

## New UV Spectrophotometric Method for the Estimation of Atazanavir Sulfate in Bulk and Pharmaceutical Dosage Form

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### Abstract

### Original Research Article

A simple, fast and reliable UV spectrophotometric method has been developed for estimation of atazanavir sulphate in bulk and pharmaceutical dosage form. Estimation was carried out at  $\lambda_{\max}$  249 nm using 0.1 N HCl as solvent. The linearity was observed in the range of 10-90  $\mu\text{g/ml}$  with correlation coefficient (r) 0.999. The percentage recovery was found to be in range of 99.84-100.18%. The proposed method was found to be simple, accurate, precise, and reproducible and gave the acceptable recovery of the analyte which could be directly and easily applied to analysis of bulk and pharmaceutical tablet dosage form of atazanavir sulphate.

**Keywords:** Atazanavir sulphate; UV spectrophotometry; Tablet dosage form.

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## INTRODUCTION

Atazanavir sulphate (ATV) is a chemically (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-{{[4-(2 pyridinyl)phenyl]methyl}-2,5,6,10,1 penta aza tetra decane dioic acid dimethyl ester, sulphate (1:1) (Figure-1). ATV is an antiretroviral agent for the treatment of HIV infection and consequently it is clinically useful in the treatment of AIDS [1]. Atazanavir sulfate, azapeptide inhibitor of HIV-1 protease, is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection [2]. Atazanavir sulfate is a white to pale yellow powder. It is slightly soluble in water [2]. The drug was approved by the United States Food and Drug Administration (USFDA) in June 2003. Structure-activity studies with a series of azadipeptides designed to mimic the transition state of the peptide-cleavage reaction catalyzed by HIV-1 protease identified lead compounds that had either potent antiviral activity against mutant HIV-1 strains or good oral bioavailability, but not both. Atazanavir is given with food by mouth as the sulfate and the usual adult dose is 400 mg once daily [3]. The drug is well absorbed when administered orally with food (bioavailability 68%). The drug is highly bound to plasma proteins (86%) and is metabolized by CYP3A isozyme. It is a moderate inhibitor of CYP3A. Literature survey revealed few spectrophotometric methods [4-6], RP-HPLC methods [7-13] and HPTLC methods [14] available for

estimation of Atazanavir sulfate in bulk and pharmaceutical formulation.

Here attempt is made to develop simple UV spectrophotometric method for estimation of atazanavir sulfate in bulk and formulation with good accuracy, simplicity, precision and economy over other chromatographic methods and which can be used for routine analysis. The method was validate as per ICH guidelines [15].

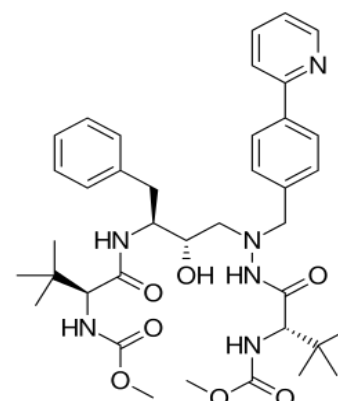


Fig-1: Structure of atazanavir sulfate

## MATERIALS & METHODS

### Instrumentation

An ELICO UV/Visible spectrophotometer model SL 244 with 10 mm matched quartz cells was

used for all spectral measurements. An electronic digital weighing balance (1 mg sensitivity, SHIMADZU AUX 220) used for weighing purpose.

### Chemicals and Reagents

All reagents and chemicals used were of Analytical Grade. Gift sample of atazanavir sulfate was supplied by Vivid labs, Hyd. Marketed formulation Atazor capsules (label claim 300 mg) was procured from local market

### Preparation of standard stock solution

Accurately weighed 10 mg of atazanavir sulfate was transferred into 100 ml volumetric flask and

the content was dissolved in 0.1 N HCl and volume was made up to the mark with 0.1 N HCl to get a stock solution containing 100 µg/ml.

### Preparation of working standard solution

From the standard stock solution 5 ml was transferred to 10 ml volumetric flask and diluting to 10 ml with 0.1 N HCl to get a concentration of 50 µg/ml. Working standard solution of atazanavir sulfate was scanned between 200-400 nm. The wavelength maximum exhibited for atazanavir sulfate was at 249 nm (Fig-2).



