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SIMULTANIOUS ESTIMATION OF COBICISTAT AND ATAZANAVIR SULPHATE IN BULK AND COMBINED DOSAGE FORM BY UV-SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple and sensitive ultra-violet spectrophotometric method was developed and validated for the determination of cobicistat (COBI) and atazanavir sulphate (ATV) simultaneously in bulk and pharmaceutical formulations. The developed method was applied to determine the stability of the drugs in the drug sample under different degradation conditions. Wavelength of maximum absorbance of COBI and ATV were found to be 239.3 and 292.5 nm respectively. The system precision and method precision expressed as % RSD of six replicate measurements were found to be 0.3471 & 0.4612 and 0.8695 & 0.8784 for COBI and ATV respectively. The mean percent of recovery for COBI and ATV were found to be in between 100.84 and 100.79. Limit of detection and limit of quantitation were found to be 0.795 & 2.651 and 1.7525 & 5.750 for COBI and ATV respectively. Absorbance was linearly proportional to concentration of COBI and ATV in the range of 7.5-37.5 and 15-75 µg/ml. The assay of the tablet dosage form Evotaz was determined and found to be 99.61 and 100.46% for COBI and ATV respectively. The developed method was adopted for the study of satiability of the drugs under different degradation conditions and percent of degradation was found to be in between 12.74-5.38 and 15.94-6.94 for COBI and ATV respectively. Therefore, the developed method was found to be simple, rapid and applied for the quality control, therefore the proposed method is

recommended for the analysis of COBI and ATV in pure and tablet dosage forms in any quality control laboratories.

KEYWORDS: Cobicistat, Atazanavir sulphate, Stability, Assay, Evotaz.

INTRODUCTION

Profile of the selected drugs

Cobicistat, a licensed drug used for the treatment of infection with the human immunodeficiency virus (HIV) 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 molecular formula and molecular weight are $C_{40}H_{53}N_7O_5S_2$ and 776.023 g/mol respectively. It is a white to pale yellow solid with a solubility of 0.1 mg/mL in water at 20°C. The molecular structure of the cobicistat was represented in Fig.-1.

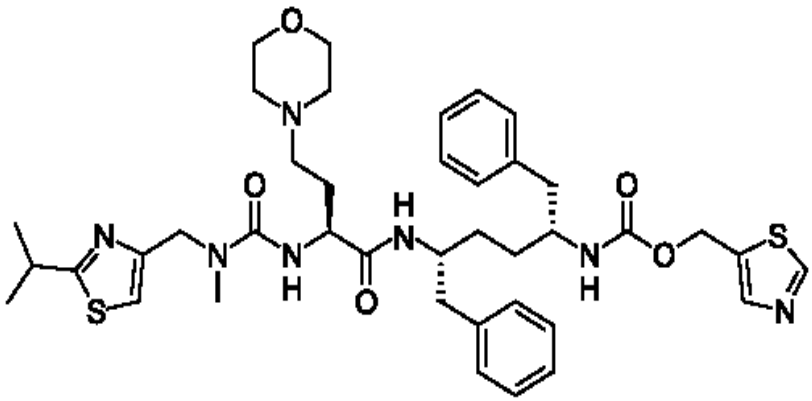

Fig.- 1: Molecular structure of Cobicistat (COBI)

Atazanavir, an antiretroviral drug of the protease inhibitor (PI) class which is used to treat infection of human immunodeficiency virus[1,2] is chemically known as methyl *N*-[(1*S*)-1-{[(2*S*,3*S*)-3-hydroxy-4-[(2*S*)-2-[(methoxycarbonyl) amino]-3,3-dimethyl-*N'*-{[4-(pyridineyl) phenyl] methyl} butanehydrazido] -1-phenylbutan-2-yl] carbamoyl} -2,2 dimethylpropyl] carbamate. The molecular formula and molecular weight of atazanavir sulphate are $C_{38}H_{52}N_6O_7$ and 802.9416 g/mol respectively. Atazanavir sulfate is a white to pale yellow crystalline powder with a solubility of 4 to 5 mg/ml free base equivalents in water at 24°C. The molecular structure of the atazanavir sulphate was represented in Fig.-2. A fixed-dose combination of cobicistat and protease inhibitor atazanavir is marketed as Evotaz, by Bristol-

Myers Squibb and was approved by the FDA in January 2015. Evotaz, a fixed-dose combination tablet for oral administration containing 300 mg atazanavir as atazanavir sulfate and 150 mg cobicistat is a once-daily fixed-dose combination of a protease inhibitor and a pharmacokinetic enhancer for the treatment of HIV-1 infection. Inactive ingredients present in Evotaz are microcrystalline cellulose, croscarmellose sodium, sodium starch glycollate, crospovidone, stearic acid, magnesium stearate, hydroxypropylcellulose and silicon dioxide in tablet core and hypromellose, titanium dioxide, purified talc, glycerol triacetate and iron oxide red in film coating.

