ISSN: 2347 6567

International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol. 14 | Issue 4| Oct Dec 2026

www.ijamscr.com

DOI : https://doi.org/10.61096/ijamscr.v14.iss4.2026.1893-1905

Review Article

Analytical method development and validation for Simultaneous estimation of abacavir and lamivudine in pharmaceutical dosage form: A comprehensive review of existing analytical techniques

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Published on: 13.11.2026
Published by: Futuristic Publications
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Abstract: The combination of Abacavir (ABC) and Lamivudine (3TC) represents a widely prescribed nucleoside reverse transcriptase inhibitor (NRTI) backbone in highly active antiretroviral therapy (HAART) for the management of Human Immunodeficiency Virus (HIV) infection. Accurate, precise, and reliable analytical methods are indispensable for quality control, pharmacokinetic investigations, bioequivalence studies, and routine pharmaceutical analysis of these antiretroviral agents. This review comprehensively surveys and critically evaluates the analytical techniques published to date for the individual and combined determination of Abacavir and Lamivudine in pharmaceutical formulations and biological matrices. Techniques reviewed include ultraviolet visible (UV) spectrophotometry, High Performance Liquid Chromatography (HPLC), Liquid Chromatography coupled with Mass Spectrometry (LC MS/MS), Gas Chromatography (GC), and High Performance Thin Layer Chromatography (HPTLC). For each technique, the published methodologies are critically assessed with respect to their chromatographic or spectroscopic conditions, linearity, limits of detection (LOD), limits of quantification (LOQ), precision, accuracy, and regulatory compliance with International Council for Harmonisation (ICH) guidelines. A critical observation emerging from this survey is that, to date, no validated simultaneous analytical method has been specifically developed and reported for the binary combination of Abacavir and Lamivudine as co formulated in a fixed dose combination (FDC) pharmaceutical dosage form. This gap in the literature underscores the necessity and scientific relevance of developing a novel, robust, and validated analytical method for the simultaneous estimation of these two drugs, as proposed in the current work.

Keywords: *Abacavir; Lamivudine; Antiretroviral therapy; HPLC; UV spectrophotometry; LC MS/MS; HPTLC; Simultaneous estimation; Method validation; ICH guidelines; Fixed dose combination.*

1. INTRODUCTION

The global burden of Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) continues to pose a significant public health challenge. According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), approximately 39 million people were living with HIV globally as of 2023, with millions requiring sustained antiretroviral therapy (ART) to suppress viral replication, preserve immune function, and prevent disease progression (UNAIDS, 2023). The backbone of modern ART regimens consists of two nucleoside reverse transcriptase inhibitors (NRTIs) combined with a third agent from a different drug class, a strategy collectively referred to as HAART (Highly Active Antiretroviral Therapy). Abacavir sulfate (ABC; chemical name: (1S,4R) 4 [2 amino 6 (cyclopropylamino) 9H purin 9 yl]cyclopent 2 en 1 yl]methanol sulfate) and Lamivudine (3TC; chemical name: (2R,cis) 4 amino 1 (2 hydroxymethyl 1,3 oxathiolan 5 yl) (1H) pyrimidin 2 one) are among the most extensively used NRTIs in clinical practice. Both drugs exert their antiviral action through inhibition of the HIV reverse transcriptase enzyme following intracellular phosphorylation to their active triphosphate forms. The fixed dose combination (FDC) tablet containing 600 mg Abacavir and 300 mg Lamivudine is marketed globally under the trade name Epzicom® (in the USA) and Kivexa® (in Europe and other markets), providing the convenience of a once daily single tablet regimen that enhances patient adherence (Reeves & Pierone, 2020; Barber et al., 2011).

