

## Reverse Phase High Performance Liquid Chromatographic Estimation of Atazanavir and Ritonavir in Pharmaceutical Dosage form

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**Abstract:** A new simple, rapid, specific, accurate, precise and novel reverse phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the simultaneous estimation of atazanavir and ritonavir in the combined pharmaceutical dosage form. The chromatographic separation for Atazanavir and Ritonavir were achieved with mobile phase containing mixed phosphate buffer (pH 4.0) and acetonitrile (45:55 % v/v), Symmetry C18 (4.6 x 100mm, 3.5 $\mu$ m, Make: ACE) at 5 $^{\circ}$  C and UV detection at 237 nm. The compounds were eluted in the isocratic mode at a flow rate of 0.9 ml min $^{-1}$ . The retention times of atazanavir 4.29  $\pm$  0.09 min and ritonavir at 5.018  $\pm$  0.09 min. The above method was validated in terms of linearity, accuracy, precision, LOD, LOQ etc. in accordance with ICH guidelines.

**Key word:** Atazanavir • Ritonavir • RP-HPLC • Validation

### INTRODUCTION

Atazanavir, chemically methyl N-[(1S)-1-{N'-(2S,3S)-2-hydroxy-3-[(2S)-2-[(methoxycarbonyl)amino]-3,3-dimethylbutanamido]-4-phenylbutyl]-N'-{[4-(pyridin-2-yl)phenyl]methyl}hydrazinecarbonyl}-2,2-dimethylpropyl]carbamate [1] (Fig.1), 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. Ritonavir, chemically, (1,3-thiazol-5-ylmethyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-[[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}) carbamoyl]amino} butanamido]-1,6-diphenylhexan-2-yl]carbamate [2] (Fig.2). Ritonavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in non infectious, immature viral particles.

Literature survey reveals that there are several analytical methods for the estimation of atazanavir and ritonavir individually or in combination with other drugs [3-9]. Although the combination use of atazanavir and

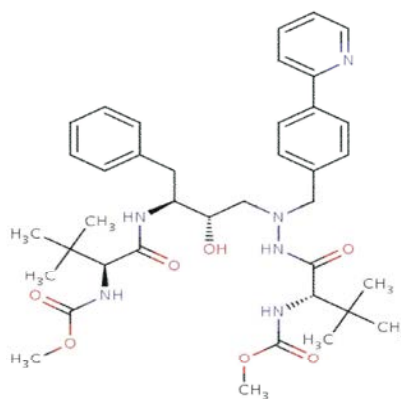


Fig. 1: Structure of Atazanavir

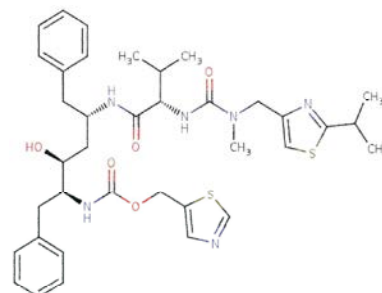


Fig. 2: Structure of Ritonavir

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ritonavir is continuously increasing, there is no simple and economical RP-HPLC method for the determination of these drugs in the combined pharmaceutical dosage form. The purpose of present study is to investigate a simple, precise, accurate and economic RP-HPLC method in simultaneous determination of atazanavir and ritonavir in the combined pharmaceutical dosage form.

## MATERIAL AND METHODS

Atazanavir and ritonavir gift samples were obtained from Sura pharma labs. Acetonitrile (HPLC grade) and dipotassium hydrogen phosphate (AR grade) were purchased from Merck Ltd, India. Water for HPLC was obtained from Qualigen fine chemicals, Mumbai, India. Analytical reagent potassium dihydrogen orthophosphate (AR grade) was obtained from Rankem Pvt. Ltd, Mumbai. Chromatographic separation was performed on Waters® HPLC system equipped with Waters 2489 UV/Visible detector and Empower software. Symmetry C18 (4.6 x 100mm, 3.5µm, Make: ACE) and constant flow pump and Auto injector with 20 µL loop were used. The composition of the mobile phase was in the ratio of mixed phosphate buffer (pH 4.0) and acetonitrile (45:55 % v/v) and was delivered at a flow rate of 0.9 ml min<sup>-1</sup>. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15 min. Analysis was performed at 5°C temperature.