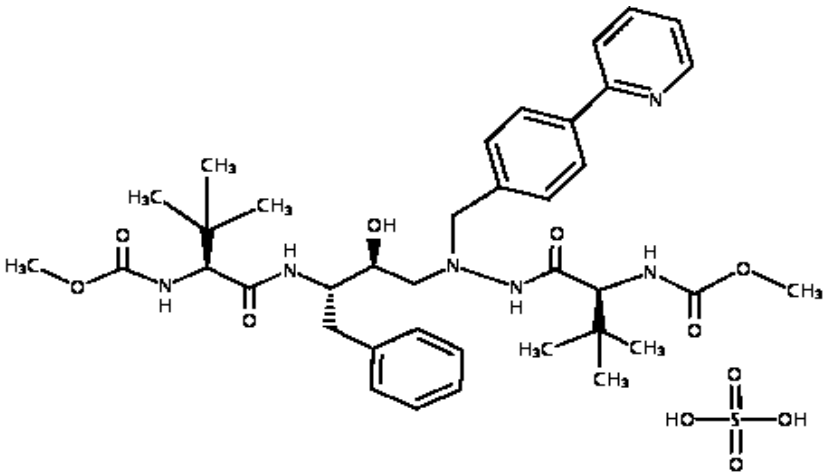

Fig.- 2: Molecular structure of Atazanavir sulphate (ATV)

Literature review

Literature survey reveals a few UV spectrophotometric methods[3-6] and RP-HPLC methods[7-16] for the determination of atazanavir sulphate alone and simultaneously with other retroviral drugs in formulations and biological fluids. In addition, one HPTLC[17] and two LC/MS/MS methods[18-19] were also reported. RP-HPLC methods[20-21] for the analysis of Cobicistat and related impurities in bulk and pharmaceutical dosage forms, Stability indicating HPLC method[22] for simultaneous estimation of emtricitabine, tenofovir disoproxyl fumarate, cobicistat and elvitegravir in pharmaceutical dosage form, A new gradient liquid chromatographic method[23] for simultaneous estimation of Tenofovir, Disoproxil fumarate, Cobicistat, Emtricitabine and Elvitegravin in bulk drug and tablet dosage form. UV-visible spectrophotometric method[3] and derivative spectrophotometric method[4] were intended to determine only atazanavir in bulk and pharmaceutical formulations, where as methods[5,6] were aimed at to determine atazanavir sulphate in combination with ritonavir simultaneously. So, there is no single UV spectrophotometric method for the simultaneous determination of

atazanavir in combination with cobicistat, hence the author attempted to develop stability indicating UV spectroscopic method and succeeded.

Objective of the investigation

The aim of the present investigation is to determine potency and to test the stability of drug sample under different degradation conditions. The main objectives of the present work are, to develop UV spectrophotometric method by choosing different solvents, validate the developed method as per the ICH guidelines to determine precision, accuracy, sensitivity, linearity, robustness and ruggedness of the developed method and finally to determine the assay of cobicistat and atazanavir sulphate simultaneously in pharmaceutical formulations. The developed method was also extended to study the stability of cobicistat and atazanavir sulphate under different degradation conditions such as acid, base, peroxide, thermal and photo light and to find out the percent of degradation of the drug moiety present in the sample.

Spectrophotometry

Spectrophotometry mainly concerned with the following regions of the electromagnetic spectrum: ultraviolet 185 to 400nm; visible 400 to 760 nm and infrared 0.76 to 15µm. When light falls upon a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium and the rest is transmitted. Two separate laws governing absorption are usually known as Lambert's law and Beer's law, in combined form they are known as Beer-Lambert law. On combining Lambert's law and Beer's law, we get $I_t=I_0 10^{-acl}$ or $A=\log_{10}(I_0/ I_t)=acl$. If c is expressed in mol/L and l in cm then 'a' is given the symbol € and is called the molar absorption coefficient or molar extinction coefficient. Finally the relationship between absorbance, transmittance and molar extinction coefficient is given by A=€cl=log (1/T) =-log (T). When the value of € is known, the concentration of an unknown solution can be calculated from the formula $c_{unknown}=A/€l$.

Molecules absorb radiation from visible or ultraviolet regions of the electromagnetic spectrum and the absorption of a given substance is greatly affected if it contains a chromophore, a functional group such as double or triple bonds, nitro and nitroso groups, the azo group, the carbonyl and thiocarbonyl groups. If the chromophore is conjugated with another group of the same kind or different kind, then the absorption is enhanced and new absorption bands appear at a higher wavelength. The absorption of a given molecule may also

be enhanced by the presence of groups such as –OH, -NH_2, -CH_3 and –NO_2 are called auxochromes.

Multi component analysis

Since absorbances are additive, it is possible to determine the concentration of two absorbing constituents X and Y by spectrophotometry if provided there is no chemical reaction between the two solutes. The complexity of the situation depends upon the absorption spectra of X and Y.

