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New Validated RP-HPLC method for the Determination of Atazanavir Sulphate in Bulk and Dosage form

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ABSTRACT

A validated RP HPLC method for the estimation of Atazanavir sulphate in capsule dosage form on Egilent TC C_{18} (2) 250 x 4.6 mm, 5 µ column using mobile phase composition of water: acetonitrile (20:80 v/v) pH adjusted to3. Flow rate was maintained at 1 ml/min at an ambient temperature. Quantification was achieved with ultraviolet (DAD) detection at 255 nm. The retention time obtained for Atazanavir sulphate was at 3.7 min. The detector response was linear in the concentration range of 10 – 80 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 sulphate in capsule dosage forms as well as in bulk drug.

Key words: Atazanavir sulphate, Reverse phase high performance liquid chromatography, Atazor capsules.

INTRODUCTION

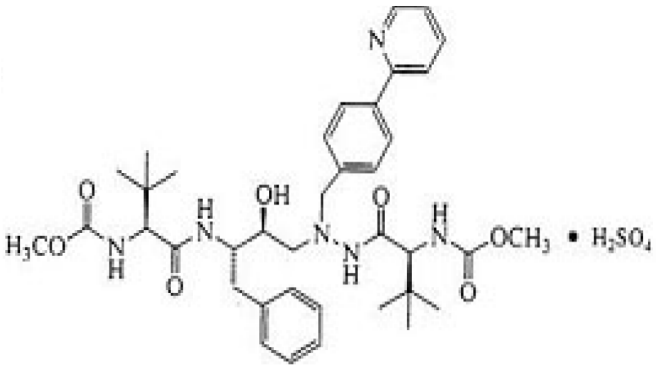
Chemically, Atazanavir Sulfate [1] is (3S,8S,9S,12S)-3,12-Bis(1,1-dimethylethyl)-8-hydroxy-4,11-dioxo-9-(phenylmethyl)-6-[[4-(2-pyridinyl) phenyl] methyl]-2,5,6,10,13 penta azatetrade anedioic acid dimethyl ester;1-[4-(pyridine-2-yl)phenyl]-5S,2,5-bis[[N (methoxy carbonyl)-L-tert- leucinyl]amino]-4S hydroxyl-6-phenyl-2-azahexane. 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. Literature survey reveals few chromatographic methods for the determination of atazanavir sulphate in combination with other retroviral drugs in biological fluids [2-8], one assay with quantification of impurities method in active pharmaceutical ingredient [9] and one assay in dosage form **[**10]. 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 Hetro Drugs Ltd., Hyderabad, India. The methanol, acetonirile (HPLC grade) were purchased from MERK and commercially available ATAZOR capsules (one equivalent to 300 mg of atazanavir sulphate) of Hetro drugs Ltd. was purchased from market for analysis.

Instruments
Egilent technologies 1200 LC system with gradient pump connected to DAD UV detector, LC-GC AGN204PO balance was used for all weighing.

Fig.1. Structure of Atazanavir sulphate



Method development
Chromatographic conditions
Chromatographic separation was achieved on Egilent TC C_{18} (2) 250 x 4.6 mm, 5 µ column using mobile phase composition of water: acetonitrile (20:80 v/v) pH adjusted to3. Flow rate was maintained at 1 ml/min with 255 nm UV detection. The retention time obtained for atazanavir sulphate was at 3.7 min. with injection volume 20 µL and the detection was made at 255 nm. Diluent was prepared by mixing 800 mL of acetonitrile with 200 mL of triple distilled water, 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 methanol was added and ultrasonicated for 5 min and diluted up to the mark with methanol.(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 methanol (100 µg/ml).

Preparation of mobile phase
The mobile phase was prepared by mixing acetonitrile: water (80:20) 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 methanol 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 [10-11].

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 10-80µg/ml. accurately measured standard working solution of ATV (1,2,3,4,5,6,7 and 8ml) were transferred to a series of 10ml

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).

Intermediate Precision (Reproducibility)
The intra-day and inter-day precision of the proposed methods were determined by the corresponding responses three times on the same day and on three different days over a period of one week for three different concentration of ATV (20,40 and 80µg/ml)

Robustness:
Robustness of the method was determined by carrying out the analysis at three different wavelengths (i.e. 255±2 nm) and three different flow rates (i.e. 1±0.1 ml/min).

Ruggedness:
Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted. The result was indicated by % RSD.

