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A Validated RP-HPLC Method for the Determination of Atazanavir in Pharmaceutical Dosage Form

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Abstract: A validated RP HPLC method for the estimation of atazanavir in capsule dosage form on YMC ODS 150 x 4.6 mm, 5 µ column using mobile phase composition of ammonium dihydrogen phosphate buffer (pH 2.5) with acetonitrile (55:45 v/v). Flow rate was maintained at 1.5 mL/min with 288 nm UV detection. The retention time obtained for atazanavir was at 4.7 min. The detector response was linear in the concentration range of 30 – 600 µg/mL. This method has been validated and shown to be specific, sensitive, precise, linear, accurate, rugged, robust and fast. Hence, this method can be applied for routine quality control of atazanavir in capsule dosage forms as well as in bulk drug.

Keywords: Atazanavir, Reverse phase high performance liquid chromatography, Atavir® capsules.

Introduction

Atazanavir is a orally administered chemotherapeutic agent used in the treatment of acute and chronic HIV infection (HIV protease inhibitor) with a chemical[1] name 2,5,6,10,13-pentaazatetradecanedioicacid,3-12-bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[-4-(2-pyridinyl)phenyl]methyl]-,dimethylester,(3*S*,8*S*,9*S*,12S) sulfate. Literature survey reveals few chromatographic methods for the determination of atazanavir in combination with other retroviral drugs in biological fluids[2-8] and one assay with quantification of impurities method in active pharmaceutical ingredient[9]. The present paper aims at reporting sensitive, selective, precise, accurate, robust and rugged isocratic validated RP-HPLC method for the estimation of atazanavir in bulk as well as capsules dosage form.

Experimental

Shimadzu HPLC with DAD detector was used with LC solution software. Milli-Q water, HPLC grade methanol and acetonitrile (Rankem Ltd) and GR grade ammonium dihydrogen phosphate and orthrophosphoric acid were used. The capsule formulation containing equivalent of 300 mg of atazanavir (Atavir®-300 mg, Cipla) was procured from local market.

Chromatographic conditions

Chromatographic separation was achieved on YMC ODS (150 x 4.6 mm i.d. 5 µ) analytical column at ambient temperature with mobile phase consisting of 2.0 g of ammonium dihydrogen phosphate in 1000 mL of Milli-Q water, adjusted pH to 2.5 with ortho-phosphoric acid and acetonitrile (55:45 v/v). The flow rate was maintained at 1.5 mL/min with injection volume 10 µL and the detection was made at 288 nm. Diluent was prepared by mixing 600 mL of methanol with 400 mL of buffer.

Preparation of standard solution

Accurately weighed 34 mg of atazanavir sulphate standard into a 100 mL volumetric flask added about 60 mL of diluent and sonicated to dissolve and then made up to the volume with diluent to get 300 µg/mL standard solution.

Preparation of sample solution

Contents of 20 capsules weighed and mixed and equivalent to 30 mg of atazanavir was taken into 100 mL volumetric flask, about 75 mL of diluent was added sonicated for 25 min at room temperature with intermediate shaking and then made up to volume with diluent. The sample solution was centrifuged at 7500 rpm for 5 min to get a clear solution to get a concentration of 300 µg/mL.

Assay

Sample solution of 10 µL was injected into HPLC (n=6) and recorded the chromatograph. The amount of drug present per capsule was calculated by comparing the peak area of the sample solution with that of the standard solution. Ruggedness of the method was checked on different days on different system following the proposed procedure. The data presented in Table 1.

Table 1. Results in assay

Parameter	[*]Assay		
	Amount Claim, mg/Capsule	Amount Found, mg/Capsule	
		Method Precision	Ruggedness
Found, mg	300	299.71	299.15
% Assay		99.9	99.7
SD		0.23	0.18
% RSD		0.2	0.2

[*]*Assay average of six determinations (n=6)*

Linearity

Several aliquots of standard stock solution (1 mL=2600 µg/mL) of atazanavir were taken in different 25 mL volumetric flasks and diluted up to the mark with diluent to obtained concentration of 30, 60, 150, 210, 300, 360, 455 and 600 µg/mL of atazanavir. Peak areas were recorded for all the peaks and a calibration curve was obtained by plotting peak areas versus concentrations of atazanavir (Figure 1).

