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Original Article

Stability indicating RP-HPLC method for the determination of Atazanavir sulphate in bulk and dosage form

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ABSTRACT

Objective: The objective of the present work is to develop a simple, precise, accurate, validated stability indicating RP-HPLC method for the determination of Atazanavir sulphate in bulk and capsule dosage form.

Method: A validated stability indicating RP-HPLC method for the estimation of Atazanavir sulphate in capsule dosage form on Agilent TC C18 (2) 250 × 4.6 mm, 5 μ column using mobile phase composition of 0.02 M ammonium dihydrogen phosphate buffer:acetonitrile:methanol (30:25:45 v/v) and pH adjusted at 2.5 with *ortho*-phosphoric acid. Flow rate was maintained at 1 ml/min at an ambient temperature. Quantification was achieved with ultraviolet detection at 288 nm.

Results: The retention time obtained for Atazanavir sulphate was at 3.0 min. The result obtained with the detector response was found to be linear in the concentration range of 5–50 μg/ml. This method has been validated and shown to be specific, sensitive, precise, linear, accurate, rugged, robust and fast. Atazanavir sulphate was subjected to different accelerated stress conditions. The degradation products, when anywhere well resolved from the pure drug with significantly different retention time values.

Conclusion: It is concluded that this method can be applied for routine quality control of Atazanavir sulphate in capsule dosage forms as well as in bulk drug.

1. Introduction

Chemically, Atazanavir sulphate 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 pentaazatetradecane dioic 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 as shown in Fig. 1.

It is an oral antiretroviral protease inhibitors used in the treatment of HIV/AIDS.[1] Atazanavir sulphate is antiretroviral drug specifically belongs to protease inhibitors class. In some literature Atazanavir sulphate is reported as poorly water soluble and is known substrate of both hepatic

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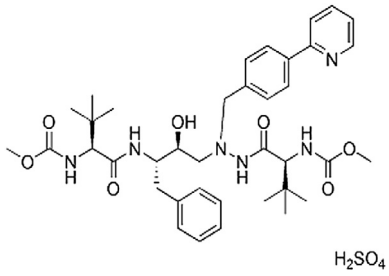

Fig. 1 – Structure of Atazanavir sulphate.

metabolizing enzyme Cytochrome 450 (CYP3A) and intestinal drug efflux pump, P-glycoprotein (Pgp) has low oral bioavailability.[2] To avoid this problem low dose of Ritonavir is administered as booster. Low dose of Ritonavir has been used to increase the bioavailability of other PI.[3,4] Literature survey reveals few UV spectrophotometric methods,[5–7] chromatographic methods for the determination of Atazanavir sulphate alone and in combination with other retroviral drugs in biological fluids.[8–11] The present paper aim is to report simple, sensitive, selective, precise, accurate, robust and rugged validated stability indicating RP-HPLC method for the estimation of Atazanavir sulphate in bulk as well as dosage form.[12–14]

2. Experimental

2.1. Materials and methods

Pharmaceutical grade Atazanavir sulphate was supplied by Hetro Drugs Ltd., Hyderabad, India. The methanol, acetonitrile (HPLC grade) were purchased from MERCK and commercially available ATAZOR capsules (one equivalent to 300 mg of Atazanavir sulphate) of Hetro drugs Ltd. was purchased from market for analysis.

2.2. Instruments

Agilent technologies 1260 LC system with gradient pump connected to DAD UV detector, LC-GC AGN204PO balance was used for all weighing.

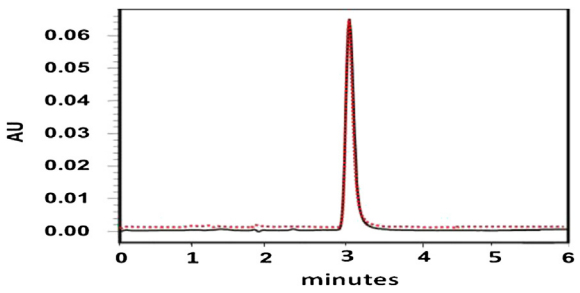

Fig. 2 – Typical chromatogram of Atazanavir sulphate at 288 nm.

2.3. Method development

2.3.1. Chromatographic conditions

Chromatographic separation was achieved on Agilent TC C18 (2) 250 × 4.6 mm, 5 μ column using mobile phase composition of 0.02 M Ammonium dihydrogen phosphate buffer:acetonitrile:methanol (30:25:45 v/v) and pH adjusted at 2.5 with *ortho*-phosphoric acid. Flow rate was maintained at 1 ml/min with 288 nm UV detection. The retention time obtained for Atazanavir sulphate was at 3.0 min as shown in Fig. 2. Diluent was prepared by mixing 300 ml of ammonium dihydrogen phosphate buffer (pH 2.5), 250 ml of acetonitrile and 450 ml of methanol, filtered through 0.45 μm and degassed before use.

2.3.2. Preparation of stock solution

Accurately weighed quantity of Atazanavir sulphate (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: 1000 μg/ml).

2.3.3. Preparation of standard working solution

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

2.3.4. Preparation of mobile phase

The mobile phase was prepared by mixing ammonium dihydrogen phosphate buffer:acetonitrile:methanol (30:25:45 v/v). The mobile phase was filtered through 0.45 μm and degassed before use.

2.3.5. Preparation of working sample solution

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

2.4. Method validation

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

2.4.1. Linearity

Calibration curve was constructed by plotting peak area Vs concentration of Atazanavir sulphate solutions, and the regression equation was calculated. The calibration curve was plotted over the concentration range 5–50 μg/ml. Accurately measured standard working solution Atazanavir sulphate (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5 ml) were transferred to a series of 10 ml volumetric flasks and diluted up to the mark with mobile phase. Aliquots (20 μl) of each solution were