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.

$$LOD = 3.3 \text{ X } \alpha / S$$
$$LOQ = 10 \text{ X } \alpha / S$$

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: water (80: 20v/v) pH adjusted to 3. Quantification was achieved with UV detection at 255nm 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 10-80µg/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 99.49 to 101.32% for ATV. The high values indicate that method is accurate (Tab-4).

Precision
The low% RSD values of intra-day and inter-day (0.413 and 0.892%) for Ataznavir sulphate reveal that the proposed method is precise (Tab-5).

Robustness and ruggedness

The low % RSD values of robustness and ruggedness (1.04 and 0.87%) for Ataznavir sulphate ATV reveal that the proposed method is robust and rugged (Tab-6).

LOD and LOQ

LOD for Ataznavir sulphate was found to be 1.31 and LOQ for Ataznavir sulphate was found to be 3.98 This data show that the method is sensitive for the determination of Ataznavir sulphate (Table-7).

Fig.2. Typical chromatogram of Atazanavir sulphate at 255nm

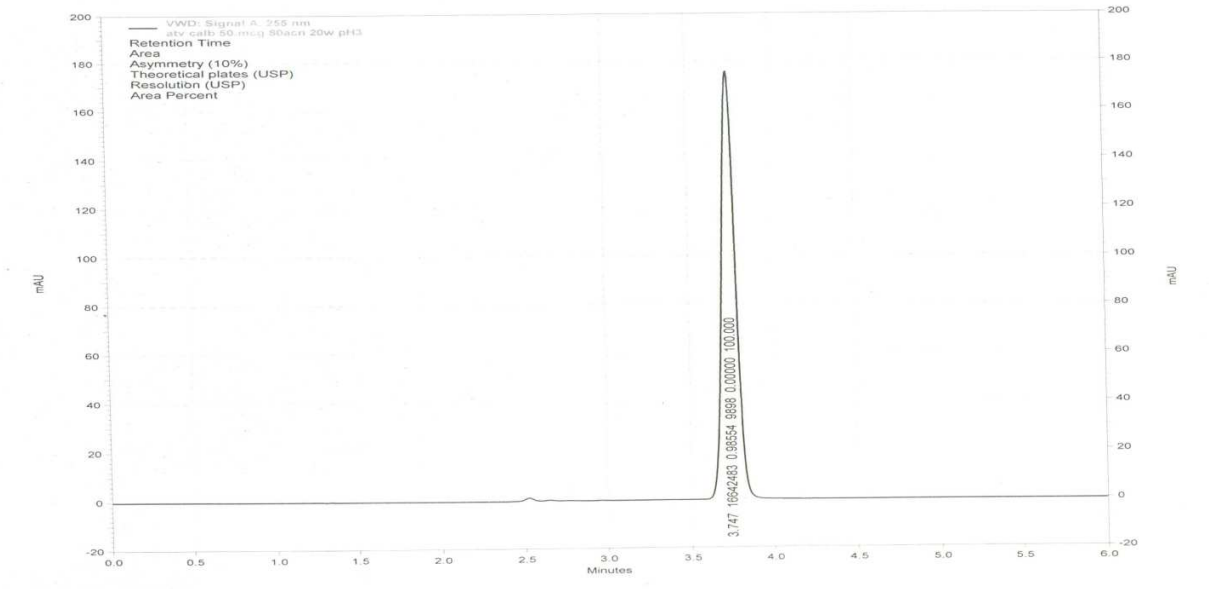


Fig.3. Calibration curve of Atazanavir sulphate

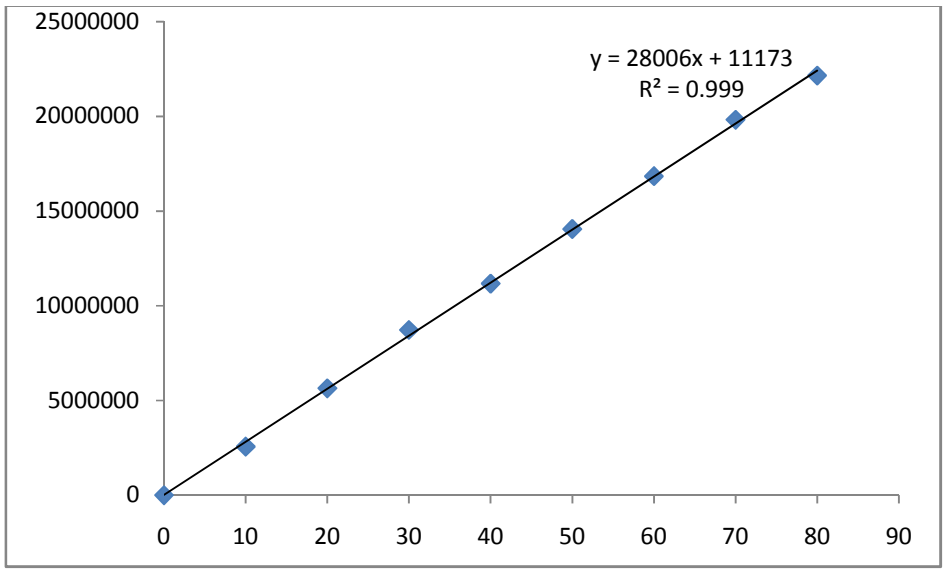


TABLE.1. linearty of Atazanavir sulphate

Parameter	Result
Linearity range	10-80µg//ml
Slope	28006
Intercept	11173
Correlation coefficient	0.9993

TABLE.2.System Suitability Parameters

Parameter	Result
Retention time	3.7min
Asymmetry (10%)	0.985
Theoretical plates	9898

TABLE.3. Assay of Atazanavir sulphate

Name of the formulation	Labled claim	Amount found(%) Mean*	% RSD
ATAZOR	300	100.28	0.854

*Assay average of six determinations (n=6)

TABLE.4. Accuracy studies of atazanavir sulphate

Amount of sample taken (mg/m)	Amount of standard added (mg/ml)	% of std added	Amount recovered (mg/ml)*	% amount recovered*	% RSD
30	24	80	24.31	101.32	0.911
30	30	100	29.84	99.49	
30	36	120	36.16	100.45	

*Aaverage of three determinations (n=3)

TABLE.5. Precision syudies of Atazanavir sulphate

