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Novel Sensitive UV Spectrophotometric Method for the Determination of Atazanavir

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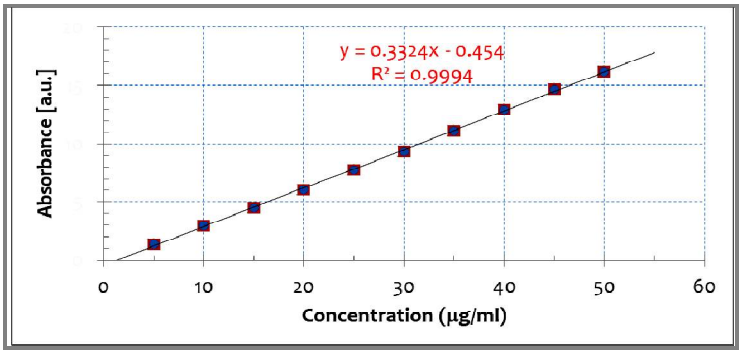
ABSTRACT

Atazanavir is an aza-dipeptide analogue with a bis-aryl substituent. A UV spectroscopic technique was developed for the estimation of atazanavir using 20% methanol as solvent and blank was demonstrated at an absorbance maxima 248 nm. This UV method is modest, precise, specific and validated. A linear trendline of (5-50 µg mL^{-1}) was plotted for all the sample absorbance at a wavelength of 248 nm with R^2, 0.9994. The proposed UV spectroscopic technique is simple, specific is accurate, precise and can be employed effectively for the approximation of ATV pharmaceutical dosage form.

Graphical Abstract



Linearity graph for Aazanavir.

Keywords: 4-hydroxy carbazole, epichlorohydrin, 4-(2,3-epoxyprpoxy) carbazole, R software, 2-(2-methoxyphenoxy) ethanamine.

INTRODUCTION

Atazanavir is an aza-dipeptide analogue with a bis-aryl substituent on the (hydroxethyl) hydrazine moiety with activity against both wild type and mutant forms of HIV protease, chemically known as methyl N-[(2S)-1-[2-[(2S,3S)-2-hydroxy-3-[[(2S)-2-(methoxy carbonyl amino)-3,3-dimethylbutanoyl] amino]-4-phenylbutyl]-2-(4-pyridin-2-ylphenyl) methyl] hydrazinyl]-3,3-dimethyl-1-oxobutan-2-yl]

carbamate. Atazanavir is an antiretroviral protease inhibitor that is used in the prevention of human immunodeficiency virus (HIV-1) infection and the acquired immunodeficiency syndrome (AIDS). Chemical structure of Atazanavir Sulphate is g=shown in figure 1.

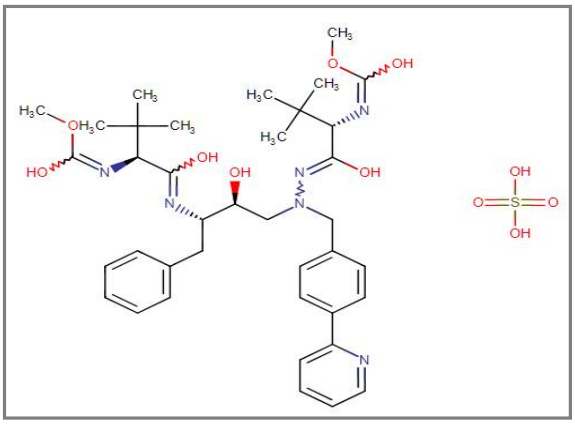


Figure 1. Chemical structure of Atazanavir Sulphate.

Atazanavir only needs to be taken once a day [**1**]. Atazanavir is in a class of medications called protease inhibitors. Atazanavir sulphate used for treating HIV infections. HIV infections are managed with combination therapy using mixtures of antiviral drugs. As the treatment mostly require taking medication for a number of times a day, with an interest in simpler regimens. Atazanavir is used along with other medications, such as ritonavir (Norvir), to treat human immunodeficiency virus (HIV) infection in adults and children. In order to increase its bioavailability, daily dose must be taken with food. Atazanavir inhibits an enzyme tangled in bilirubin conjugation, due to this patients continuously have higher bilirubin concentrations and up to 10.5% may even progress to jaundice while taking atazanavir dose of 400 mg daily. Other antagonistic effects reported in clinical trials include nausea, rashes and cardiac block. The literature presents several methods to determine ATV [**2-3**], the determination of ATV in biological fluids either separately or in combination with additional retroviral drugs in bulk and pharmaceutical dosage forms include liquid chromatography (LC) [**4-6**], high-performance liquid chromatography (HPLC) [**7-10**], reversed-phase high performance liquid chromatography (RP-HPLC) [**11-14**], high performance thin layer chromatographic (HP-TLC) [**15-16**], high performance liquid chromatography in tandem with mass spectrometry (HPLC-MS/MS) [**17**], ultra-high performance liquid chromatography in tandem with mass spectrometry (UPLC-MS/MS) [**18**], ultra-violet (UV)-Visible (VIS) spectrophotometric method [**19-21**], etc.