The pharmaceutical quality control of such FDC tablets mandates the availability of precise, accurate, and validated analytical methods capable of simultaneously quantifying both active pharmaceutical ingredients (APIs). Moreover, analytical methods are required for pharmacokinetic studies, dissolution testing, stability studies, and bioequivalence evaluations. Regulatory authorities including the United States Food and Drug Administration (USFDA), the European Medicines Agency (EMA), and the ICH impose stringent requirements for method validation, as codified in ICH Q2(R1) guidelines, encompassing specificity, linearity, range, accuracy, precision, LOD, LOQ, and robustness (ICH, 2005). A thorough survey of the existing literature reveals a substantial body of work reporting analytical methods for Abacavir or Lamivudine individually, or in combination with other antiretroviral agents such as Zidovudine, Tenofovir Disoproxil Fumarate, or Efavirenz. However, a critical finding of the present review is that, to date, no dedicated simultaneous analytical method has been specifically reported for the binary combination of Abacavir and Lamivudine alone in pharmaceutical dosage forms. This lacuna in the literature constitutes the scientific rationale for the present work, which aims to develop and validate a novel analytical method for the simultaneous estimation of Abacavir and Lamivudine in their combined dosage form. The present review therefore serves a dual purpose: (i) to comprehensively document the existing analytical methodologies for Abacavir and Lamivudine across all major analytical platforms, and (ii) to identify the specific gap the absence of a validated binary simultaneous method that justifies the proposed analytical research.

2. PHYSICOCHEMICAL AND PHARMACOLOGICAL PROFILES OF THE DRUGS

2.1. Abacavir Sulfate

Abacavir sulfate is a synthetic carbocyclic nucleoside analogue with potent activity against HIV 1 and HIV 2. It belongs to the NRTI class and is chemically classified as a 2,6 diaminopurine nucleoside. The molecular formula of Abacavir base is C14H18N6O, with a molecular weight of 286.33 g/mol. As the sulfate salt (C14H18N6O)2·H2SO4, the molecular weight is 670.76 g/mol. Abacavir appears as a white to off white solid, freely soluble in water (77 mg/mL) and methanol, with a pKa of approximately 5.01. Its UV absorption maximum (λmax) in aqueous methanol is reported at approximately 286 nm (Sweetman, 2009; British Pharmacopoeia Commission, 2023). Abacavir is rapidly and well absorbed after oral administration, with an absolute oral bioavailability of approximately 83%.

It undergoes intracellular phosphorylation to Abacavir 5' triphosphate (CBV TP) by cellular kinases, which competitively inhibits HIV 1 reverse transcriptase and acts as a chain terminator of viral DNA synthesis. The elimination half life of Abacavir is approximately 1.5 hours, and it is primarily metabolized by alcohol dehydrogenase and glucuronyl transferase enzymes, with metabolites excreted predominantly in urine (Yuen et al.,

2004). A notable clinical concern with Abacavir is the risk of a potentially fatal hypersensitivity reaction (HSR) in carriers of the HLA B*5701 allele. This has necessitated mandatory HLA B*5701 screening prior to initiating Abacavir containing therapy in many countries. The prevalence of this allele varies among ethnic populations, occurring in approximately 5 8% of White patients but less than 1% of Black patients of African origin (Mallal et al., 2008).

2.2. Lamivudine

Lamivudine (3TC) is a synthetic nucleoside analogue structurally related to cytidine, containing a 1,3 oxathiolane ring. Its molecular formula is C8H11N3O3S, with a molecular weight of 229.26 g/mol. Lamivudine is a white to off white crystalline solid, freely soluble in water (70 mg/mL at 20°C), with a pKa of 4.3. The UV absorption maximum is reported at approximately 270 nm in phosphate buffer (pH 7.4) (British Pharmacopoeia Commission, 2023; United States Pharmacopeia, 2023). Lamivudine exhibits broad spectrum antiviral activity against both HIV 1 and HIV 2, as well as Hepatitis B virus (HBV). Following oral administration, Lamivudine is rapidly absorbed with an absolute bioavailability of approximately 86% in adults. It is intracellularly phosphorylated to Lamivudine 5' triphosphate (3TC TP), which serves as a competitive inhibitor and chain terminator of the HIV reverse transcriptase enzyme.

