

METHOD DEVELOPMENT AND VALIDATION OF ATAZANAVIR SULFATE BY VARIOUS ANALYTICAL TECHNIQUES - A REVIEW

C. Divya*, A. Ajitha, T. Rama Mohana Reddy, V. Umamaheswara Rao

Department of Pharmaceutical Analysis and Quality Assurance, CMR College of Pharmacy, Kandlakoya, Hyderabad, Telangana, India

*Corresponding author e-mail: kaladivya450@gmail.com

ABSTRACT

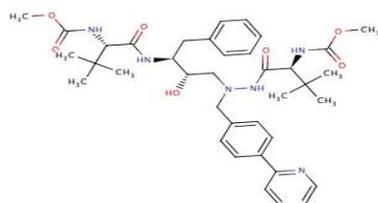
Atazanavir Sulfate is a sulfate salt form of atazanavir, an aza-dipeptide analogue with a bis-aryl substituent on the (hydroxethyl)hydrazine moiety with activity against both wild type and mutant forms of HIV protease. Atazanavir does not elevate serum lipids, a common problem with other protease inhibitors. In this review, we discussed various analytical methods like UV, HPLC, LC-MS for the estimation of Atazanavir Sulfate in pharmaceutical dosage forms.

Key words: Atazanavir Sulfate, HPLC, LCMS

INTRODUCTION

Atazanavir is Methyl N-[(1S)-1-[[[(2S,3S)-3-hydroxy-4-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-N'-[[4-(pyridin-2-yl)phenyl]methyl]butanehydrazido]-1-phenylbutan-2-yl]carbonyl]-2,2-dimethyl propyl]carbamate. Atazanavir is an antiretroviral drug of the protease inhibitor (PI) class. Like other antiretrovirals, it is used to treat infection of human immunodeficiency virus (HIV). Atazanavir is distinguished from other PIs in that it can be given once-daily (rather than requiring multiple doses per day) and has lesser effects on the patient's lipid profile (the amounts of cholesterol and other fatty substances in the blood). Like other protease inhibitors, it is used only in combination with other HIV medications.

Fig 1: Chemical Structure of Atazanavir:



Indication: Used in combination with other antiretroviral agents for the treatment of HIV-1 infection, as well as postexposure prophylaxis of HIV infection in individuals who have had occupational or nonoccupational exposure to potentially infectious body fluids of a person known to be infected with HIV when that exposure represents a substantial risk for HIV transmission.

Pharmacodynamics: Atazanavir (ATV) is an azapeptide HIV-1 protease inhibitor (PI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Atazanavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs. Atazanavir is pharmacologically related but structurally different from other protease inhibitors and other currently available antiretrovirals.

Mechanism of action: Atazanavir selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells by binding to the active site of HIV-1 protease, thus preventing

the formation of mature virions. Atazanavir is not active against HIV-2.

Absorption: Atazanavir is rapidly absorbed with a T_{max} of approximately 2.5 hours. Administration of atazanavir with food enhances bioavailability and reduces pharmacokinetic variability. Oral bioavailability is 60-68%.

Volume of distribution : Not Available

Protein binding : 86% bound to human serum proteins (alpha-1-acid glycoprotein and albumin). Protein binding is independent of concentration.

Metabolism: Atazanavir is extensively metabolized in humans, primarily by the liver. The major biotransformation pathways of atazanavir in humans consisted of monooxygenation and dioxygenation. Other minor biotransformation pathways for atazanavir or its metabolites consisted of glucuronidation, N-dealkylation, hydrolysis, and oxygenation with dehydrogenation. In vitro studies using human liver microsomes suggested that atazanavir is metabolized by CYP3A.

Half life : Elimination half-life in adults (healthy and HIV infected) is approximately 7 hours (following a 400 mg daily dose with a light meal). Elimination half-life in hepatically impaired is 12.1 hours (following a single 400 mg dose).

Various analytical techniques:

Atazanavir having some review article about HPTLC⁽⁵⁾, UV⁽⁶⁻⁹⁾ and HPLC⁽¹⁰⁻¹⁴⁾ methods.

