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Research Article

# DEVELOPMENT OF NOVEL AND SIMPLE ANALYTICAL METHOD FOR THE ESTIMATION OF ATAZANAVIR SULPHATE IN PHARMACEUTICAL FORMULATION BY RP-HPLC

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**ABSTRACT**

Reversed phase high performance liquid chromatographic method was developed and validated for estimation of Atazanavir Sulphate in tablet dosage form. A Zodiac C18 , 250x4.6 mm i.d , 5 µm partical size , with mobile phase consisting of a buffer of 1.85 g ammonium acetate in 1000 ml water and acetonitrile in the ratio of 60:40 v/v was used. The flow rate was 1.0 ml/min and the effluents were monitored at 205 nm. The retention time was 2.840 min. The detector response was linear in the concentration of 18-42 mcg/ml , with the regression coefficient of 0.999. The percentage assay of Atazanavir Sulfate was 98.81 %. The method was validated by determining its accuracy , precision and system suitability. The results of the study showed that the proposed RP-HPLC method can be applied for the determination of Atazanavir Sulphate in quality control samples and formulations without interferences of the excipients present.

**Keywords**: Atazanavir sulphate , RP-HPLC , Estimation and Atazor capsules.

## INTRODUCTION

Chemically , Atazanavir Sulfate is (3S , 8S , 9S ,12S) - 3, 12 – Bis (1,1-dimethylethyl ) – 8 – hydroxyl -4 , 11 – dioxo – 9 - ( phenylmethyl ) – 6 - [ [ 4-( 2 – pyridinyl ) phenyl ] methyl ] – 2 , 5 , 6 , 10 , 13 penta azatetrade anedioic acid dimethyl ester;1 - [ 4 - ( pyridine – 2 – y l ) phenyl ] - 5S , 2 , 5-bis [ [ N (methoxy carbonyl ) – L – tert – leucinyl ] amino ] - 4S hydroxyl – 6 –phenyl – 2 – azahexane (Sean C. Sweetman; Martindale – The Complete Drug Reference , 34th Edition , Pharmaceutical Press , London , Chicago , Pg .629 (2005) , with a molecular formula $C_{38}H_{52}N_6O_7.H_2SO_4$ and a molecular weight of 802.93 (The Merck Index , XIV edition , Merck Research Laboratories , (Monograph No : 858 ) 142 (2006). It is an oral antiretroviral Protease inhibitors used in the treatment of HIV / AIDS. ATV is a antiretroviral drug specifically belongs to protease inhibitors class (Sean C . Sweetman ; Martindale - The Complete Drug Reference, 34th Edition , Pharmaceutical Press , London , Chicago , Pg . 629 (2005). Literature survey reveals few chromatographic methods for the determination of atazanavir sulphate in combination with other retroviral drugs in biological fluids [Cateau E, Tournier N, Dupuis A, Gwenael Le M and Venisse N, J Pharm Biomed Anal., 2005, 39(3-4), 791-795.] , one assay with quantification of impurities method in active pharmaceutical ingredient [K.Srinivasu. J, Venkateswara Rao, N. Appala Raju and K. Mukkanti, E-Journal of Chemistry,

2011, 8(1), 453-456.] and one assay in dosage form [International conference on Harmonization guidance for Industry In: Q2A Text on Validation of Analytical methods. Switzerland, IFPMIA: 1994, 1, 4.] . The present paper aims at reporting sensitive , selective , precise , accurate , robust and rugged validated RP-HPLC method for the estimation of atazanavir sulphate in bulk as well as dosage form.

## MATERIALS AND METHODS

Pharmaceutical grade atazanavir sulphate was supplied by Chandra labs , Hyderabad , India , ammonium acetate of analytical grade , acetonirile was of HPLC grade (Qualigens) and commercially available ATAZOR capsules ( one equivalent to 300 mg of atazanavir sulphate ) of Hetro drugs Ltd. was purchased from market for analysis .

## INSTRUMENTS

Schimadzu system with gradient pump connected to UV – Visible detector, Sartorius CP2250 balance was used for all weighing .

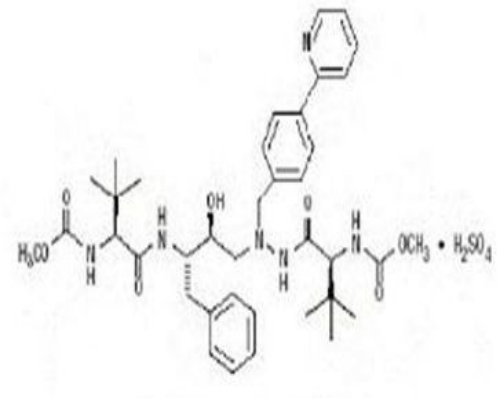

**Fig. 1: Structure of atazanavir sulphate**

### Method development

### Chromatographic conditions

Chromatographic separation was achieved on Schimadzu C18 (2) 250 x 4.6 mm , 5 µ column using mobile phase composition of buffer : acetonitrile (60:40 v/v) pH adjusted to 4. Flow rate was maintained at 1 ml/min with 205 nm UV detection . The retention time obtained for atazanavir sulphate was at 2.8 min . with injection volume 20 µL and the detection was made at 205 nm . Diluent was prepared by mixing 400 mL of acetonitrile with 400 mL of buffer , filtered through 0.45µm and degassed before use .