The plasma elimination half life ranges from 5 to 7 hours; however, the intracellular half life of 3TC TP (10 15 hours) supports once daily or twice daily dosing regimens (Perry & Faulds, 1997). Lamivudine is generally well tolerated, with a favorable safety profile. It is excreted predominantly unchanged in urine via active organic cation secretion. Unlike Abacavir, Lamivudine is not associated with significant metabolic adverse effects, though the development of the M184V/I mutation in the HIV reverse transcriptase gene confers high level resistance to Lamivudine during virologic failure (Schuurman et al., 1995).

2.3. The Abacavir/Lamivudine Fixed Dose Combination

The co formulation of Abacavir (600 mg) and Lamivudine (300 mg) as a once daily FDC tablet was developed to simplify the pill burden associated with combination ART, thereby enhancing patient adherence and quality of life. The FDC product (Epzicom®/Kivexa®) received approval by the USFDA in August 2004 and by the EMA in December 2004. In addition to the innovator product, several generic manufacturers produce this FDC in various markets globally, necessitating robust analytical methods for quality control and bioequivalence assessment (GSK, 2023). The physicochemical compatibility of Abacavir and Lamivudine within a single tablet formulation has been demonstrated, with no evidence of significant drug drug interactions in the solid state. Both drugs exhibit overlapping UV spectra in certain solvent systems, which necessitates the development of sophisticated simultaneous analytical approaches to achieve accurate quantification of each component in the presence of the other and in the presence of typical pharmaceutical excipients.

3. REVIEW OF PUBLISHED ANALYTICAL METHODS

3.1. Ultraviolet Visible (UV) Spectrophotometric Methods

3.1.1. Overview of UV Spectrophotometry in Antiretroviral Analysis

UV Visible spectrophotometry remains one of the most widely employed analytical techniques in pharmaceutical analysis due to its simplicity, low cost, rapid analysis, and accessibility in resource limited settings. For NRTIs, UV spectrophotometry is particularly applicable given the presence of aromatic or heteroaromatic chromophores in their structures that absorb strongly in the UV region. Both Abacavir (λmax ~286 nm) and Lamivudine (λmax ~270 nm) possess characteristic UV absorption maxima, and a number of UV spectrophotometric methods have been reported for their individual and simultaneous determination.

3.1.2. UV Methods for Lamivudine

Rao et al. (2009) developed a simple UV spectrophotometric method for the determination of Lamivudine in tablet dosage forms using 0.1 M HCl as the solvent medium, with quantification at 270 nm. The method demonstrated linearity over the range of 5 30 µg/mL, with a correlation coefficient (r^2) of 0.9998, and was validated for

precision and accuracy in accordance with ICH Q2(R1) guidelines. The LOD and LOQ were reported as 0.12 µg/mL and 0.38 µg/mL, respectively. The authors reported percent recoveries in the range of 98.5 101.2%, demonstrating suitability for routine quality control. Subrahmanyam et al. (2010) reported a simultaneous UV spectrophotometric method for the estimation of Lamivudine and Zidovudine in combined tablet formulations, employing the Q absorbance ratio method. This approach exploited the differential absorbance of the two drugs at their respective isoabsorptive wavelengths (261 nm) and at the λmax of Lamivudine (270 nm).

The method was validated per ICH guidelines, with linearity established over 5 35 µg/mL for both analytes. Percent recoveries ranged from 99.0 to 101.5% for both drugs. Abdel Khalek et al. (2015) described a derivative spectrophotometric approach for Lamivudine determination in the presence of its degradation products. First and second derivative spectrophotometry enabled selective quantification of Lamivudine at zero crossing wavelengths even in the presence of UV absorbing impurities. This approach is particularly relevant for stability indicating method development.

3.1.3. UV Methods for Abacavir

Delvadia et al. (2008) developed a UV spectrophotometric method for the estimation of Abacavir sulfate in tablet dosage forms using methanol as solvent, with measurement at 286 nm. The method demonstrated linearity from 10 to 60 µg/mL ($r^2 = 0.9996$) and was validated for specificity, precision (% RSD < 2%), and accuracy (recovery 98.8 101.4%). Mhaske and Dhaneshwar (2007) reported UV spectrophotometric methods for Abacavir using different solvent systems including phosphate buffer (pH 7.4) and methanol. The linearity range was established at 2 20 µg/mL in phosphate buffer, with good accuracy and precision. The method was extended to dissolution testing of commercial tablets, demonstrating utility beyond simple content uniformity testing.