Manoj Gadhvi, Anil Bhandari, et al., reported as A new, simple, sensitive, precise and accurate High-performance thin-layer chromatographic method for simultaneous determination of Ritonavir and Atazanavir in their combined tablet dosage form has been developed, validated and used for determination of the compounds in commercial pharmaceutical products. Chromatographic separation was achieved on aluminium plates precoated with silica gel 60 F254 as the stationary phase and chloroform: ethyl acetate: acetone (5:2:3, v/v/v) as a mobile phase. Densitometric measurements of their spots were achieved at 244 nm over the concentration ranges of 800-2800 ng spot-1 and 2400-8400 ng spot-1, with mean recoveries of 98.57 ± 0.35 and 99.16 ± 0.20 for ritonavir and atazanavir respectively. Limit of detection for ritonavir and atazanavir were found to be 300 ng spot-1 and 200 ng spot-1 respectively.

Minal R. Ghante, Manoj M. Kadam et al., reported as Two simple, accurate, precise and cost effective UV-Spectrophotometric methods have been developed for estimation of Atazanavir sulphate (ATV), an anti-HIV drug, in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum

wavelength, 247nm. Method B is Area under Curve (AUC), in wavelength range of 240-254nm. The linear responses were observed in the range of 5- 40 µg/ml for both the methods, with the regression coefficient of 0.9996 and 0.9997 respectively. The accuracy of the methods was assessed by recovery studies and was found to be 100.56% and 100.86% respectively. The developed methods were validated for different parameters like linearity, accuracy (recovery), precision and specificity, as per the ICH Q2 R1 (International Conference for Harmonization) guidelines and were found to be satisfactory. These methods can be used for the determination of Atazanavir sulphate in bulk and formulation without interference of the excipients.

Sathish Kumar Konidala, Sujana. K et al., reported as The present study describes a simple, accurate, precise and cost effective UV-Spectrophotometric method for the estimation of Atazanavir sulphate, an Anti-HIV drug, in bulk and pharmaceutical dosage form. The drug was first dissolved in 20% glacial acetic acid and final volume was made up with distilled water. The λ_{max} or the absorption maxima of the drug was found to be 299nm. A linear response was observed in the range of 10- 50µg/ml with the regression coefficient of 0.999. The method was validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Atazanavir sulphate in quality control of formulation without interference of the excipients.

Suddhasattya Dey, Y.Vikram Reddy, Thirupathi Reddy et al., reported as the present study describes a simple, accurate, precise and cost effective UV-VIS Spectrophotometric method for the estimation of Atazanavir, an anti-HIV drug, in bulk and pharmaceutical dosage form. The solvent used was methanol and the λ_{max} or the absorption maxima of the drug was found to be 250nm. A linear response was observed in the range of 10- 50µg/ml with a regression coefficient of 0.999. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines. This method can be used for the determination of Atazanavir in quality control of formulation without interference of the excipients. Atazanavir sulphate was subjected to stress degradation under different conditions recommended by ICH. The samples so generated were used for degradation studies using the developed method.

S.G. Khanage, V.K. Deshmukh, P.B. Mohite, V.M. Dhamak et al., reported as A simple, precise and

economical spectrophotometric method has been developed for the estimation of Atazanavir sulfate in bulk and pharmaceutical formulations. The quantitative determination of the drug was carried out using the first order derivative method. Atazanavir sulfate shows a sharp peak at 254.0 nm in first order derivative spectrum with $n = 1$. The drug follows Beer-Lambert's law in the concentration range of 10-50 $\mu\text{g mL}^{-1}$ with correlation coefficient of 0.9986. Results of the analysis were validated statistically and found to be satisfactory. The method was validated as per ICH guideline.

Sathish Kumar Konidala et al., reported as A validated RP HPLC method for the estimation of Atazanavir sulphate in capsule dosage form on Agilent TC C18 (2) 250 x 4.6 mm, 5 μ column using mobile phase composition of water: acetonitrile (20:80 v/v) pH adjusted to 3. 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 $\mu\text{g/ml}$. 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.