### Preparation of stock solution

Accurately weighed quantity of ATV (10 mg) was transferred to 10.0 ml volumetric flask . Then small amount mobile phase was added and ultrasonicated for 5 min and diluted up to the mark with mobile phase. (Concentration:1000µg/ml).

### Preparation of standard working solution

From the stock solution pipette out 1ml into 10 ml volumetric flask and makeup the final volume with mobile phase (concentration : 100 µg/ml).

### Preparation of mobile phase

The mobile phase was prepared by mixing acetonitrile : buffer (40 : 60) the mobile phase was filtered through 0.45µm and degassed before use.

### Preparation of working sample solution

Twenty capsules of ATAZOR (containing 300mg of ATV) were weighed and powder equivalent to 10mg of ATV was transferred to 10ml standard flask and small amount mobile phase was added . The solution was sonicated for 15min , and the final volume was made with same to obtain solution of ATV (1000µg/ml) . The mixture was then filtered through a nylon 0.45mm membrane filter . The above solution was suitably diluted with mobile phase to obtain final dilution of ATV (30µg/ml).

### Method validation

The method was validated for its linearity range , accuracy , precision , sensitivity and specificity . Method validation is carried out as per ICH guidelines .

### Linearity

Calibration curve was constructed by plotting peak area Vs concentration of ATV solutions , and the regression equation was calculated . The calibration curve was plotted over the concentration range 18-42µg/ml . Accurately measured standard working solution of ATV (0.6ml ,0.8ml, 1.0ml, 1.2ml and 1.4ml) were transferred to a series of 25ml volumetric flasks and diluted up to the mark with mobile phase . Aliquots (20 µl) of each solution were injected under the operating chromatographic condition described above .

### Accuracy

The accuracy of the methods was determined by calculating recoveries of ATV by the standard addition methods . The accuracy of the method was determined by

preparing solutions of different concentrations that is 80 %, 100% and 120% in which the amount of marketed formulation (ATAZOR-300mg) was kept constant (30mg) and the amount of pure drug was varied that is 24mg , 30mg and 36mg for 80% , 100% and 120% respectively. The solutions were prepared in triplicates and the accuracy was indicated by % recovery was shown in table .

**Method precision**

The precision of the instruments was checked by repeatedly injecting (n=6) solutions of ATV (30µg/ml) .

**Limit of detection and limit of quantification**

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines .

$$LOD = 3.3 \times \alpha / S$$

$$LOQ = 10 \times \alpha / S$$

**RESULTS AND DISCUSSION**

To optimize the RP-HPLC parameters , several mobile phases of different compositions were tried . A satisfactory separation and good peak symmetry for ATV were obtained with a mobile phase consisting of acetonitrile : buffer (40: 60 v/v) pH adjusted to 4 . Quantification was achieved with UV detection at 205nm based on peak area . Complete resolution of the peaks with clear baseline was obtained. System suitability parameters was calculated and compared with the standard limit as per ICH .

**Validation of the proposed method**

**Linearity**

Linear correlation was obtained between peak area used absorbance Vs concentration of ATV in the range of 18 - 42 µg/ml . The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression (Tab-1).

**Accuracy**

The accuracy experiments were carried out by the standard addition method . The recoveries obtained by 98.81 to 101.40% for ATV . The high values indicate that method is accurate (Tab-4) .

**Precision**

The low % RSD values of for Ataznavir sulphate was 0.89% which reveal that the proposed method is precise (Tab-5) .

**LOD and LOQ**

LOD for Ataznavir sulphate was found to be 0.94 and LOQ for Ataznavir sulphate was found to be 0.31 . This data show that the method is sensitive for the determination of Ataznavir sulphate (Table-6).