**Preparation of Standard Solution:** Accurately weigh and transfer 10 mg of Atazanavir and 10mg of Ritonavir working standard into a 10ml clean dry volumetric flask add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.6ml of Atazanavir and Ritonavir the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. A typical chromatogram obtained from the analysis of drugs using the developed method was shown in Fig.3.

**Preparation of Sample Solution:** Accurately weigh and transfer 568.2 mg of Atazanavir and Ritonavir Tablet powder into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.1ml of atazanavir and ritonavir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

## RESULT AND DISCUSSION

Symmetry C18 (44.6 x 100mm, 3.5µm, Make: ACE), column maintained at ambient temperature 5° C was used for the separation and the method was validated for the estimation of atazanavir and ritonavir in tablets. The composition, pH and the flow rate of the mobile phase were optimized. A mobile phase consisting of phosphate buffer (pH 4.0): acetonitrile (45:55 % v/v) set at a flow rate of 0.9 ml min<sup>-1</sup> was selected for use of further studies after several preliminary investigatory chromatographic runs. Under the described experimental conditions, all peaks were well defined and free from tailing. The effects of small deliberate changes in the mobile phase composition and flow rate were evaluated as a part of testing for method robustness.

**Method Validation:** The proposed method was validated as per International Conference on Harmonization (ICH) guidelines.

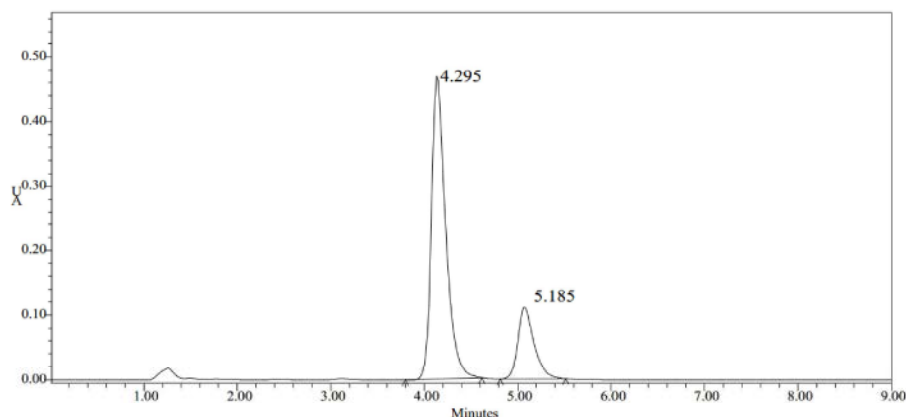


Fig. 3: A typical chromatogram of standard atazanavir and ritonavir measured at 237 nm.