Dnyaneshwar sukhadev pawar et al., reported as The assay involved an isocratic elution of these two component on Hi-Q Sil C-18 Column (250 mm \times 4.6 mm, 5 μm) using a mobile phase composition of Acetonitrile: Methanol: Phosphate Buffer (40:40:20) adjusted to pH 3.1 with orthophosphoric acid. The flow rate was 1.0 mL/min and the analytes monitored at 238 nm using photodiode array (PDA) detector. The performance of the method was validated according to the present ICH guidelines for specificity, linearity, accuracy, precision and robustness. Retention time of Atazanavir sulphate and Ritonavir were found to be 3.7 min and 7.5 min, respectively. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 30 to 180 $\mu\text{g/ml}$ of Atazanavir Sulphate and 10 to 60 $\mu\text{g/ml}$ for Ritonavir respectively. Typically the regression equation for the calibration curve was found to be $y = 12938x - 27927$ ($R^2 = 0.999$) for ATV and $y = 13050x - 2083$ ($R^2 = 0.999$) for RTV.

Charushila H. Bhirud et al., reported as A validated stability indicating RP-HPLC method for the estimation of Atazanavir sulphate in capsule dosage

form on Agilent TC C18 (2) 250 \times 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. 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 $\mu\text{g/ml}$. 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.

P. Anupama et al., reported as Reversed phase high performance liquid chromatographic method was developed and validated for estimation of Atazanavir Sulphate in tablet dosage form. A Zodiac C18, 250 \times 4.6 mm i.d., 5 μm particle 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.

K. Srinivasu et al., reported as A validated RP HPLC method for the estimation of atazanavir in capsule dosage form on YMC ODS 150 \times 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 $\mu\text{g/mL}$. 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.

REFERENCES

1. Palella, F.J. Jr. N. Engl. J. Med., 338(13), 1998,853.
2. Nolan, D. Drugs, 63(23), 2003, 2555.
3. Musial, B.L. Am. J. Health Syst. Pharm. 61, 2004, 1365.
4. Wood, R. J. Acquir. Immune Defic. Syndr., 36, 2004,684
5. Manoj Gadhvi, Anil Bhandari. Simultaneous Determination of Ritonavir and Atazanavir in Combined Tablet Dosage Form by HPTLC. *Asian J Biomedical and Pharma Sci* 2(15) 2012, 15-19.
6. Minal R. Ghante*, Manoj M. Kadam. Development And Validation Of Uv Spectrophotometric Methods For Estimation Of Atazanavir Sulphate In Bulk And Tablet Dosage Form. *Int J Pharm Pharm Sci*, 6(7)2014, 351-353
7. Sathish Kumar Konidala,Sujana. K. Development And Validation Of Uv Spectroscopic Method For Determination Of Atazanavir Sulphate In Bulk And Formulation. *Int J Pharm Pharm Sci*, 4(3)2012, 614-617.
8. Suddhasattya Dey, Y.Vikram Reddy, Thirupathi Reddy. Method Development And Validation For The Estimation Of Atazanavir In Bulk And Pharmaceutical Dosage Forms And Its Stress Degradation Studies Using Uv-Vis Spectrophotometric Method *Int Jof Pharma and Bio Sci*; 1(3)2010,1-8.
9. S.G. Khanage , V.K .Deshmukh, P.B Mohite , V.M Dhamak Development of Derivative Spectrophotometric Estimation of Atazanavir sulfate in Bulk Drug and Pharmaceutical Dosage Forms *Int.J.Pharm. & Health Sci*. 1(3), 2010,149-154.
10. Sathish Kumar Konidala. New Validated RP-HPLC method for the Determination of Atazanavir Sulphate in Bulk and Dosage form. *Der Pharma Chemica*, 2012, 4 (3):1305-1310
11. Dnyaneshwar sukhadev pawar. development and validation of rp-hplc method for the simultaneous estimation of atazanavir sulphate and ritonavir in bulk and formulations. *int j pharm pharm sci*, 5(3), 2013 905-909.
12. Charushila H. Bhirud. Stability indicating RP-HPLC method for the determination of Atazanavir sulphate in bulk and dosage form. *Drug Invention Today* 5(2), 2013, 81–86.
13. P. Anupama. development of novel and simple analytical method for the estimation of atazanavir sulphate in pharmaceutical formulation by rp-hplc *ijrpc*, 3(3), 2013,645-649.
14. K.Srinivasu. Validated RP-HPLC Method for the Determination of Atazanavir in Pharmaceutical Dosage Form *E-J Chem* 8(1), 2011, 453-456.