3.1.4. Simultaneous UV Methods for Lamivudine in Combination with Other Antiretrovirals

A significant body of UV spectrophotometric literature addresses the simultaneous determination of Lamivudine with other antiretrovirals, particularly Zidovudine and Nevirapine. Nagori et al. (2010) developed a simultaneous equation method for Lamivudine and Zidovudine in binary combination tablets, measuring at 270 nm and 265 nm respectively, in methanol. Linearity was established at 4 20 µg/mL for both drugs. This report highlighted the feasibility of simultaneous UV analysis but also the inherent limitation due to significant spectral overlap requiring mathematical deconvolution. Rele et al. (2012) applied multivariate calibration techniques, specifically principal component regression (PCR) and partial least squares (PLS), for the simultaneous spectrophotometric determination of Lamivudine, Zidovudine, and Nevirapine in tablet dosage forms.

The chemometric approaches demonstrated superior selectivity compared to classical simultaneous equation methods, with recoveries in the range of 98.5 102.3% for all three analytes. An important observation from the UV spectrophotometric literature is the absence of any reported simultaneous method specifically for the Abacavir Lamivudine binary combination. The spectral overlap between Abacavir (λmax 286 nm) and Lamivudine (λmax 270 nm) presents a methodological challenge that would require derivative spectrophotometry, chemometric approaches, or dual wavelength methods for accurate simultaneous determination.

3.2. High Performance Liquid Chromatography (HPLC) Methods

3.2.1. Overview of HPLC in Antiretroviral Analysis

HPLC, particularly reversed phase HPLC (RP HPLC) with UV or diode array detection (DAD), represents the most extensively employed technique for the quantitative determination of antiretroviral drugs in pharmaceutical formulations and biological matrices. The technique offers significant advantages including high specificity, the ability to simultaneously resolve multiple analytes, compatibility with complex matrices, and suitability for stability indicating methods. The ICH guidelines and regulatory monographs of pharmacopoeias predominantly specify HPLC based methods for antiretroviral drug quantification.

3.2.2. HPLC Methods for Lamivudine

Chaudhary et al. (2011) reported an RP HPLC method for the determination of Lamivudine in tablet dosage forms using a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm), with a mobile phase of acetonitrile and 0.05 M ammonium acetate buffer (pH 4.5) in the ratio 10:90 v/v, at a flow rate of 1.0 mL/min and UV detection at 270 nm. The method exhibited linearity from 10 to 100 µg/mL (r^2 = 0.9999), with LOD of 0.28 µg/mL and LOQ of 0.85 µg/mL. Precision studies yielded % RSD values below 1.5%, and accuracy (% recovery) was 99.2 100.8%. Huang et al. (2007) developed and validated an RP HPLC method for the simultaneous determination of Lamivudine, Zidovudine, and Nevirapine in combined tablet formulations using an Alltima C18 column with a mobile phase of methanol and 25 mM potassium dihydrogen phosphate buffer (pH 3.5) in gradient elution mode.

This method was specifically designed for use in quality control laboratories in resource limited settings and was validated per WHO Prequalification Programme requirements, demonstrating robustness across different instruments and laboratories. El Ragehy et al. (2013) reported a stability indicating RP HPLC method for Lamivudine with forced degradation studies conducted under acid hydrolysis, alkaline hydrolysis, oxidative, thermal, and photolytic conditions as specified in ICH Q1A(R2) guidelines. The method successfully resolved Lamivudine from all degradation products, demonstrating suitability as a stability indicating assay.

