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# Simultaneous determination of abacavir and zidovudine from rat tissues using HPLC with ultraviolet detection

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**Abstract**

A simple high-performance liquid chromatography (HPLC) method has been developed and validated for the simultaneous determination of abacavir and zidovudine (AZT) in rat plasma, amniotic fluid, fetal, and placental tissues. Extraction of abacavir, AZT, and the internal standard, azidouridine (AZDU) in amniotic fluid was carried out by protein precipitation. Extraction from plasma, fetal and placental homogenates was achieved by using a salting out technique. Chromatographic separation was performed using a $C_8$ column (150 mm × 4.6 mm, 5 μm). The mobile phase consisted of 12% acetonitrile in 25 mM sodium phosphate buffer (adjusted to pH 7 with sodium hydroxide) for the fetus, placenta, plasma and amniotic fluid samples at a flow rate of 0.8 mL/min. The method was validated over the range from 0.05 to 50 μg/mL for both abacavir and AZT in the four biological matrices. The absolute recovery of abacavir ranged from 79 to 94%, while AZT recoveries ranged from 79 to 90% in the different biological matrices. The internal standard recovery ranged from 90 to 92%. Acceptable intra- and inter-day assay precision (<10% R.S.D.) and accuracy (<10% error) were observed over 0.05–50 μg/mL for all four matrices.


*Keywords:* Abacavir; Zidovudine; Validation; Bioanalytical; HPLC

## 1. Introduction

In 2005, an estimated 700,000 children were newly infected with HIV worldwide and 540,000 children died from AIDS [1]. Vertical transmission of HIV may occur early or late in pregnancy, during birth or postnatally through breastfeeding. In the absence of preventative measures, about 35% of children born to HIV-positive women will contract the virus. With the advent of potent antiretroviral therapies, a great reduction in morbidity and mortality has been achieved. Combination therapy or highly active antiretroviral therapy, generally consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI), is currently the recommended standard treatment of HIV-infected non-pregnant adults in industrialized countries [2]. In resource-poor settings, the approach to antiretroviral prophylaxis of vertical transmission of HIV is dramatically different in that the goal is to obtain effective, shorter, and less expensive antiretroviral regimens [3,4].

Zidovudine (AZT) belongs to the NRTI class of antiretroviral agents and was the first anti-HIV agent to be approved by the FDA for the treatment of HIV-1 infection. In 1994, results from the PACTG protocol 076 clinical trial demonstrated that short-course AZT therapy reduced the maternal–fetal transmission of HIV-infected pregnant women from 26 to 8% [5]. As resistance to zidovudine begins to increase, emphasis has shifted to combination therapies. Combination therapies involving AZT can further reduce vertical transmission to less than 2% [6].

Abacavir is a novel nucleoside reverse transcriptase inhibitor (NRTI) used for the treatment of HIV and is a synthetic analogue of guanine. It differs structurally from other NRTIs in that it is a carbocyclic nucleoside analogue rather than a dideoxynucleoside analogue. Abacavir sulfate was approved by the FDA in 1998 for use in combination with other antiretroviral agents for the treatment of HIV-1 infection in adults and children [7]. It is also prescribed in combination with lamivudine (3TC) and AZT, and marketed as Trizivir® for use in combination with a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor.

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Abacavir is classified by the FDA as a pregnancy category C drug, meaning that animal studies have shown an adverse effect on the fetus and there are no well-controlled studies in humans, but potential benefits may warrant use of this drug in pregnant women despite potential risks [8].

While the use of combinations of antiviral drugs is becoming increasingly common, the impact of such combination therapies on placental transport is largely unknown. A series of studies have reported the lack of interaction between several anti-HIV drugs, suggesting passive diffusion as the primary mechanism of placental transfer [9,10]. However, the combination of abacavir and AZT was not studied. Recent studies of other antiviral compounds found substantial interactions between antiviral compounds in placental transport [11,12]. Continued study of these compounds is necessary to gain further understanding of the mechanism of placental transport for this important class of therapeutic agents.