Two simple, sensitive, rapid spectrophotometric methods developed for simultaneous estimation of atazanavir sulphate (ATVs) and ritonavir (RTV) in tablets [**22**]. The concentration range of 10-50 µg mL^{-1} and 10-50 µg mL^{-1} for ATV and RTV was obtained respectively. Method involves solving concurrent equations based on the measurement of absorbance at two wavelengths 249.5 nm (ATVs) and 238.5 nm (RTV). Method-2 was based on the area under the curve (AUC) and the wavelength ranges selected for analysis were from 254.5 to 244.5 nm for ATVs and from 243.5 to 233.5 nm for RTV. Literature also reports the development of a simple, accurate and precise absorbance correction method by using UV spectrophotometry for the simultaneous estimation of ATV and RTV in combined tablet dosage. The method was also validated for different parameters like linearity, accuracy, precision, and robustness, the limit of detection (LOD) and limit of quantification (LOQ) [**23**]. A recent study [**24**] described a sensitive and selective method for simultaneous determination of three PIs, atazanavir, darunavir and ritonavir in human plasma by UPLC-MS/MS. Chromatographic separation of analytes was performed on Waters Acquity UPLC C18 (50 × 2.1 mm, ID 1.8 µm) column under gradient environments using 10 mM NH_4HCO_2, pH 4.0, and ACN as a mobile phase. In this research study, a UV spectroscopic technique was developed for the estimation of atazanavir using 20% methanol as solvent and blank. This UV method is modest, precise, specific and validated.

MATERIALS AND METHODS

A smart UV-Vis Double Beam Spectrophotometer, 2203, Systronics, with a spectral bandwidth of 1 nm and wavelength accuracy ±0.5 nm with paired quartz cells of 1cm matched was used for measuring the absorbance. Reference standards were used as required (obtained as gift samples) from pharmaceutical laboratories, India.

The primary aim of this research work was to develop a simple, sensitive spectrophotometric method for estimation of Atazanavir sulphate with good accuracy, reproducible, precision and economical over other techniques and that are used for routine analysis.

RESULTS AND DISCUSSION

Absorbance (λ_{max}): 100 mg of pure ATV drug was accurately weighed and dissolved in 50% MeOH and final volume made up to 100 mL with double distilled water to obtain a standard stock solution (1000 µg mL^{-1}). Aliquots of stock solution pipetted out and appropriately diluted with double distilled water to get the final concentration of 5-50 µg mL^{-1} of the standard solution. The solutions were scanned from 200 to 400 nm UV wavelength ranges, with blank as methanol and the maximum absorbance (λ_{max}) shown at 248 nm. The UV spectrum of Atazanavir with absorption maxima at 248 nm is shown in figure 2. The optical characteristics and precision are given in table 1.

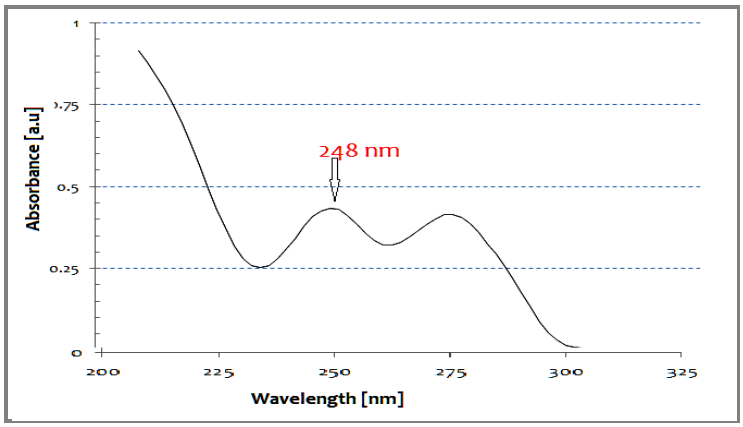


Figure 2. UV spectrum of Atazanavir with absorption maxima at 248 nm.

Validation of the UV method was completed with respect to the ensuing parameters. The technique validation parameters like linearity, repeatability, precision, accuracy, the limit of detection were checked as per ICH guidelines [25].