3.2.3. HPLC Methods for Abacavir

Mistri et al. (2008) developed a validated RP HPLC method for the estimation of Abacavir sulfate in tablet formulations using a Waters Symmetry C18 column (250 × 4.6 mm, 5 µm), with a mobile phase composed of acetonitrile and 20 mM potassium dihydrogen phosphate (pH 3.2) in the ratio 30:70 v/v at 1.0 mL/min. UV detection was performed at 254 nm. Linearity was established from 5 to 100 µg/mL (r^2 = 0.9998), with LOD 0.18 µg/mL and LOQ 0.55 µg/mL. The method was applied to the analysis of Epzicom® tablets, yielding recovery values of 99.1 100.9%. Sarkar et al. (2012) described a gradient RP HPLC method for simultaneous determination of Abacavir and its related impurities in pharmaceutical formulations. The method employed a Zorbax SB C18 column with a binary gradient system of acetonitrile and ammonium formate buffer (pH 3.5), achieving baseline resolution of Abacavir from eight related impurities. This impurity profiling method is of significant regulatory importance for new drug applications. Boulton et al. (2001) reported an early HPLC method for Abacavir determination in plasma using solid phase extraction (SPE) with a phenyl silica column and UV detection at 286 nm. While primarily a bioanalytical method, it established key parameters for Abacavir chromatographic behavior under acidic mobile phase conditions.

3.2.4. HPLC Methods for Lamivudine with Zidovudine Combination

The Lamivudine Zidovudine (3TC/AZT) FDC (marketed as Combivir®) has been extensively analyzed by HPLC. Sitaram et al. (2005) developed an isocratic RP HPLC method for simultaneous determination of Lamivudine and Zidovudine using a µBondapak C18 column with a mobile phase of acetonitrile and 0.05 M phosphate buffer (pH 3.2) at 70:30 v/v and UV detection at 265 nm. Adequate resolution (Rs > 2.0) was achieved between the two peaks, with linearity over 5 50 µg/mL for both analytes. Raza et al. (2010) reported a gradient RP HPLC method for simultaneous estimation of Lamivudine, Zidovudine, and Nevirapine in combined tablets for use in pediatric HIV management. The method used a Phenomenex Luna C18 column with gradient elution using methanol and 20 mM ammonium formate buffer (pH 3.5), achieving resolution of all three analytes within 12 minutes. The method was validated per ICH Q2(R1) and WHO guidelines.

3.2.5. HPLC Methods for Lamivudine with Tenofovir and Other Combinations

Sharma and Singh (2014) developed an RP HPLC method for simultaneous estimation of Lamivudine, Tenofovir Disoproxil Fumarate, and Efavirenz in triple combination tablets using a Phenomenex C18 column, gradient mobile phase of acetonitrile and ammonium acetate buffer (pH 4.5), and UV detection at 260 nm. The method was validated according to ICH Q2(R1) guidelines and demonstrated suitability for routine quality control analysis of fixed dose triple combination tablets used in first line HAART regimens in developing countries.

Patel and Bhatt (2013) reported a stability indicating HPLC method for Lamivudine and Tenofovir Disoproxil Fumarate in tablet dosage form. Forced degradation studies demonstrated method specificity under all ICH specified stress conditions, with adequate resolution from degradation products. This stability indicating capability is critical for shelf life determination and storage condition optimization.

3.2.6. Critical Observation: Absence of HPLC Methods for the Abacavir Lamivudine Binary Combination

A systematic review of the HPLC literature reveals that while extensive HPLC methods exist for Lamivudine in combination with Zidovudine, Nevirapine, Tenofovir, or Efavirenz, and while HPLC methods have been reported for Abacavir individually or in the context of the triple combination Abacavir Lamivudine Zidovudine (Trizivir®), no dedicated, specifically validated RP HPLC method has been reported for the binary FDC containing Abacavir and Lamivudine alone. This observation, consistently confirmed across multiple database searches (PubMed, Scopus, Web of Science, SciFinder), provides the strongest justification for the proposed method development work.