No overlap of spectra

The spectra do not overlap, or at least it is possible to find a suitable wavelength (λ_1) where X absorbs and Y does not and a similar wavelength (λ_2) for measuring Y. The constituents X and Y are simply measured at wavelength (λ_1) and (λ_2).

One-way Overlap spectra

In this case, Y does not interfere with the measurement of X at λ_1, but X does absorb appreciably along with Y at λ_2. In this case the concentration of X is determined directly from the absorbance of the solution at λ_1. Then the absorbance contributed at λ_2 by this concentration of X is calculated from the previously known molar absorptivity of X at λ_2. This contribution is subtracted from the measured absorbance of the solution at λ_2, yielding the absorbance due to Y, whose concentration is then calculated in the usual manner.

Two-way Overlap spectra

When no wavelength can be found where either X or Y absorbs exclusively, it is necessary to solve simultaneous equations in two unknowns. Since the total absorbance is the sum of the contributions of the individual absorbing constituents of the solution, then by measuring the absorbance of the mixture at λ_1 and λ_2, the concentrations of the two components can be calculated by solving these simultaneous equations. $A_1 = \epsilon_{x1} C_x + \epsilon_{y1} C_y$ and $A_2 = \epsilon_{x2} C_x + \epsilon_{y2} C_y$. The solution of these simultaneous equations gives $C_x = (\epsilon_{y2}A_1 - \epsilon_{y1}A_2) / (\epsilon_{x1} \epsilon_{y2} - \epsilon_{y1} \epsilon_{x2})$ and $C_y = (\epsilon_{x1}A_2 - \epsilon_{x2}A_1) / (\epsilon_{x1} \epsilon_{y2} - \epsilon_{y1} \epsilon_{x2})$. The values of ϵ_x and ϵ_y can be deduced from the measurements of pure solutions of X and Y respectively. Where A_1= Measured absorbance at λ_1, A_2= Measured absorbance at λ_2, ϵ_{x1}= Molar absorptivity of X at λ_1, ϵ_{x2}= Molar absorptivity of X at λ_2, ϵ_{y1}= Molar absorptivity of Y at λ_1, ϵ_{y2}= Molar absorptivity of Y at λ_2, C_x= Concentration of X and C_y= Concentration of Y

EXPERIMENTAL

Standards and reagents

Cobicistat (potency of 99.8) and atazanavir sulphate (potency 99.8) drug products were obtained as a gift samples from Mylan Laboratories and Hetero Drugs Ltd. Hyderabad, Telangana state India respectively. The Evotaz tablets (150 mg of cobicistat and 300mg atazanavir sulphate, Bristol-Myers Squibb Company) were procured from the local pharmacy. Analytical grade reagents such as methanol, hydrochloric acid, sodium hydroxide, hydrogen peroxide and water were procured from Merck India.

Preparation of solutions

Preparation of individual stock solutions

Exactly 15 mg of cobicistat and 30 mg of atazanavir sulphate working standards were accurately weighed, transferred into two separate 100 ml volumetric flasks, 70 ml of methanol was added to each flask and sonicated for five minutes to dissolve completely and filtered through 4.5 µ filter under vacuum filtration and then made up to the mark with water**.** The concentration of COBI and ATV were found to be 150 and 300 µg/ml respectively.

Preparation of standard mixture solution

Exactly 15 mg of cobicistat and 30 mg of atazanavir sulphate working standards were accurately weighed, transferred into a 100 ml volumetric flasks, 70 ml of methanol was added and sonicated for five minutes to dissolve completely and filtered through 4.5 µ filter under vacuum filtration and then made up to the mark with water**.** The concentration of COBI and ATV were found to be 150 and 300 µg/ml respectively.

Preparation of sample stock solution

Average weight of ten tablets was determined, made them into a fine powder and an amount of the powder equivalent to (150 mg of Cobicistat and 300 mg Atazanavir sulphate) the average weight of ten tablets was accurately weighed and transferred into a 100 ml clean dry volumetric flask, dissolved in 70 ml of methanol and sonicated for five minutes and filtered through 4.5 µ filter under vacuum filtration and made up to the mark with water. The concentration of COBI and ATV were found to be 150 and 300 µg/ml respectively.

Instrumentation

Elico-SL159 model, 2nm high resolution, double beam, 1cm length quartz coated optics and wavelength range190-1100nm is used for all the spectral measurements. Schematic diagram of double beam UV-visibale spectrophotometer was shown in Fig.-3.