Fig-2: UV spectra of atazanavir sulfate

### Procedure for Calibration Curve

Appropriate volume of aliquot (1-9 ml) from standard stock solution was transferred to volumetric flask of 10 ml capacity. The volume was adjusted to the mark with 0.1 N HCl to give solutions concentrations in the range of 10-90 µg/ml. The absorbance measurements of these solutions were carried out against 0.1 N HCl as blank at 249 nm. Calibration curve was constructed by plotting absorbance versus concentrations. Linear regression equation was obtained from this calibration curve.

### Procedure for Pharmaceutical Formulation

Ten capsules (ATAZOR) were weighed and average weight was calculated. Capsule content powder equivalent to 10 mg atazanavir sulfate was accurately weighed and transferred to 100 ml volumetric flask. To this 60 ml of 0.1 N HCl was added and sonicated for 10 min. The flask was shaken and volume was made up to the mark with 0.1 N HCl. The above solution was filtered through whatmann filter paper No.41. From the

above solution 5 ml was transferred to 10 ml volumetric flask. Volume was made up to the mark with 0.1 N HCl to give a solution containing 50 µg/ml. The content in the capsule was calculated from the calibration curve.

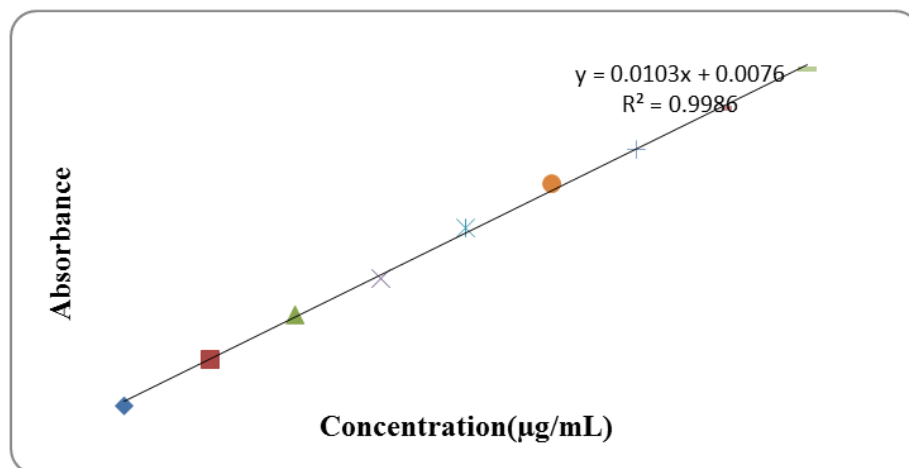
## RESULTS AND DISCUSSION

### Method Validation

The developed method was validated in terms of Linearity, precision, accuracy, Limit of detection (LOD) and Limit of Quantitation (LOQ), robustness and ruggedness.

### Linearity

Nine points calibration curve were obtained in a concentration range from 10-90 µg/ml for atazanavir sulfate. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was  $y=0.0103x+0.0076$  with correlation coefficient 0.999 (Table 1 and 2, Fig-2).

**Table-1: Linearity Data for Atazanavir sulfate**

S. NO	Concentration (µg/ml)	Absorbance
1	10	0.1001
2	20	0.2131
3	30	0.3234
4	40	0.4128
5	50	0.5376
6	60	0.6458
7	70	0.7300
8	80	0.8305
9	90	0.9259

**Table-2: Optical Characteristics of atazanavir sulfate**

S. No	Parameters	Method
1.	$\lambda_{max}$ (nm)	249
2.	Beers law limit (µg/ml)	10-90
3.	Sandell's sensitivity(µg/cm <sup>2</sup> /0.001 A.U)	0.0102
4.	Molar absorptivity (L mol <sup>-1</sup> cm <sup>-1</sup> )	7.89 x 10 <sup>3</sup>
5.	Correlation coefficient (r)	0.9986
6.	Regression equation(Y=mX+c)	Y=0.0103X+0.076
7.	Slope(m)	0.0103
8.	Intercept(c)	0.0076
9.	LOD (µg/ml)	0.21
10.	LOQ (µg/ml)	0.65
11.	Standard error of mean of Regression line	0.00494

**Precision**

Precision was checked in terms of repeatability, inter and intraday precision. It was expressed in percentage RSD.

**Repeatability**

The repeatability was evaluated by assaying six times of sample solution prepared for assay determination. Percentage RSD was calculated (Table-3).

