# Optimization of HPLC Quantization of the Protease Inhibitor, Atazanavir

The aim of this study is to determine a reliable method for therapeutic drug monitoring of the protease inhibitor atazanavir (ATV). We compared two reverse-phase high-performance liquid chromatography methods for the quantization of ATV. The first method involved liquid-liquid extraction from plasma using .1 mol/L $NH_4OH$, methyl-tert-butyl ether, hexane, and a mobile phase of $KH_2PO_4$. In the second method we used acetonitrile to extract the drug and used the mobile phase with the same column and wavelength but extending the time to 38 minutes. The second method proved to be simple, accurate, and useful for therapeutic drug monitoring of ATV.

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## Introduction

The protease inhibitors are potent antiretroviral drugs that have been associated with improved management of HIV infection. An important objective for treatment of HIV-infected patients is to bring the plasma viral load to the lowest level, preventing disease progression. Serum monitoring of antiretroviral therapy may play a role in improving treatment of HIV patients. Atazanavir (ATV) is the most recently introduced protease inhibitor for the suppression of HIV. For this drug, only validated assays using high-performance liquid chromatography (HPLC) tandem mass spectrometry have been described. We present a method to quantify serum concentrations of ATV that is easy to perform and does not require specialized equipment.

## Materials and Methods

Atazanavir (ATV) was obtained from Bristol-Myers Squibb (New Brunswick, NJ), and the internal standard (IS) A860930 was obtained from Abbot Laboratories (North Chicago, Ill). Acetonitrile and HPLC-quality water and methanol were purchased locally.

### Standard Preparation

Stock solutions of ATV were prepared in methanol and kept at −20°C. For the preparation of standards and quality control (QC) samples, the stock solution was added to blank plasma to achieve concentrations of .05 to 10 μg/mL.

### Chromatographic Equipment and Conditions

The HPLC system consisted of a model LC-10AT VP solvent delivery pump, a model SIL-10AD VP auto sampler, a model SPD-10A VP UV Detector, and a Discovery C18 analytical column. The chromatographic separation was performed at ambient temperature. For the first method, the mobile phase components were 36% acetronitrile and 64% 50 mmol/L potassium phosphate adjusted to pH 5.75 with 50 mmol/L sodium hydroxide. We ran the samples for 25 minutes. In the second method, the mobile phase consisted of 40% acetonitrile, 55% tetramethylammonium solution, and 5% methanol. The flow rate was set at 1.0 mL/min. Atazanavir (ATV) and the IS were detected at 215 nm. The injection volume was 50 μL.

### Sample Preparation

For the first method, a 500 μL aliquot of plasma of each standard and QC was spiked with 50 μL of IS and mixed with 500 μL 0.1 mol/L $NH_4OH$ and 5 mL methyl-tert-butyl ether. After vortexing for one minute and centrifuging for five minutes, the organic layer was transferred and evaporated to dryness with nitrogen at 37°C. The residue was dissolved in 300 μL mobile phase and washed with 3 mL hexane and vortexed for five minutes. After centrifuging for five minutes, 50 μL of each standard and QC and a blank plasma with IS were injected into the chromatograph.

### Accuracy, Precision, Recovery Calibration Curves

Two replicates of three different concentrations of QC samples were analyzed in three different runs for each method determining the accuracy and precision. Average recovery of ATV was

determined by comparing responses with those obtained by direct injection of the same amount of drug in mobile phase at seven different concentrations in three different runs. To calculate the linear regression, the ratio of peak sizes was plotted against the drug concentration in micrograms per milliliter.

## RESULTS

Under the described chromatographic conditions, retention time for ATV was 10.34 minutes at the detection wavelength of 215 nm. An assay performed on drug-free human plasma spiked with internal standard did not show any interfering peak at the retention time of interest.

A four-point calibration curve in human plasma ranging from 0 to 10,000 μg/mL for ATV was analyzed for each run. The weighted peak height ratio values of the calibration standards were proportional to the concentration level.

## DISCUSSION

The HPLC conditions were adapted for ATV from previous studies for the detection of single or multiple protease inhibitors. We observed a good baseline resolution between ATV and the detected metabolites at pH 3.59. From the results we concluded that our conditions are suitable for quantization. We performed peak purity testing and library matching with the described procedure and showed that no analytic interference was encountered from extracted metabolites of ATV or from other substances. An advantage of this method is that ATV can be monitored at 215 nm of wavelength, which means that this HPLC method does not require the use of an expensive photodiode detector. This assay was developed for the purpose of therapeutic drug monitoring of ATV in HIV-infected patients.