Amount of std taken (µg/ml)	Intra-day precision Mean*±% RSD	Inter-day precision Mean*±% RSD
30	101.64 ±0.413	100.35±0.892

*Average of six determinations (n=6)

TABLE.6. Robustness and Ruggedness

Parameter		Mean*±% RSD
Robustness	Change in flow rate (±0.1ml/min)	98.84 ±1.04
	Change in λmax (± 2nm)	99.47±1.21
Ruggedness	1st analyst	100.97±0.87
	2nd analyst	99.85±0.73

*Aaverage of three determinations (n=3)

TABLE.6. LOQ and LOQ of Atazanavir sulphate

STD solution	LOD (µg/ml)	LOQ (µg/ml)
Ataznavir sulphate	1.31	3.98

CONCLUSIONS

A simple, precise, selective and sensitive RP- HPLC assay method with DAD 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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REFERENCES

[1]. Aarti Raja, John lebbos, Peter Kirkpatric, *Nature Review Drug Discovery*, **2003**, 2, 857-858.
[2]. Cateau E, Tournier N, Dupuis A, Gwenael Le M and Venisse N, *J Pharm Biomed Anal*., **2005**, 39(3-4), 791-795.
[3]. Loregian A, Pagni S, Ballarin E, Sinigalia E and Saverio G P and Palu G, *J Pharm Biomed Anal*., **2006**, 42(4), 500-505.
[4]. Choi S Ok, Rezk N L and Kashuba Angela D M, *J Pharm Biomed Anal*., **2007**, 43(4), 1562-1567.
[5]. Rebiere H, Mazel B, Civade C and Bonnet Pierre-Antoine, *J Chromatography B*, **2007**, 850(1-2), 376-383.
[6]. Weller Dennis R, Brundage Richard C, Balfour Henry H and Vezina Heather E, *J Chromatography B*, **2007**, 848(2), 369-373.
[7]. D'Avolio A, Siccardi M, Sciandra M, Lorena B, Bonora S, Trentini L and Di Perri G, *J Chromatogr B*, **2007**, 859(2), 234-240.
[8]. Cattaneo D, Maggiolo F, Ripamonti D and Perico N, *J Chromatographic Science*, **2008**, 46(6), 485-489.
[9]. K.Srinivasu. J, Venkateswara Rao, N. Appala Raju and K. Mukkanti, *E-Journal of Chemistry*, **2011**, 8(1), 453-456.
[10]. International conference on Harmonization guidance for Industry In: Q2A Text on Validation of Analytical methods. Switzerland, IFPMIA: **1994**, 1, 4.
[11]. International conference on Harmonization guidance for Industry In: Q2B Text on validation of Analytical methods. Switzerland, IFPMIA: **1996**, 1-8.