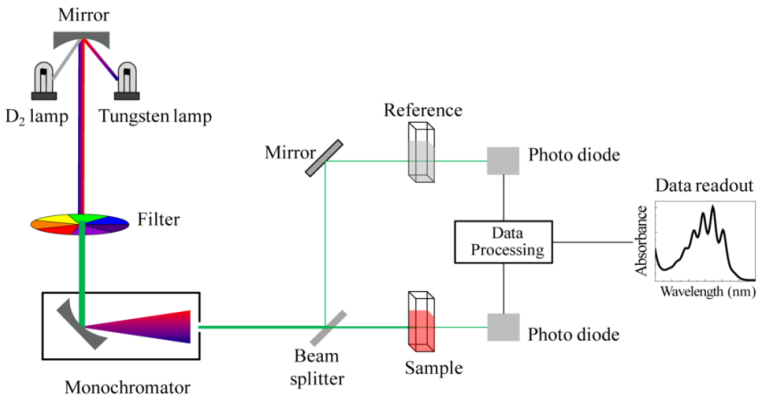

Fig.-3: Schematic diagram of a double beam UV-Visible spectrophotometer

Method development

Choice of solvent

Solvent used for the spectrophotometric determination of a substance must be a good and pure solvent which should not interact with the solute and must not show significant absorption at the wavelength employed in the determination. For the majority of the analysis of organic compounds and drugs it is necessary to use polar solvents such as water, methanol, ethanol etc. Cut-off wavelengths of some commonly used solvents are 190 nm for water, 207 nm for ethanol and 210 nm for methanol.

Determination of absorption spectra

The development of UV-spectrophotometric method was based on selection of wavelength of maximum absorbance of the selected drugs in different solvents. Since the selected drugs were completely soluble in water, methanol, diethyl ether and ethanol, wavelength of maximum absorbance and stability of the absorbance was determined in these solvents and found that methanol was a good choice for the method development. Exactly 2 ml of the standard stock solutions was introduced into two separate 10 ml volumetric flasks and diluted up to the mark with water and final individual concentrations of COBI and ATV were found to be 30 and 60 µg/ml respectively. Reagent blank is also prepared in similar manner without COBI/ATV. Into two cuvettes, reagent blank and standard solutions COBI/ATV was taken,

absorbance of the solutions were determined as a function of wavelength against reagent blank. The absorption spectra of COBI and ATV were represented in Fig.-4 and Fig.-5 respectively. The wavelength of maximum absorbance of COBI and ATV were found to be 239.3 and 292.5 nm respectively. The over line absorption spectra were shown in Fig.-6.