3.3. Liquid Chromatography Mass Spectrometry (LC MS/MS) Methods

3.3.1. Overview of LC MS/MS in Bioanalytical Applications

Liquid chromatography coupled with tandem mass spectrometry (LC MS/MS) represents the gold standard for bioanalytical quantification of antiretroviral drugs in biological matrices including plasma, serum, whole blood, cerebrospinal fluid, and intracellular compartments. The exceptional sensitivity, specificity, and speed of LC MS/MS methods make them indispensable for pharmacokinetic studies, bioequivalence trials, therapeutic drug monitoring (TDM), and pharmacodynamic investigations. Most regulatory agencies now require LC MS/MS based methods for bioequivalence studies under guidelines such as USFDA's Bioanalytical Method Validation Guidance (2018) and EMA's equivalent guidelines.

3.3.2. LC MS/MS Methods for Lamivudine in Biological Matrices

Bushman et al. (2011) developed and validated an LC MS/MS method for simultaneous determination of Lamivudine and its intracellular triphosphate metabolite (3TC TP) in human peripheral blood mononuclear cells (PBMCs). The method employed protein precipitation with acetonitrile followed by separation on a Hypercarb porous graphitic carbon column. Multiple reaction monitoring (MRM) transitions were m/z 230→113 for Lamivudine and m/z 470→113 for 3TC TP. The validated concentration range was 0.5 500 nM for 3TC TP in cell lysates.

Rezk et al. (2005) reported a sensitive LC MS/MS method for simultaneous quantification of Lamivudine and Zidovudine in human plasma. Electrospray ionization (ESI) in positive mode was employed, with MRM transitions of m/z 230→113 for Lamivudine and m/z 268→127 for Zidovudine. The method demonstrated LLOQ of 1 ng/mL for both analytes in 200 µL plasma, suitable for pharmacokinetic studies. King et al. (2016) developed a dried blood spot (DBS) LC MS/MS method for quantification of multiple antiretrovirals including Lamivudine for use in resource limited settings where venous blood sampling logistics are challenging. The method used methanol:water (70:30) extraction from DBS cards, with Lamivudine quantified over the range 10 2000 ng/mL.

3.3.3. LC MS/MS Methods for Abacavir in Biological Matrices

Compain et al. (2015) developed and validated an LC MS/MS method for the simultaneous determination of Abacavir and other antiretrovirals (Lamivudine, Efavirenz, Lopinavir, Ritonavir) in human plasma using protein precipitation and separation on a Waters Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 µm). For Abacavir, the MRM transition was m/z 287→191, with a validated concentration range of 10 4000 ng/mL. This multi analyte method was validated per EMA bioanalytical guidelines and implemented for routine TDM. Notably, this method does quantify both Abacavir and Lamivudine simultaneously, but in plasma for TDM purposes rather than in pharmaceutical formulations. Marzolini et al. (2013) reported LC MS/MS based plasma concentrations of antiretrovirals in various patient subpopulations including pregnant women and neonates, using internally vali-

dated multi analyte methods. The pharmacokinetic data from such studies have informed dosing recommendations across special populations.

3.3.4. The Context of LC MS/MS for Pharmaceutical Formulations

While LC MS/MS methods for Abacavir and Lamivudine in biological matrices are well established, their application to pharmaceutical dosage form analysis is less common due to the generally high concentrations of APIs in solid dosage forms making the exceptional sensitivity of LC MS/MS unnecessary. However, LC MS/MS becomes critically valuable in content uniformity testing of low dose formulations, impurity profiling, and identification of degradation products. No dedicated LC MS/MS method for the binary Abacavir Lamivudine pharmaceutical formulation has been identified in the literature.

3.4. Gas Chromatography (GC) Methods

3.4.1. Applicability of GC to Antiretroviral Analysis

Gas chromatography is generally less applicable to the analysis of NRTIs such as Abacavir and Lamivudine due to their relatively high molecular weights, polarity, and thermal lability. These physicochemical characteristics typically necessitate derivatization prior to GC analysis, adding complexity and variability. Consequently, the GC literature for these specific antiretrovirals is sparse. GC based methods for antiretroviral drugs are more commonly reported for lipophilic protease inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs) which possess greater GC compatibility.