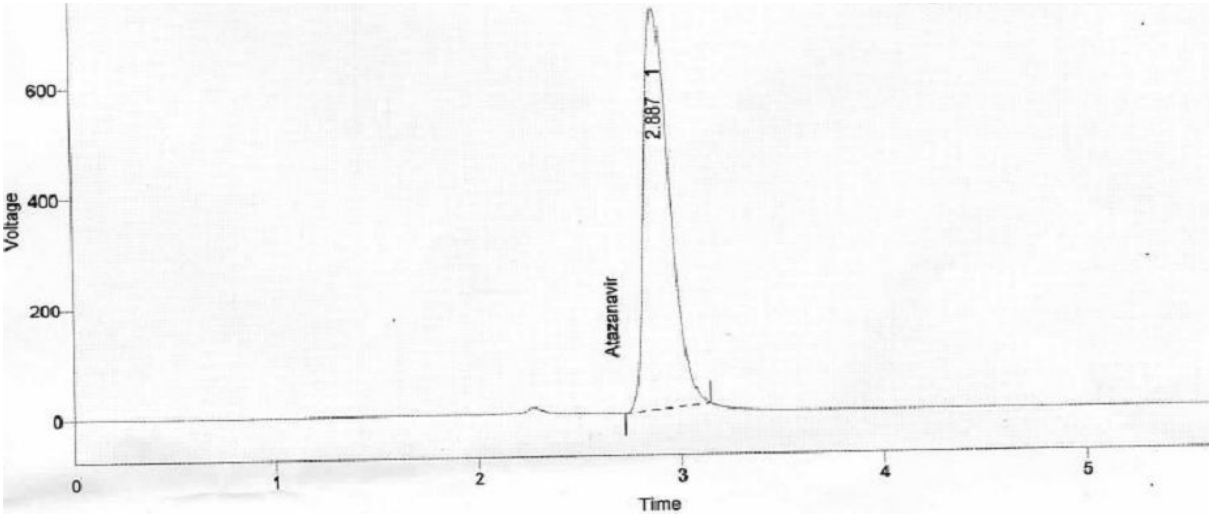


**Fig. 2: Typical chromatogram of atazanavir sulphate at 205nm**

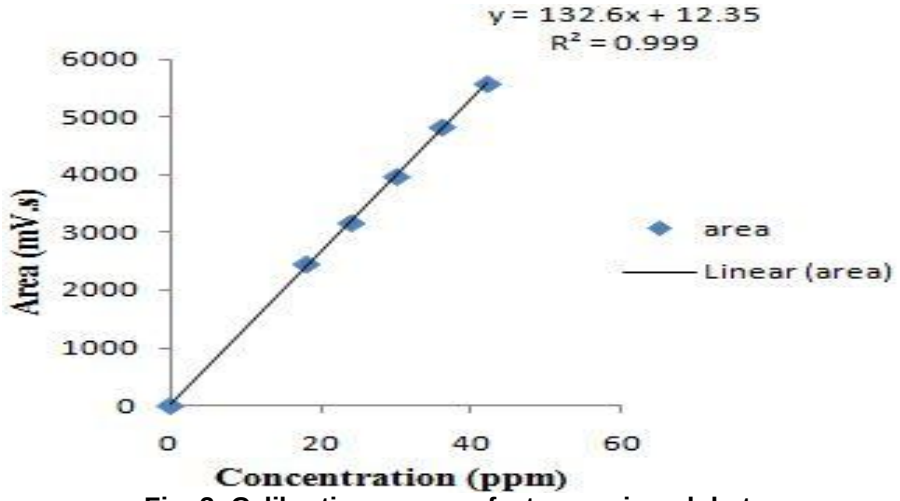


**Fig. 3: Calibration curve of atazanavir sulphate**

## CONCLUSIONS

A simple , precise , selective and sensitive RP - HPLC assay method with UV – Visible detection for ATV in pharmaceutical dosage form has been developed and validated . The method will be extensively used for the estimation of Atazanavir sulphate in bulk and pharmaceutical formulation .

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**Table 1: Linearty of Atazanavir sulphate**

| Parameter | Result |
|---|---|
| Linearity range | 18 – 42 µg/ml |
| Slope | 132.6 |
| Intercept | 12.35 |
| Correlation co-efficient | 0.999 |

**Table 2: System Suitability Parameters**

| Parameter | Result |
|---|---|
| Retention time | 2.887 |
| Assymetry | 1.704 |
| Theoritical plates | 2472 |

**Table 3: Assay of Atazanavir sulphate**

| Name of the formulation | Labeled claim | Amount found (%) Mean | %RSD |
|---|---|---|---|
| ATAZOR | 300 | 100.45 | 0.861 |

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

**Table 4: Accuracy studies of atazanavir sulphate**

| Amount of sample (mg/ml) | Amount of standard Added (mg/ml) | % of standard added | Amount recovered (mg/ml) | % Amount recovered | %RSD |
|---|---|---|---|---|---|
| 30 | 24 | 80 | 28.66 | 98.81 | 0.874 |
| 30 | 30 | 100 | 34.95 | 99.86 | |
| 30 | 36 | 120 | 41.57 | 101.40 | |

*Average of three determinations (n=3)

**Table 5: Precision studies of Atazanavir sulphate**

| Amount of standard taken (µg/ml) | Intra-day precision Mean ± %RSD | Inter-day precision Mean ± %RSD |
|---|---|---|
| 30 | 101.72 ± 0.425 | 100.37 ± 0.901 |

*Average of six determinations (n=6)

**Table 6: LOQ and LOQ of Atazanavir sulphate**

| Standard solution | LOD(µg/ml) | LOQ(µg/ml) |
|---|---|---|
| Atazanavir sulphate | 0.37 | 1.12 |

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