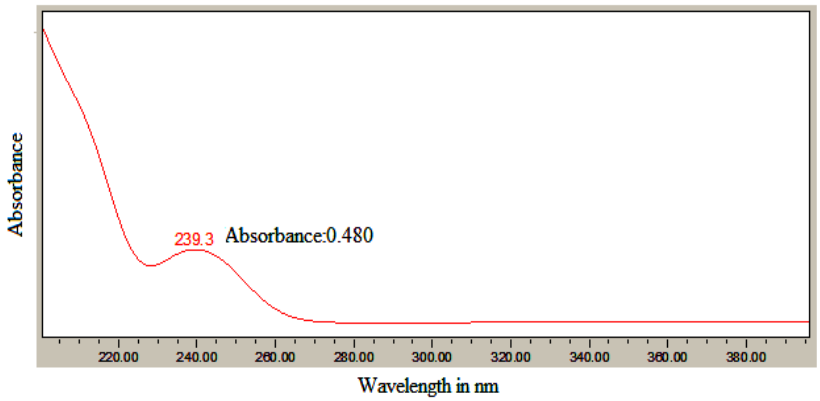

Fig.-4: Absorption spectrum of cobicistat (30µg/ml) in methanol solvent

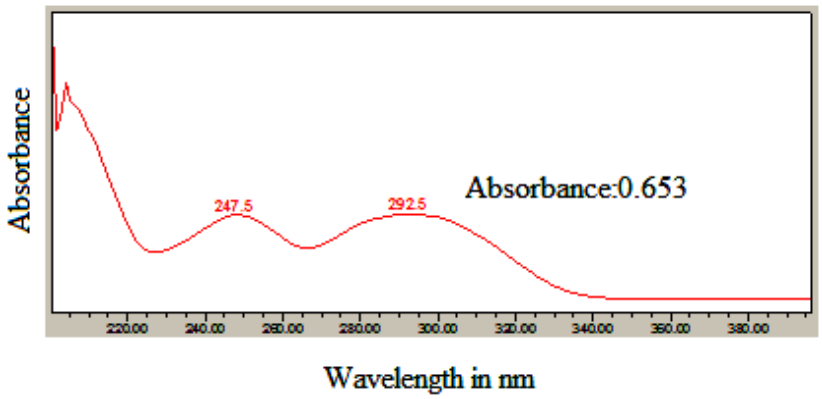

Fig.-5: Absorption spectrum of atazanavir sulphate (60µg/ml) in methanol solvent

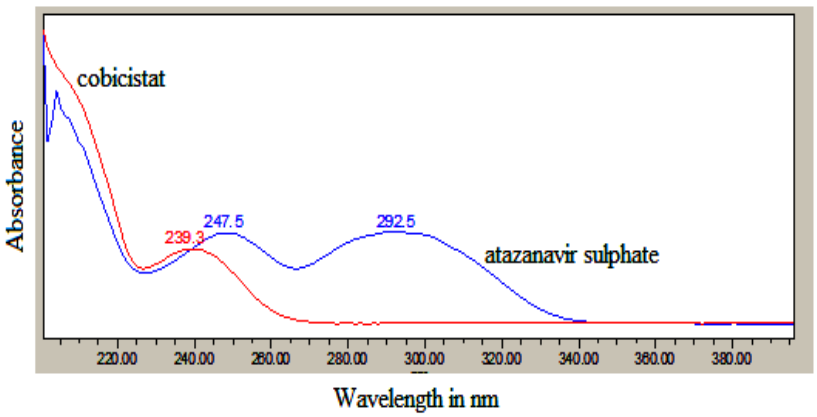

Fig.-6: Over line absorption spectrum of cobicistat and atazanavir sulphate in methanol solvent

From the observation of the over line absorption spectra of COBI and ATV it was found that, COBI does not interfere with the measurement of ATV at λ_1=292.5 nm, but ATV does absorb appreciably along with COBI at $\lambda_{2=}$239.3 nm. In this case the concentration of ATV is determined directly from the absorbance of the solution at 292.5 nm. The molar extinction coefficient of ATV at λ_1=292.5 nm and $\lambda_{2=}$239.3 nm is determined and found to be $1.689x10^4$ and $6.169x10^3$ respectively. The molar extinction coefficient of COBI at $\lambda_{2=}$239.3 nm is determined and found to be $1.25x10^4$. Then the absorbance contributed at 239.3 nm by this concentration of ATV is calculated from the previously known molar absorptivity of ATV at 239.3 nm i.e. $6.169x10^3$. This contribution is subtracted from the measured absorbance of the solution at 239.3, yielding the absorbance due to COBI, whose concentration is then calculated in the usual manner.

METHOD VALIDATION

Validation of an analytical procedure is demonstration of new analytical method whether it is suitable for its intended purpose or not. Assay procedures are intended to determine the content of analyte present in a given sample i.e. quantitative measurement of the active pharmaceutical ingredients in the sample.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Statistically precision was expressed as %RSD and it can be determined from mean and standard deviation of replicate measurements.

To determine repeatability, precisely 2 ml of standard mixture solution was introduced into a 10 ml volumetric flasks and diluted up to the mark with water and final individual concentrations of COBI and ATV were found to be 30 and 60 µg/ml respectively. Reagent blank is also prepared in similar manner without COBI/ATV. Absorbance of the solution against reagent blank was determined six times at 239.3 and 292.5 nm over a short interval of time. In the determination of method precision, working standard mixture solution of COBI and ATV were prepared six times as described in experimental section; absorbance of these solutions were measured against reagent blank at 239.3 and 292.5 nm. Mean, standard deviation and then % RSD were determined and % RSD values were found to be less than

2.0%. The system precision and method precision were presented in Table-1 and Table-2 respectively.

Intermediate precision is the precision determined within same laboratories but different days, different analysts, different equipment, etc. To evaluate the intermediate precision (ruggedness) of the method, absorption measurements were carried out for standard solution in similar manner. In the present investigation, the author determined intra-day and inter-day precision. Working standard solution of concentration of COBI and ATV 30 and 60 µg/ml was prepared, absorption measurements against reagent blank at 239.3 and 292.5 nm were carried out in two different days, mean, standard deviation and then % RSD were determined.

Accuracy

The accuracy of an analytical procedure may be defined as the concordance between found value and the true or most probable value. For analytical methods, there are two possible ways of determining the accuracy, absolute method and comparative method. In the analysis of pharmaceutical formulations (or solid laboratory prepared samples of desired composition), the content of the constituent sought (expressed as percent recovery) has been determined by two or more (proposed and official or reference) supposedly "accurate" methods of essentially different character can usually be accepted as indicating the absence of an appreciable determinate error. Accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations / three replicates each of the total analytical procedure). Accuracy should be reported as percent recovery of known added amount of analyte into the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

Preparation of 50% / 100% and 150% sample solution

Exactly 7.5 / 15 / 22.5 mg of cobicistat and 15.0 / 30.0 / 45.0 mg of atazanavir working standard was accurately weighed and transferred into three 100 ml volumetric flasks containing an amount of sample, about 70 ml of diluents was added to each flask and sonicated to dissolve the components completely for five minutes and made volume up to the mark with the same solvent. Then precisely 2.0 ml of above solution was introduced into a 10 ml volumetric flask and diluted up to the mark with water and final individual concentrations of standard COBI and ATV were found to be 15 / 30 / 45 and 30 / 60 / 90 µg/ml respectively. Reagent blank is also prepared in similar manner without COBI/ATV.