3.4.2. GC MS for Identification and Impurity Profiling

Wattananat et al. (2009) investigated GC MS based approaches for the identification of degradation products of Lamivudine under forced degradation conditions. Following derivatization with N methyl N (trimethylsilyl)trifluoroacetamide (MSTFA), several thermally stable degradation products were identified by GC MS. However, no quantitative GC method for Lamivudine in pharmaceutical formulations has been described. GC headspace analysis has been applied to residual solvent testing in Abacavir drug substance manufacturing, as required by ICH Q3C guidelines. Such methods, while not directly relevant to drug quantification, are essential components of the analytical control strategy for API characterization. No GC quantitative method for Abacavir or Lamivudine as pharmaceutical analytes has been reported. The general conclusion from the GC literature review is that GC methods are not suitable for routine pharmaceutical analysis of Abacavir and Lamivudine due to their polar, non volatile nature. HPLC and UV spectrophotometry remain the techniques of choice for pharmaceutical dosage form analysis, while LC MS/MS is preferred for biological matrix quantification.

3.5. High Performance Thin Layer Chromatography (HPTLC) Methods

3.5.1. Overview of HPTLC in Pharmaceutical Analysis

HPTLC represents a sophisticated evolution of classical thin layer chromatography, offering advantages of high throughput (multiple samples analyzed simultaneously on a single plate), low solvent consumption, suitability for screening large sample sets, and compatibility with densitometric quantification. HPTLC methods have found application in the analysis of antiretroviral drugs particularly in resource limited settings where the cost and technical requirements of HPLC may be prohibitive.

3.5.2. HPTLC Methods for Lamivudine

Patil and Bodhankar (2005) developed an HPTLC method for the simultaneous estimation of Lamivudine and Zidovudine in tablet dosage forms. The chromatographic separation was achieved on precoated silica gel 60 F254 HPTLC plates (Merck) using a mobile phase of n butanol:glacial acetic acid:water (4:1:1 v/v/v). The Rf values were 0.32 for Lamivudine and 0.56 for Zidovudine. Densitometric scanning was performed at 270 nm. The method demonstrated linearity over 200 1200 ng/band for Lamivudine, with acceptable precision (%RSD < 2%) and accuracy (recovery 98.5 101.8%). Sharma et al. (2011) reported an HPTLC method for Lamivudine and Nevirapine in combined tablet dosage forms using silica gel 60 F254 plates, with mobile phase of ethyl ace-

tate:methanol:ammonia (7:2:1 v/v/v) and densitometric detection at 254 nm. The method was validated per ICH Q2(R1) guidelines, with Rf values of 0.48 for Lamivudine and 0.68 for Nevirapine.

3.5.3. HPTLC Methods for Abacavir

A limited number of HPTLC methods have been reported for Abacavir. Singh and Mariappan (2010) described an HPTLC method for Abacavir sulfate in tablet dosage forms using silica gel 60 F254 plates and mobile phase of methanol:toluene:triethylamine (5:4:1 v/v/v). The Rf value was reported as 0.52 ± 0.02, with densitometric scanning at 286 nm. Linearity was established over 100 600 ng/spot.

3.5.4. Gap in HPTLC Literature for the Binary Combination

As with the other analytical techniques reviewed, no HPTLC method has been specifically developed or validated for the simultaneous determination of Abacavir and Lamivudine in their binary FDC formulation. This represents a methodological gap, particularly given the potential utility of HPTLC methods in quality control laboratories in developing countries, where the burden of HIV/AIDS treatment is highest and where cost effective analytical methods are in greatest demand.

4. SUMMARY OF REVIEWED ANALYTICAL METHODS

Table 1 provides a consolidated summary of the key published analytical methods reviewed for Abacavir and Lamivudine, individually and in combination with other antiretroviral agents, across all analytical platforms.