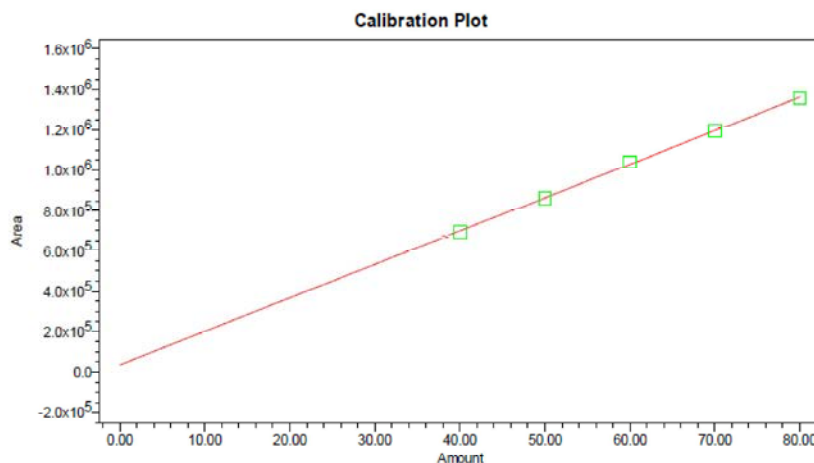


Fig. 4: Calibration curve of Atazanavir

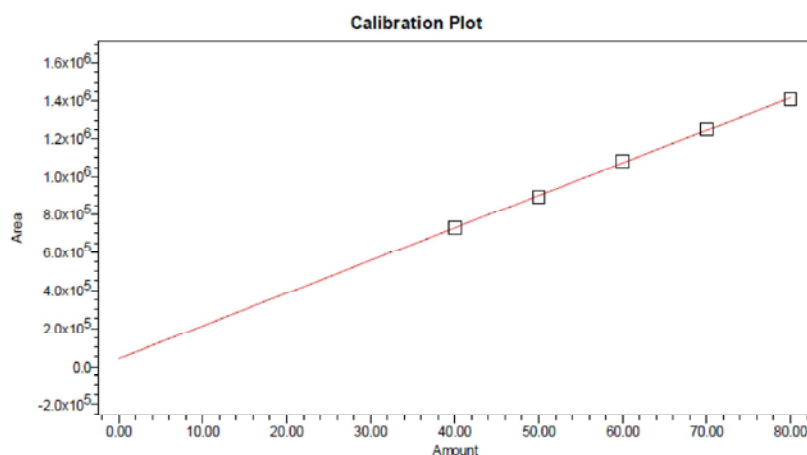


Fig. 5: Calibration curve of Ritonavir

Table 1: summary of validation parameters

Parameters (units)	Ramipril	Amlodipine besylate
Linearity range ( $\mu\text{g/ml}$ )	40-80	40-80
Correlation coefficient	0.999	0.999
LOD ( $\mu\text{g/ml}$ )	0.084	.09
LOQ ( $\mu\text{g/ml}$ )	0.288	0.114
Recovery (%)		
50	100.08	100.59
100	100.95	100.92
150	99.09	99.07
Precision (% RSD)		
Intraday (n=3)	0.17	0.40
Inter day(n=3)	0.47	0.71

**Linearity and Range:** Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 40-80  $\mu\text{g mL}^{-1}$  for atazanavir, 40-80  $\mu\text{g mL}^{-1}$  for ritonavir. Peak areas were plotted versus respective

concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficient values were found to be 0.999 and 0.999 for ramipril and Amlodipine besylate respectively, (Figs. 4, 5). The results are given in Table 1.

**Precision:** Intra-day precision was investigated by injecting five replicate samples of each of the samples on the same day. The % RSD obtained for atazanavir and ritonavir were found to be 0.17 and 0.40 respectively. Inter-day precision was assessed by injecting the same two samples over six consecutive days. The % RSD obtained for atazanavir and ritonavir were found to be 0.47 and 0.71 respectively. The results are given in Table 1.

**Accuracy:** Recovery studies were carried out by applying the method to drug sample to which known amount of standard corresponding atazanavir and ritonavir to 50, 100 and 150% of label claim had been added. At each level of

the amount six determinations were performed. The mean recoveries obtained for atazanavir and ritonavir were 100.0% and 100.2%, respectively. The results are given in Table 1.

**Specificity:** The method specificity was assessed by comparing the chromatograms obtained from the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in water without drug). The method was specific as none of the excipients interfered with the analytes of interest.

**LOD and LOQ:** LOD and LOQ of atazanavir and ritonavir were determined by calibration curve method. LOD and LOQ for atazanavir were 0.084 and 0.288  $\mu\text{g mL}^{-1}$ , for ritonavir were 0.09 and 0.114  $\mu\text{g mL}^{-1}$ . The results are given in Table 1.

**Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

**Ruggedness:** The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for atazanavir and ritonavir that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method

## CONCLUSION

The proposed RP-HPLC method is simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Moreover the shorter duration of analysis for atazanavir and ritonavir make the reported method suitable for routine quantitative analysis in pharmaceutical dosage forms.

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