Absorbance of the solution against reagent blank was determined triplicate at 239.3 and 292.5 nm. The results of recovery were presented in Table-3 and Table-4.

Linearity studies

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. To determine linearity of the developed method, stock solution of cobicistat and atazanavir was prepared as explained in experimental section. The stock solution was used for further dilutions in the study of linearity. Into a series of six 10 ml volumetric flasks, different aliquots of stock solution 0.5 to 3.0 ml were accurately transferred, diluted up to the mark. Absorbance of these solutions was measured against reagent blank in duplicate at 239.3 and 292.5 nm. Since the absorption spectrum of COBI does not interfere with the measurement of ATV at λ_1=292.5 nm, the mean absorbance values at 292.5nm were plotted against the concentration of ATV. But ATV does absorb appreciably along with COBI at λ_2=239.3 nm, the absorbance contributed at 239.3 nm by this concentration of ATV is calculated from the previously known molar absorptivity of ATV at 239.3 nm and this contribution is subtracted from the measured absorbance of the solution at 239.3, yielding the absorbance due to COBI. Then linearity plot (Fig.-7 and Fig.-8) was drawn by taking mean peak area against concentration of COBI. Linearity, slope, intercept and correlation coefficient were determined in each case and the values were reported in Table-5.

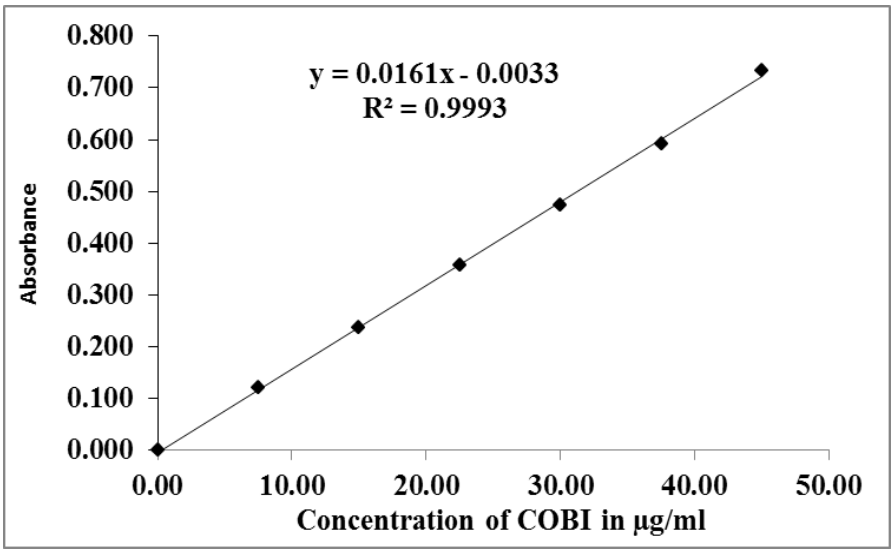

Fig.7-: Linearity plot between peak area and concentration of COBI

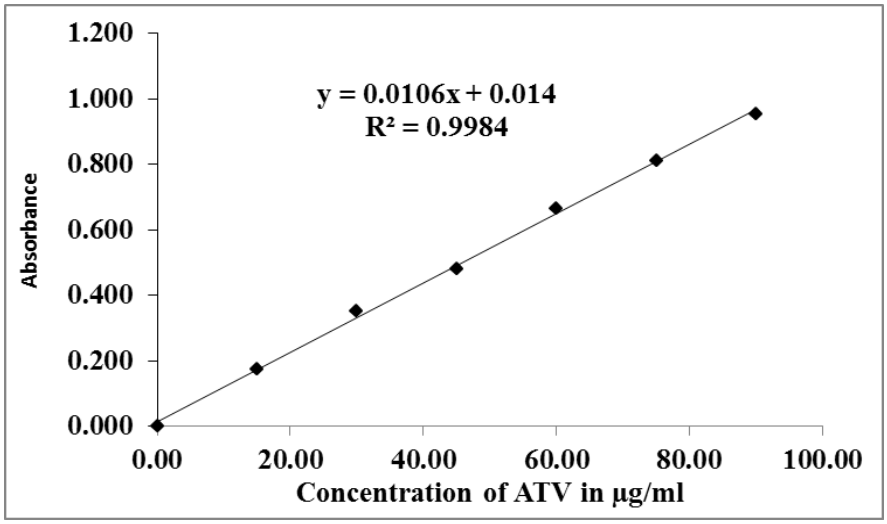

Fig.8-: Linearity plot between peak area and concentration of ATV

Sensitivity

Sensitivity of an analytical technique is defined as the instrument response to the low concentration of the analyte. The sensitivity is expressed in terms of limit of detection (LOD) and limit of quantization (LOQ) and were determined from the signal-to-noise ratio or the standard deviation of the response (σ) and the slope (s). Limit of detection and limit of quantitation may be expressed as LOD=3σ/s and LOD=10 σ/s. The calculated values of LOD and LOQ were presented in Table-6.

