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Original Article

Analytical Method Development and Validation of Simultaneous Estimation of Atazanavir in Bulk and Pharmaceutical Dosage form by using RP-HPLC

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ABSTRACT

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. A simple and reproducible HPLC procedure was developed and validated as per ICH guidelines for the estimation of Atazanavir and Ritonavir. Quantitative estimation of Atazanavir and Ritonavir was estimated by RP-HPLC using ACN: 0.1% Ortho phosphoric acid (45:55 %v/v) as a mobile phase and Hypersil column (250mm×4.6mm, 5µ) as a stationary phase and the peaks were observed at 240nm which was selected as a wavelength for quantitative estimation. After development of the method it was validated for specificity, system suitability, accuracy, linearity, precision, ruggedness and robustness. The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits, hence it is concluded that the system is suitable to perform assay. The method was found to be specific because it did not show any interference with placebo and blank..

Keywords: Ritonavir and Atazanavir, pharmaceutical preparation, RP-HPLC, limit of drug product.

1. INTRODUCTION

It is well known in the pharmaceutical industry that pharmaceutical analyst in research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality

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control (QC) or quality assurance department[1] . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions[6].

By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements[7].

2. MATERIALS AND METHODS

Drug profile

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). Like other protease inhibitors, it is used only in combination with other HIV medications. The U.S. Food and Drug Administration (FDA) approved atazanavir on June 20, 2003[8]. Atazanavir is the first PI approved for once-daily dosing, and also appears to be less likely to cause lipodystrophy and elevated cholesterol as side effects. It may also not be cross-resistant with other PIs. When boosted with ritonavir it is equivalent in potency to lopinavir for use in *salvage* therapy in patients with a degree of drug resistance, although boosting with ritonavir reduces the metabolic advantages of atazanavir and marketed under the trade name Reyataz by Bristol Myers,

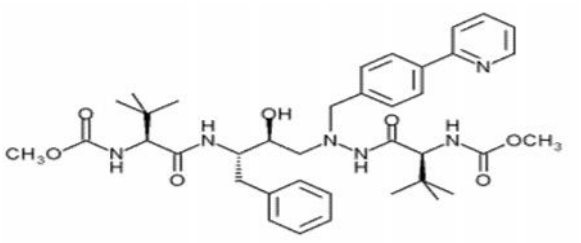

Fig 1: Atazanavir Structure

IUPACName: methylN-[(2S)-1-[2-[(2S,3S)-2-hydroxy-3-[[(2S)-2,-(methoxycarbonylamino)-33-dimethylbutanoyl]amino]-4-phenylbutyl]-2-[(4-pyridin-2-ylphenyl)methyl]hydrazinyl]-3,3-dimethyl-1-oxobutan-2-yl]carbamate

Molecular Formula: $C_{38}H_{52}N_6O_7$

Molecular Weight :704.9

Catagory: Atazanavir is an HIV protease inhibitor.

Antiviral Activity: Atazanavir has activity against HIV-1.

METHODOLOGY

Selection of wavelength:

An ideal wavelength is one that uses good response for the drugs to be detected. Atazanavir and Ritonavir in diluents the spectra were scanned on UV- Visible spectrophotometer in the range of 200 nm to 400 nm against diluent as blank. The Maximum absorbance of Atazanavir and Ritonavir was found to be 261 nm and 208 nm respectively. From the UV Visible spectrophotometric results, the iso-absorptive point of the combined spectrum of both drugs at 240 nm was chosen for detections of Atazanavir[9].

Selection of chromatographic method:

Selection of chromatographic method in general is done taking into consideration several parameters like the nature of the drugs, molecular weight and solubility. Since both the drugs selected are polar in nature, reversed phase chromatography has been used. C_{18} and C_4 columns were chosen as stationary phase and a mixture of organic solvents and buffers are used to develop a method for the simultaneous estimation ofAtazanavir[10].

Source

Method development for the product was initiated based on the individual chemical characteristics and their methods given in some journals.

Trail-1

Preparation of 0.1 % Ortho Phosphoric acid solution:

Add 0.5 mL of O-phosphoric acid in 500 mL of water. Mix well filter and degas through 0.45µm membrane filter.