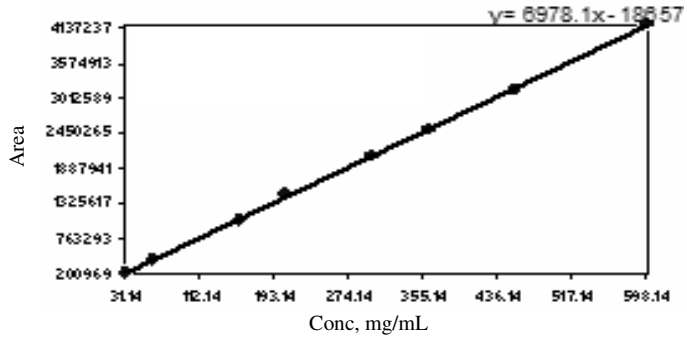

Figure 1. Calibration curve of atazanavir by HPLC

Recovery studies

Accuracy was determined by spiking the known amount of atazanavir drug substance to the pre analyzed samples and subjected to the proposed HPLC Method. The study was done at 20%, 50% and 150% of test concentration levels (*i.e.* 6, 15 and 45 mg respectively) in triplicates. Results of recovery study are shown in Table 2.

Table 2. Results of recovery studies.

	**Accuracy					
Sample ID	Amount Added, mg			Amount Found, mg		
1	6.05	15.10	45.68	6.02	15.08	45.78
2	6.05	15.05	45.50	5.99	15.07	45.73
3	6.02	15.10	45.61	6.00	15.13	45.72

***Accuracy performed in triplicates*

Forced degradation

Degradation studies are performed on drug product under acidic, alkali, oxidative, thermal and photolytic stress conditions. Each stress condition samples are analyzed in the proposed method and peak purity data is recorded to check the homogeneous nature of the drug.

Robustness

Standard solution was prepared and injected into HPLC following the proposed method in different variable conditions such as Flow (±10%), pH (±0.2units), wavelength (±5 nm) and organic composition in mobile phase (± 2% absolute) and checked system suitability criteria in the each variable condition.

Results and Discussion

Atazanavir was freely soluble in methanol and has λ_{max} at 288 nm, shown in Figure 2. System suitability parameters were carried out on freshly prepared standard stock solution of atazanavir as per the USP-XXVIII and reported in Table 3. Retention time achieved at 4.7 min. Regression equation y=6978.1x-18657 with concentration range of 30 - 600 µg/mL and the correlation coefficient (r=0.9999) obtained for atazanavir shows that the method is Linear. The assay results (n=6) of atazanavir in capsules were found to be 99.9% for method precision, 99.7% for ruggedness and 99.9% for recovery studies. System suitability parameters are within the acceptance criteria in robustness study indicates the method is robust. All the stress condition samples were analyzed for 30 min and checked for co elution - peak purity passes in each stress condition indicates there is no co-elution with the main analyte. Above parameters demonstrates that the developed HPLC method was specific, fast, simple, precise, accurate, sensitive, linear, accurate, robust and rugged. Thus the developed method can be easily used for the routine quality control of bulk and capsule dosage form of atazanavir within a short analysis time.

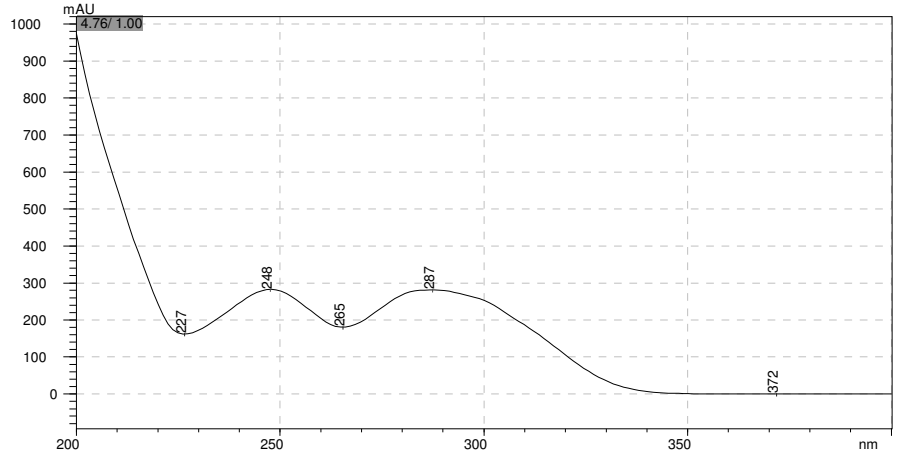

Figure 2. Spectrum of atazanavir standard by HPLC

Table 3. Validation and system suitability parameters

Parameter	Atazanavir
Retention time, min	4.7
Asymmetry	1.1
Theoretical plates	7929
Capacity factor	4.0
Minimum peak purity index	10
Wavelength, nm	288
Calibration range, µg/mL	30 – 600
Correlation coefficient	0.9999
Regression coefficient	0.9998
% RSD of standard	0.1

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