Due to ethical concerns, pregnant women are excluded from clinical trials, making it difficult to study placental and fetal distribution in humans [13]. Therefore, an animal model must be utilized that will provide clinically useful information. The pregnant rat model has been proved successful for the investigation of the basic mechanisms involved in placental transfer of nucleoside analogs due to the structural similarities between rat and human placenta [14]. The large litter size allows for serial sampling, providing a complete concentration versus time profile. The pregnant rat model has been utilized in maternal–fetal drug transfer studies of a variety of compounds, including nucleoside analogs [12,15–21].

Several HPLC methods have been developed for abacavir and AZT analysis [22–25]. However, none of these methods deal with the analysis of these compounds from complex matrices such as maternal plasma, amniotic fluid, placental and fetal homogenates. Also, some of the methods use long run times and large sample volumes. This report describes the development and validation of a rapid HPLC assay for the simultaneous determination of abacavir and AZT in pregnant rat plasma, amniotic fluid, fetal and placental tissues. This method will be used in support of a comparative pharmacokinetic study to investigate the impact of abacavir and AZT combination therapy on placental transport.

## 2. Experimental

### 2.1. Chemicals and reagents

Abacavir was obtained from GlaxoSmithKline. AZT and internal standard, 3′-azido-3′-deoxythymidine (AZDU), were obtained from Sigma (St. Louis, MO). HPLC-grade acetonitrile, sodium phosphate monobasic, and ammonium sulfate were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

### 2.2. Preparation of stock and standard solutions

Stock solutions of 1.0 mg/mL abacavir, AZT, and AZDU were individually prepared in deionized water. Standard solutions of abacavir and AZT were prepared by mixing and diluting the appropriate amounts from the individual stock solutions. The final concentrations of the standard solutions were 500, 250, 50, 25, 5, 2.5 and 0.5 μg/mL. A 25 μg/mL standard solution of AZDU was prepared with deionized water from the 1.0 mg/mL stock. Precision and accuracy standards with concentrations of 400, 35, 2, and 0.5 μg/mL were also prepared in the same manner. Stock solutions were kept refrigerated when not in use and replaced on a bi-weekly basis. Fresh standard solutions were prepared for each day of analysis or validation.

### 2.3. Chromatographic system

The chromatographic analyses were performed using an HPLC system consisting of an Agilent 1100 Series components including a quaternary pump, degasser, autosampler, and variable-wavelength UV detector (Palo Alto, CA, USA). Chromatographic separations were achieved using an Agilent Eclipse XDB $C_8$ column (150 mm × 4.6 mm, 5 μm) (Palo Alto, CA, USA) with a Phenomenex Security Guard $C_{18}$ guard column (Torrance, CA, USA).

### 2.4. Chromatographic conditions

The mobile phase used for all biological matrices was 12% acetonitrile in 25 mM sodium phosphate buffer (adjusted to pH 7 with sodium hydroxide). The mobile phase flow rate was 0.8 mL/min and the detection wavelength was set at 270 nm. Under the chromatographic conditions described, abacavir, AZT and AZDU eluted at 8.3, 13.5, and 5.7 min, respectively.

### 2.5. Calibration curves

Blank plasma, amniotic fluid, placenta, and fetal tissue were collected from untreated anesthetized animals. The placental and fetal tissues were homogenized with two volumes of deionized water (w/v) using an Ultra-Turbax T8 tissue grinder (IKA Labortechnik, Germany). Plasma, placenta and fetus calibration points were prepared by spiking 100 μL of the biological matrices with 10 μL of each abacavir-AZT and AZDU standard solution. Amniotic fluid calibration points were prepared by spiking 50 μL of the biological matrix with 5 μL of each abacavir-AZT and AZDU standard solution. The calibration curves of all four matrices were in the range of 0.05–50 μg/mL, with an internal standard concentration in each sample of 2.5 μg/mL. After each matrix was spiked, it was subject to further sample preparation before analysis.

### 2.6. Precision and accuracy

This method was validated using four QC points for each calibration curve. Five replicates of each QC point were analyzed every day to determine the intra-day accuracy and precision. This process was repeated three times over 3 days in order to determine the inter-day accuracy and precision. The concentrations of the QC points for all four matrices were 0.05, 0.2, 3.5, and 40 μg/mL.