Chromatographic conditions:

Mobile phase : ACN: 0.1% OPA (70:30 % v/v)
Column : Hypersil ODS column (150mm×4.6mm, 5µ)
Wavelength : 240 nm
Flow rate : 1 mL/min
Column temperature : ambient
Sample temperature : ambient
Injection volume : 20µL
Run time : 15 min

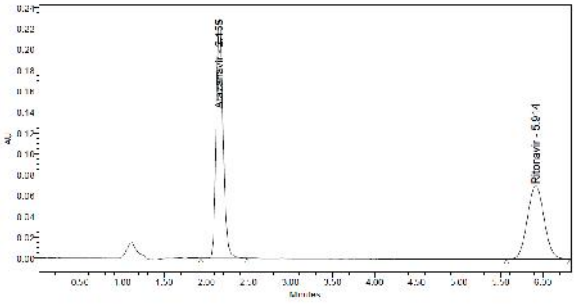

Fig 2: Chromatogram for Trail-1

3. RESULTS AND DISCUSSION

By injecting the standard mix solution which contains Atazanavir, retention time was found to be at 2.15 for Atazanavir was 5.91.The tailing factor of both drugs is satisfactory. Hence it is said to be the finalized method. The optimized mobile phase is ACN: 0.1% OPA (45:55 % v/v), at a flow rate of 1mL/min at 240 nm, under these conditions Atazanavir and Ritonavir were eluted at 2.15 and 5.9 min respectively. (Table 1)

Limit of detection (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental[11].

1. Based on Signal-to-Noise - This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 3:1 is generally considered acceptable for estimating the detection limit [12].

2. Based on the Standard Deviation of the Response and the Slope:

The detection limit (DL) may be ex pressed as:

DL = 3.3 s/S

Where,s = the standard deviation of y-intercepts of regression lines

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Limit of quantitation (LOQ):

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified[13].

1. A typical signal-to-noise ratio is 10:1.

2. Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

QL = 10 s/S

Where, s = the standard deviation of the response.

S = the slope of the calibration curve.

Chromatograms for the above discussed solutions were recorded as shown in the tables 2And figure 1 & 2.

Table 1: system suitability

Systemsuitability parameters	Atazanavir
%RSD for six replicate injections of standard	0.7
Tailing factor	1.19
Theoretical plates	3425

Table 2: linearity

Parameters	Atazanavir
Slope	8618
Intercept	11808
Correlation coefficient	0.99

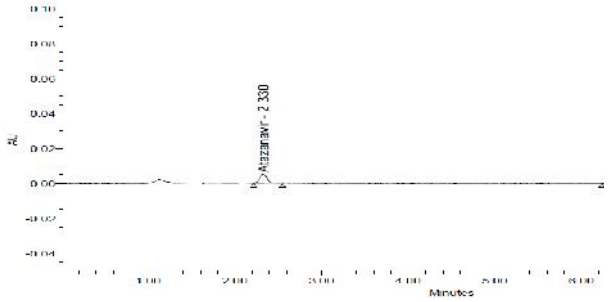

Fig 2: Chromatogram for LOD of Atazanavir

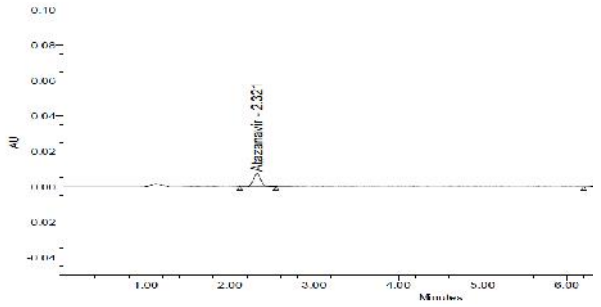

Fig 3: Chromatogram for LOQ of Atazanavir

4. CONCLUSION

A simple and reproducible HPLC procedure was developed and validated as per ICH guidelines for the estimation of Atazanavir .

Quantitative estimation of Atazanavir was estimated by RP-HPLC using ACN: 0.1% Ortho phosphoric acid (45:55 %v/v) as a mobile phase and Hypersil column (250mm×4.6mm, 5µ) as a stationary phase and the peaks were observed at 240nm which was selected as a wavelength for quantitative estimation[3]. After development of the method it was validated for specificity, system suitability, accuracy, linearity, precision, ruggedness and robustness. The value of theoretical plates, tailing factor, retention time and peak area was found to be within limits, hence it is concluded that the system is suitable to perform assay[4]. The method was found to be specific because it did not show any interference with placebo and blank[5].

The linearity studies were performed for the standard and found to be linear. From the linearity studies, the specified range was found to be 75µg/mL to 300µg/mL for Atazanavir and 25µg/mL to 100µg/mL for Ritonavir. The precision was checked and found to be within limits, hence the method is precise. The accuracy has been determined from 50% to 150% and the prescribed limits for recovery are 85%-115%. From accuracy studies, % recovery was calculated and found to be within limits. The ruggedness of the method was checked on different systems and by different columns and

standard was able to give same results which indicate that the method is rugged. The robustness of the method was checked by changing flow rate and temperature, and standard was able to give system suitability parameters within limit, which indicates that the method is robust.
Therefore it was concluded that the proposed method can be used for routine analysis of Atazanavir tablet dosage forms.

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