Table 1. Optical characteristics and precision

λ_{max}	248
Beer's law Range (µg m:$^{-1}$)	5-40
R^2	0.9994
Sandell's sensitivity (µg Sq.cm^{-1} 0.001^{-1})	0.044568
Molar absorptivity (L $mol^{-1}cm^{-1}$)	18060.47
Slope	0.0222
Intercept	0.0123

Linearity and range: By the method of dilution of the stock solution, required standard solutions were prepared to reach a concentration range equivalent mixture MeOH and double-distilled water. The obtained absorbance was plotted against the corresponding concentrations to acquire the

calibration curve. The linearity for ATV was determined at diverse concentration levels for ATV 5-50 µg mL^{-1} as working standards.

A linear trendline was plotted for all the sample absorbance at a wavelength of 248 nm. The R^2 was 0.9994. The calibration curve with a trendline was drawn as shown in figure 3.

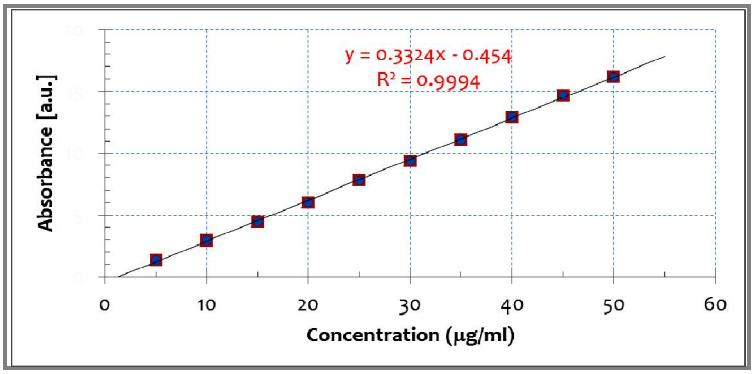


Figure 3. Linearity graph for Aazanavir.

Accuracy: A known amount of ATV corresponding to 80, 100, 120% of label claim has been added (standard addition method) and recovery studies were done by applying the method. The recovery studies give the accuracy of the method. By using the standard addition method, recovery studies were performed at 80%, 100% and 120% level and the % recoveries were calculated and are shown in table 2.

Table 2. Calculated percentage recoveries

Excess drug added (%)	% Recovery	%RSD	SE
80	99.90	0.1545	0.00102
100	100.14	0.2011	0.001294
120	100.12	0.1988	0.00038

RSD: Relative Standard deviation, SE: Standard error

Precision: Repeatability and intermediate precision studies give the precision of the method. Intra-day repeatability studies of the method were done on the same day. Intermediate precision (inter-day) of the method was checked by repeating analysis of ATV on different days. % relative standard deviation (% RSD) for the method was calculated.

The precision of the method was evaluated by inter-day and intra-day variation studies. In intra-day studies, working solutions of standard and sample were analyses thrice in a day and percentage % RSD was calculated. In the inter-day variation studies, working solution of standard and sample were analysed on three consecutive days and percentage % RSD was calculated. The data is given in table 3.

Table 3. Table shows the results of intra-day and inter-day precision

Intra-day precision	SE	0.0022
	SD	0.002026
	%RSD	0.5625
Inter-day precision	SE	0.000474
	SD	0.000582
	%RSD	0.1754

SE: Standard error, SD: Standard deviation, RSD: Relative Standard deviation

Limit of Detection and Limit of Quantitation: The smallest concentration of the analyte that gives the measurable response is Limit of Detection (LOD), calculated using the formula

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of the calibration curve, σ = standard deviation of the response.

The smallest concentration of the analyte, which gives a response is Limit of Quantification (LOQ), that can be accurately quantified, calculated using the formula

$$LOQ = 10 (\sigma / S)$$

Where, S = slope of the calibration curve, σ = standard deviation of the response.

APPLICATION

This method is useful for routine estimation of drugs in pharmaceutical industry.

CONCLUSION

The UV spectroscopic estimation analysis for ATV presented here in this research provides a simple, appropriate and accurate means for analysis of ATV in its drug dosage forms. Absorbance maxima for ATV were at 248 nm were selected for the analysis. Linearity for detector response was witnessed in the concentration range of 5-50 μg mL^{-1}. A linear trend line was plotted for all the sample absorbance at a wavelength of 248 nm with R^2, 0.9994. The proposed UV spectroscopic technique is simple, specific, accurate and precise and hence can be used in routine for estimation of ATV. The % RSD for all parameters was established to be <2, indicates the validity of the method and assay results obtained by this method are in fair agreement. The results obtained show a high degree of accuracy of the recovery studies. In conclusion, the developed technique is accurate, pr and can be employed effectively for the approximation of ATV pharmaceutical dosage form.

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