ASSAY STUDIES

Standard Solution Preparation

Accurately weighed and transfer 15 mg of cobicistat & 30 mg of atazanavir sulphate into a 100ml clean dry volumetric flask added methanol and sonicated to dissolve it completely and made up to the mark with the same solvent.

Sample Solution Preparation

An amount of fine powder of tablets equivalent to 15 mg of cobicistat & 30mg atazanavir sulphate was accurately weighed and transferred into a 100ml flask, added methanol and sonicated to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 2.0 ml standard stock/sample stock was taken into a 10ml volumetric flask and dilute up to the mark with diluents. Absorbance of the solution against reagent blank was determined triplicate at 239.3 and 292.5 nm. Since the absorption spectrum of COBI does not interfere with the measurement of ATV at λ_1=292.5 nm, the absorbance values at 292.5 nm

were directly used for the assay of ATV. Whereas the absorbance contributed at 239.3 nm by ATV is calculated from the previously known molar absorptivity of ATV at 239.3 nm and this contribution is subtracted from the measured absorbance of the solution at 239.3, yielding the absorbance due to COBI. Then assay and percent of assay of ATV and COBI was determined from absorbance values of standard and sample and the results were presented in Table-7.

STABILITY TESTING

The objective of degradation studies is to determine the percent of the sample found to be stable when it was subjected to different chemical and physical degradation such as acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH), oxidation in the presence of hydrogen peroxide (3% H_2O_2), thermal and radiation decomposition for specified period of time. Then absorbance of freshly prepared working standard solution and degradation solution was determined against reagent blank in triplicate at 239.3 and 292.5 nm. The percent of degradation was calculated from the absorbance values of ATV and COBI for standard and sample. The results of stability were presented in Table-8.

Acid / base hydrolysis

An amount of the fine tablet powder equivalent to 15 mg of cobicistat and 30 mg of atazanavir sulphate was accurately weighed and transferred into 100 ml of round bottom flask and 50 ml of freshly prepared 0.1 N HCl or 0.1N NaOH was added and kept aside, after 24 hours the solution was filtered through 0.45µfilter into a 100 ml standard flask and neutralized with 0.1N NaOH or 0.1 N HCl respectively and made up to the mark. Then precisely 2.0 ml of the filtrates were transferred into three different volumetric flasks and diluted to 10 ml with mobile phase.

Peroxide degradation

In case of peroxide degradation, the sample powder equivalent to 15 mg of cobicistat and 30 mg of atazanavir sulphate was added to 50 ml of freshly prepared 3%H_2O_2 and allowed for 24 hours, then filtered and made up to the mark. Then precisely 2.0 ml of the filtrates were transferred into three different volumetric flasks and diluted to 10 ml with mobile phase.

Thermal/Photo light degradation

In the study of thermal decomposition or UV degradation an amount of tablet powder equivalent to 15 mg of cobicistat and 30 mg of atazanavir sulphate was accurately transferred

into a clean and dry watch glass, placed in an oven which was maintained at 80°C or UV chamber for 24hrs. Then the watch glass was removed and allowed to cool to room temperature. The substance was accurately transferred into 100 ml volumetric flask and dissolved in diluents and made up to the mark, filtered and about 2.0 ml of this filtrate was diluted to 10 ml with mobile phase.

RESULT AND DISCUSSION

UV spectrophotometric method was developed for the assay of atazanavir sulphate (ATV) and cobicistat (COBI) simultaneously in pure and dosage forms and to the study of stability of the drug sample under different degradation conditions such as acid, base and peroxide hydrolysis and photo and light decomposition. Absorption spectra of individual drugs were recorded and wavelength of maximum absorbance was found to be 239.3 nm and 292.5 nm for COBI and ATV respectively. From the observation of the over line absorption spectra of COBI and ATV it was found that, COBI does not interfere with the measurement of ATV at λ_1=292.5 nm, but ATV does absorb appreciably along with COBI at λ_2=239.3 nm (one way over lapping spectra).

The molar extinction coefficient of ATV at λ_1=292.5 nm and λ_2=239.3 nm is determined and found to be 1.689×10^4 and 6.169×10^3 respectively. The molar extinction coefficient of COBI at λ_2=239.3 nm is determined and found to be 1.25×10^4. In case of one way over lapping spectra, the concentration of ATV is determined directly from the absorbance of the solution at 292.5 nm, where as the concentration of COBI was determined by calculating absorbance due to COBI at 239.3 nm. The absorbance contributed at 239.3 nm by the concentration of ATV is calculated from the previously known molar absorptivity of ATV at 239.3 nm i.e. 6.169×10^3 and this contribution is subtracted from the measured absorbance of the solution at 239.3, yielding the absorbance due to COBI.

