	25	837022

6	187.5	946286	31.25	1049477
7	225	1125673	37.5	1273064

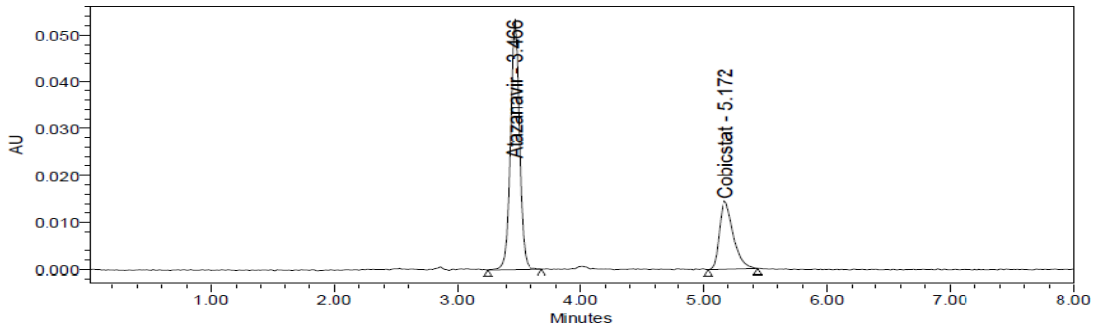

Fig-1 Linearity 25% Chromatogram of Cobicistat and Atazanavir method

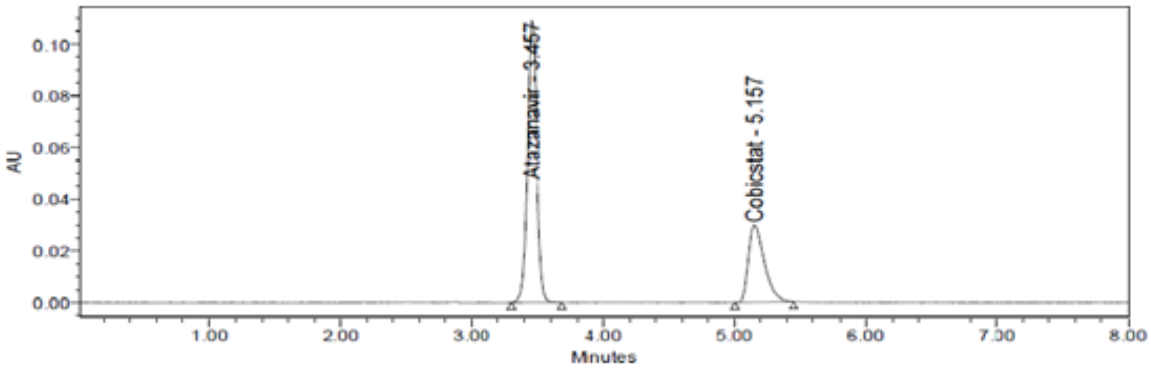

Fig-2 Linearity 50% Chromatogram of Cobicistat and Atazanavir method

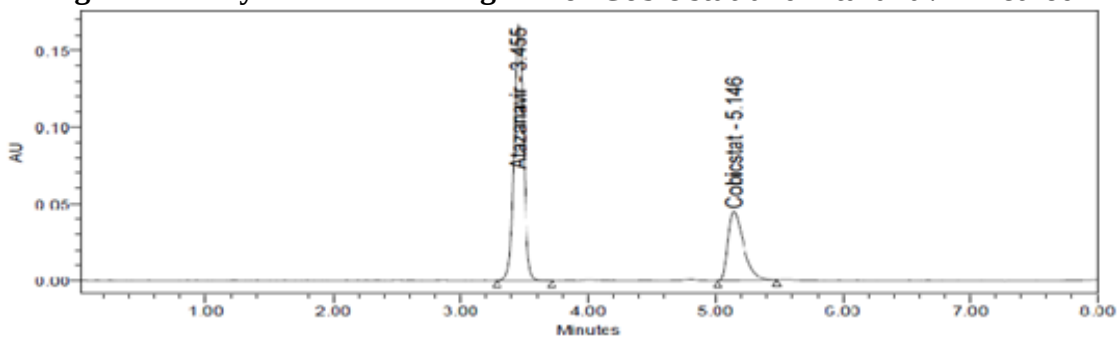

Fig-3 Linearity 75% Chromatogram of Cobicistat and Atazanavir method

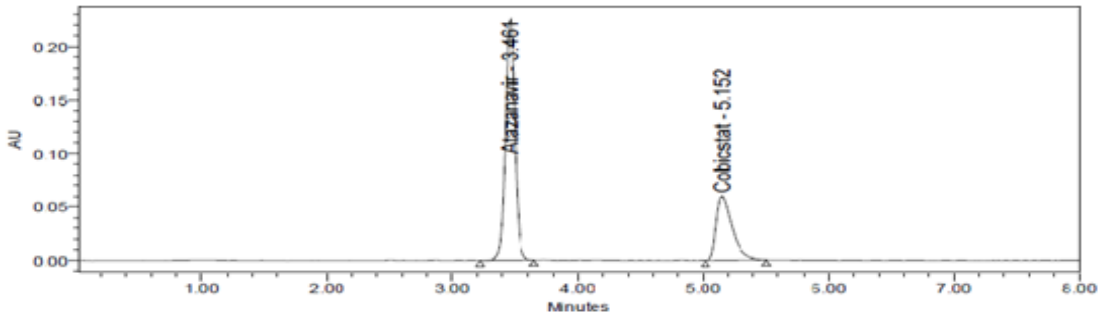

Fig-4 Linearity 100% Chromatogram of Cobicistat and Atazanavir method

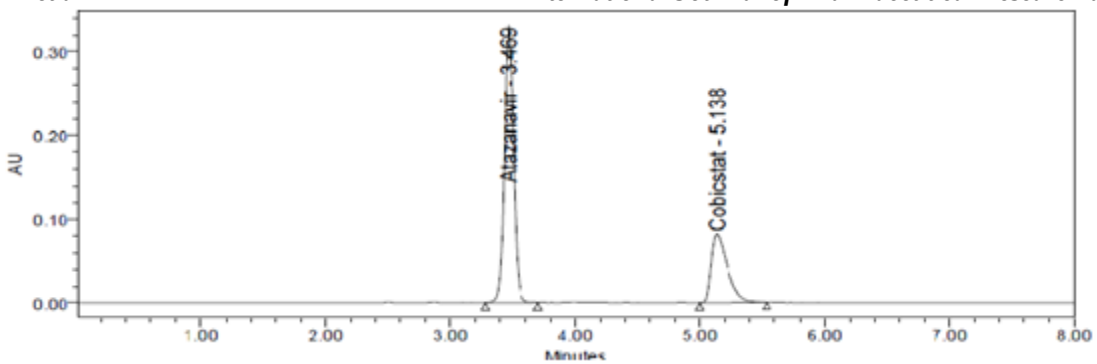

Fig-5 Linearity 125% Chromatogram of Cobicistat and Atazanavir method

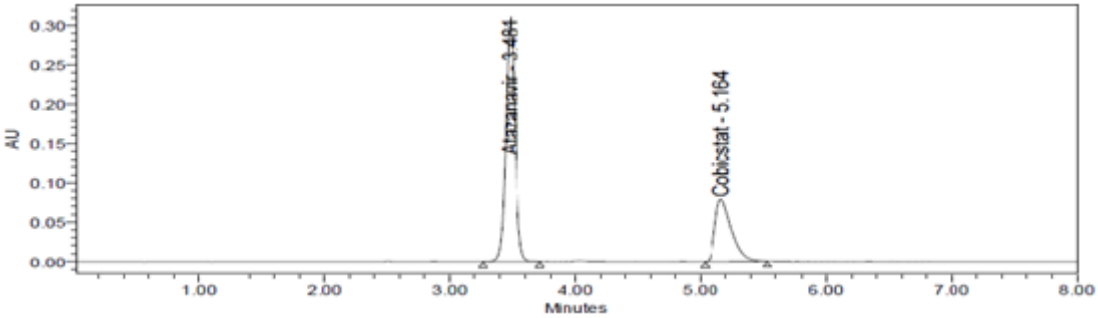

Fig-6 Linearity 150% Chromatogram of Cobicistat and Atazanavir method

Intraday precision (Repeatability)- Intraday Precision was performed and % RSD for Cobicistat and Atazanavir were found to be 1.6 % and 0.31% respectively.

Inter day precision- Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Cobicistat and Atazanavir were 1.3% and 0.3%.

Accuracy- Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table-3.

Table-3 Accuracy results of Cobicistat and Atazanavir

Sample	Amount added (µg/ml)	Recovery (%)	% RSD
Cobicistat	75	101.52	1.43
	150	99.21	0.83
	225	100.64	0.61
Atazanavir	150	99.32	0.98
	300	101.65	1.17
	450	100.09	0.56

LOD- Limit of detection was calculated by inteCobicistat and Atazanavirpt method and LOD for Cobicistat was found to be 0.87 and Atazanavir was 1.64 respectively.

LOQ- Limit of Quantification was calculated by inteCobicistat and Atazanavirpt method and LOQ for Cobicistat and Atazanavir wre found to be 1.33 and 4.17 respectively.

Assay- Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.3 % and 100.21 % for Cobicistat and Atazanavir respectively.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Cobicistat and Atazanavir in Tablet dosage form. Retention time of Cobicistat and Atazanavir were found to be 5.023 min and 3.579 min. %RSD of the Cobicistat and Atazanavir were and found to be 1.6 and 0.31 respectively. %Recover was Obtained as 100.3% and 100.21% for Cobicistat and Atazanavir. LOD, LOQ values were obtained from regression equations of Cobicistat and Atazanavir were 0.87 ppm, 1.64 ppm and 1.33ppm, 4.17 ppm respectively. Regression equation of Cobicistat is y = 4985.x + 1820, and of Atazanavir is y = 33657x + 698.2. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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