

Determination of Protease Inhibitors Used in the Treatment of HIV

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Protease inhibitors are a class of anti-HIV drugs used in combination therapy to block replication of the HIV virus in a person's blood. It is important to monitor the levels of these drugs in the patient since resistance can develop at low levels and at high levels the drug can exhibit toxic effects.

Introduction

In 2003, nearly thirty-eight million people were estimated to be contaminated by the HIV virus, almost two thirds of these being on the African continent. Since HIV was identified in 1981, almost 20 million people have died from HIV related illnesses and it is estimated that almost 14000 people are infected every day.¹

There are several categories of drugs that are used to fight HIV infection. These include entry inhibitors, nucleoside reverse transcriptase inhibitors (NRTIs), which stop the viral DNA from being formed, and protease inhibitors (PIs) which prevent the final proteins from producing the new HIV virus.²

There are several PIs available for treatment, all of which are metabolized in the liver by the same group of enzymes. Most are used alone or with other classes of anti-HIV drugs, but a combination of ritonavir and lopinavir has shown to increase the body concentration of lopinavir. Fosamprenavir, a recent 'pro-drug', is metabolized to produce amprenavir in the body at concentrations higher than amprenavir dosed alone.³

The level of drug present in the body must be monitored. The determination of compounds at low levels is not only dependent on the sensitivity of the detection method, but also on that of the chromatographic separation itself. In such separations, we aim for narrow, symmetric analyte peaks which in turn provide us with a

strong response and the ability to detect compounds at low concentrations.

Experimental Conditions

A series of 6 protease inhibitors (courtesy of Dr. John Tjia, University of Liverpool) were screened using 5 different HPLC column chemistries including Hypersil™ GOLD, HyPURITY™ AQUASTAR, HyPURITY ADVANCE, HyPURITY Cyano and Fluophase™ PFP (Thermo Electron, Pennsylvania, USA) at pH 2 and 10 in order to help establish the optimum conditions for separation. The columns were all 150 × 4.6mm. The analyses were performed on a Finnigan™ Surveyor™ HPLC with UV photodiode array detection (Thermo Electron, California, USA).

Results and Conclusions

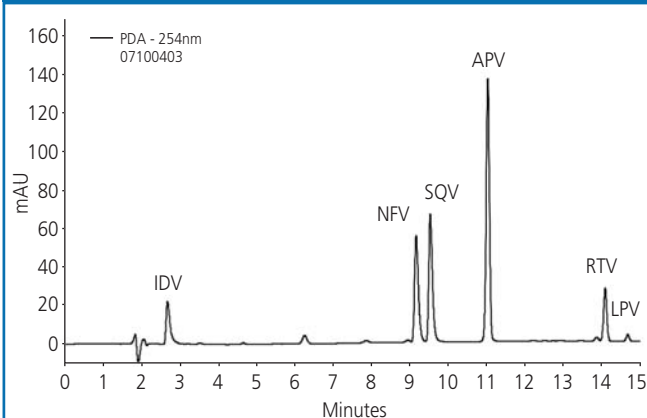
The Hypersil GOLD column used at pH 2 was found to provide the best separation and peak shape for the PIs tested; this was further optimized using a step gradient as shown in Figure 1. Hypersil GOLD columns have advanced bonding and end-capping technology which demonstrates excellent peak shape for low level analyses. Peak asymmetry was determined to be 1.00 for amprenavir with USP peak widths in the order 0.2 minutes, thereby providing excellent sensitivity for this compound. The linear range of this method for the determination of amprenavir was demonstrated between 100 and 1000 ppb (ng/mL). The sensitivity of the method could be further enhanced using a 3 µm particle size, smaller column geometries and mass spectrometric detection.

References

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2. Treatment for HIV & AIDS, Dr Howard Grossman, AIDSmeds.com. Available at <http://www.aidsmeds.com/List.htm>
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Figure 1: Chromatogram of protease inhibitors (10 ppm) using an optimized gradient method.

Column: Hypersil GOLD, 5 µm, 150 × 4.6 mm; mobile phase A: 0.1% formic acid; mobile phase B: acetonitrile; gradient: 30% B for 4 min, 30–35% B by 5 min, then 35–70% B by 15 min; flow-rate: 1.0 mL/min; detection: UV at 254 nm.



Peaks: IDV = indinavir, NfV = nelfinavir, SQV = saquinavir, APV = amprenavir, RTV = ritonavir, LPV = lopinavir.

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