System precision was determined for six replicates of the measurements and %RSD was found to be 0.3471 & 0.4612 for COBI and ATV respectively. Method precision was determined for by preparing working standard solution six times and measured absorbance and %RSD was determined and found to be 0.8695 & 0.8784 for COBI and ATV respectively. Accuracy of the developed method was determined at three different concentration levels, calculated the percent of recovery from the amount added and amount

recovered and then mean percent of recovery was calculated, and found to be 100.84 and 100.79 for COBI and ATV respectively.

Absorbance was linearly proportional to concentration of COBI and ATV in the range of 7.5-37.5 and 15-75 µg/ml. Assay of COBI and ATV was found to be 99.61 and 100.46% respectively. The developed method was adopted for the study of satiability of the drugs under different degradation conditions and % of degradation was found to be in between 12.74-5.38 and 15.94-6.94 for COBI and ATV respectively. The stability of the drug sample under different degradation conditions was determined and found to be within the range of 94.62-87.26 and 93.06-84.06 for COBI and ATV respectively. The developed method was found to be simple, rapid and applied for the analysis of drug samples, therefore the proposed method is recommended for the analysis of COBI and ATV in pure and tablet dosage forms in any quality control laboratories.

Table-1: System precision of the developed method

S.No.	Absorbance of ATV at 292.5 nm	Absorbance of COBI at 239.3 nm
1	0.660	0.480
2	0.657	0.484
3	0.659	0.482
4	0.654	0.485
5	0.657	0.482
6	0.655	0.486
Mean	0.657	0.483
Standard deviation	0.0023	0.0022
%RSD	0.3471	0.4612

Table-2: Method precision of the developed method

S.No.	Absorbance of ATV at 292.5 nm	Absorbance of COBI at 239.3 nm
1	0.663	0.478
2	0.658	0.481
3	0.653	0.479
4	0.667	0.485
5	0.659	0.487
6	0.668	0.488
Mean	0.661	0.483
Standard deviation	0.0058	0.0042
%RSD	0.8695	0.8784

Table-3: Accuracy studies (%recovery) of COBI at three spiked levels

Spiked level	Amount added	Amount found	%Recovery	Mean % Recovery
50%	15.0	15.76	105.07	
	15.0	15.12	100.80	101.89

	15.0	14.97	99.80	
100%	30.0	29.47	98.23	
	30.0	29.86	99.53	99.57
	30.0	30.28	100.93	
150%	45.0	45.98	102.18	
	45.0	44.97	99.93	101.09
	45.0	45.52	101.16	
Mean				100.84

Table-4: Accuracy studies (%recovery) of ATV at three spiked levels

Spiked level	Amount added	Amount found	%Recovery	Mean % Recovery
50%	30.0	30.94	103.13	
	30.0	30.45	101.50	101.48
	30.0	29.94	99.80	
100%	60.0	59.72	99.53	
	60.0	60.42	100.70	100.48
	60.0	60.73	101.22	
150%	90.0	90.84	100.93	
	90.0	90.35	100.39	100.43
	90.0	89.97	99.97	
Mean				100.79

Table-5: Results of linearity studies of COBI and ATV

S.No	Linearity Level	Cibicistat		Atazanavir	
		Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
1	I	7.5	0.121	15.0	0.174
2	II	15.0	0.236	30.0	0.351
3	III	22.5	0.3571	45.0	0.481
4	IV	30.0	0.474	60.0	0.667
5	V	37.5	0.593	75.0	0.813
Correlation coefficient		0.9990		0.998	
Slope		0.016		0.010	
Intercept		0.003		0.014	

Table-6: Results of LOD and LOQ of COBI and ATV

Constituent	Property	Concentration
COBI	LOD	0.795 µg/ml
	LOQ	2.651 µg/ml
ATV	LOD	1.725 µg/ml
	LOQ	5.750 µg/ml

Table-7: Assay of COBI and ATV in Evotaz formulation

Brand name	Name of the drug	Label claimed	Amount found ± SD	% of assay±%RSD
Evotaz	COBI	150	149.5±0.429	99.61±1.024
	ATV	300	301.4±0.618	100.46±1.612

Average of three determinations

Table-8: Results of study of degradation, purity angle and purity threshold

Degradation condition	% Degradation of COBI	% Degradation of ATV
Acid	10.54	15.94
Base	12.74	15.72
Peroxide	5.38	6.94
Thermal	7.30	7.98
Photo light	7.52	9.94

CONCLUSIONS

The developed UV spectrophotometric method was found to be simple, selective and sensitive and was adopted for the determination of assay of pharmaceutical formulations and extended to study the was the degradation under different forced degradation conditions, hence the reported method was suggested to use in any quality control laboratories for the studies of quality control of cobicistat (COBI) and atazanavir sulphate (ATV) in pure and pharmaceutical formulations.

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