**Table-3: Repeatability**

Concentration(µg/ml)	Absorbance
50	0.5350
50	0.5352
50	0.5350
50	0.5394
50	0.5306
50	0.5352
Mean	0.5350
Standard Deviation	0.0027
% RSD	0.50

**Interday and Intraday precision**

The intraday and interday precision study of atazanavir sulfate was carried out by estimating different concentrations of atazanavir sulfate three times

on the same day (intraday precision) and on three different days (interday precision) and the results were reported in terms of Percentage RSD (Table-4).

**Table-4: Interday & Intraday Precision**

S. No	Concentration ( $\mu\text{g/ml}$ )	Interday		Intraday	
		SD	%RSD	SD	%RSD
1	40	0.00057	1.07	0.001	1.72
2	50	0.0025	1.63	0.0015	0.98
3	60	0.003	1.17	0.0026	1.03

**Accuracy**

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the known amount of marketed formulation at three

different concentration levels 80, 100 and 120 % taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed three times and average recoveries were measured (Table-5).

**Table-5: Accuracy Studies**

Name of Drug	Spiked Level	Conc. Added ( $\mu\text{g/ml}$ )	Conc. Recovered ( $\mu\text{g/ml}$ )	%Recovery $\pm$ SD*
Atazanavir Sulfate	80%	40	40.07	100.18 $\pm$ 0.0256
	100%	50	49.92	99.84 $\pm$ 0.0582
	120%	60	59.97	99.95 $\pm$ 0.0421

\*Average of three determinations

**Robustness**

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and assay was evaluated. The effect of detection wavelength was studied at  $\pm 2$  nm. The effect of temperature was studied at  $\pm 2$  °C. The effect of solvent strength at  $\pm$

0.02 N HCl concentration. For changes of conditions, the sample was assayed in triplicates. When the effect of altering one set of conditions was tested, the other conditions were held constant at the optimum values. Assay for all deliberate changes of conditions should be within 98.0–102.0 % for the proposed method (Table-6).

**Table-6: Robustness results**

S.No	Concentration ( $\mu\text{g/ml}$ )	wavelength		Temperature( $^{\circ}\text{c}$ )		Concentration	
		247 nm	251 nm	26	30	0.08 HCl	0.12N HCl
1	50	0.532	0.532	0.537	0.547	0.547	0.543
2	50	0.533	0.532	0.532	0.540	0.547	0.540
3	50	0.534	0.532	0.523	0.542	0.547	0.542
4	50	0.530	0.532	0.533	0.540	0.549	0.543
5	50	0.532	0.532	0.530	0.547	0.547	0.524
6	50	0.537	0.523	0.532	0.545	0.547	0.540
AVG		0.532	0.530	0.531	0.543	0.547	0.538
SD		0.001	0.003	0.004	0.003	0.0009	0.007
%RSD		0.2	0.7	0.8	0.5	0.1	1.3

**Ruggedness**

Ruggedness of the proposed method is determined by analysis of aliquots from homogeneous

slot by two analysts using same operational and environmental conditions (Table-7).

**Table-7: Ruggedness Studies**

Formulation	Amount of drug taken from tablet(mg)	Analyst 1	Analyst 2
		(n=3)%Assay $\pm$ %RSD	(n=3)%Assay $\pm$ %RSD
Atazor(capsules)	300	99.83 $\pm$ 0.243	99.86 $\pm$ 0.324

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ were calculated according to below equation given by

$$\text{LOD} = 3.3 \sigma/s$$

$$\text{LOQ} = 10 \sigma/s$$

Where  $\sigma$  is the standard deviation of  $y$  intercepts of regression lines and  $s$  is the slope of the calibration curve (Table-2).

### Application of method to formulation

The proposed was applied to pharmaceutical formulation of atazanavir sulfate (Table-8).

**Table-8: Assay**

Formulation	Labeled Amount(mg)	Amount* Obtained(mg)	%Purity $\pm$ SD
Atazor (Capsules)	300	299.86	99.86% $\pm$ 0.685

\*Average of three determinations

## CONCLUSION

The method proposed in the above study was found to be simple, specific, economic, precise and rapid for the determination of Atazanavir sulfate in bulk as well as in its dosage form. Sample recoveries in all formulations were in good agreement with their respective label claims without interference of excipients and additives. Being economic and precise, the developed method may be preferred as an alternative method for the routine analysis of the Atazanavir sulfate in bulk and pharmaceutical dosage form.

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