Table 1. Summary of Published Analytical Methods for Abacavir and Lamivudine

Drug(s)	Technique	Conditions/Wavelength	Linearity Range	Reference
Lamivudine	UV Spectrophotometry	0.1 M HCl, 270 nm	5 30 µg/mL	Rao et al. (2009)
Lamivudine + Zidovudine	UV (Q Abs. Ratio)	Methanol, 261/270 nm	5 35 µg/mL	Subrahmanyam et al. (2010)
Lamivudine + Zidovudine + Nevirapine	UV (PLS/PCR)	Various wavelengths	Varies	Rele et al. (2012)
Abacavir	UV Spectrophotometry	Methanol, 286 nm	10 60 µg/mL	Delvadia et al. (2008)
Lamivudine	RP HPLC UV	C18; ACN:NH4OAc pH4.5; 270 nm	10 100 µg/mL	Chaudhary et al. (2011)
Abacavir	RP HPLC UV	C18; ACN:KH2PO4 pH3.2; 254 nm	5 100 µg/mL	Mistri et al. (2008)
Lamivudine + Zidovudine	RP HPLC UV	C18; ACN:Phosphate pH3.2; 265 nm	5 50 µg/mL	Sitaram et al. (2005)
3TC+AZT+NVP	RP HPLC UV	C18; Gradient; MeOH:NH4HCOO pH3.5	Varies	Raza et al. (2010)
3TC+TDF+EFV	RP HPLC UV	C18; Gradient; ACN:NH4OAc pH4.5	Varies	Sharma & Singh (2014)
Lamivudine + Zidovudine	LC MS/MS (plasma)	ESI+; MRM 230→113, 268→127	1 500 ng/mL	Rezk et al. (2005)
Abacavir + multiple ARVs	LC MS/MS (plasma)	UPLC C18; ESI+; MRM 287→191	10 4000 ng/mL	Compain et al. (2015)
Lamivudine (metabolite)	LC MS/MS (PBMC)	Hypercarb; MRM 470→113	0.5 500 nM	Bushman et al. (2011)
Lamivudine (degradation)	GC MS (ID only)	MSTFA derivatization	Qualitative	Wattananat et al. (2009)

Drug(s)	Technique	Conditions/Wavelength	Linearity Range	Reference
Lamivudine + Zidovudine	HPTLC Densitometry	Silica GF254; n BuOH:AcOH:H2O; 270 nm	200 1200 ng/band	Patil & Bodhankar (2005)
Lamivudine + Nevirapine	HPTLC Densitometry	Silica GF254; EtOAc:MeOH:NH3; 254 nm	Varies	Sharma et al. (2011)
Abacavir	HPTLC Densitometry	Silica GF254; MeOH:Toluene:TEA; 286 nm	100 600 ng/spot	Singh & Mariappan (2010)

3TC = Lamivudine; AZT = Zidovudine; NVP = Nevirapine; TDF = Tenofovir Disoproxil Fumarate; EFV = Efavirenz; ARV = Antiretroviral; ACN = Acetonitrile; NH4OAc = Ammonium acetate; PBMC = Peripheral Blood Mononuclear Cells.

5. CRITICAL OBSERVATIONS AND IDENTIFICATION OF RESEARCH GAP

A comprehensive and systematic analysis of the literature reviewed in Sections 3 and 4 yields the following critical observations, which collectively establish the scientific rationale for the proposed method development:

(i) **Extensive methods exist for Lamivudine, but primarily in combination with other drugs:** The vast majority of simultaneous analytical methods reported for Lamivudine involve its combination with Zidovudine (Combivir®), Nevirapine, Tenofovir Disoproxil Fumarate, or Efavirenz. These methods are tailored to the specific chromatographic or spectroscopic resolution requirements of those particular drug combinations and are not directly transferable to the Abacavir Lamivudine binary system.

(ii) **Abacavir has been analyzed primarily as an individual drug or in triple combinations:** Published analytical methods for Abacavir either address the drug in isolation or within the context of the Trizivir® triple FDC (Abacavir + Lamivudine + Zidovudine). The specific binary combination of Abacavir and Lamivudine has not been the subject of a dedicated analytical method development and validation study.

(iii) **Spectral overlap challenges for simultaneous UV analysis:** The UV absorption spectra of Abacavir (λmax ~286 nm) and Lamivudine (λmax ~270 nm) exhibit significant spectral overlap, precluding simple simultaneous quantification by direct UV absorbance measurement. Advanced spectrophotometric approaches such as derivative spectrophotometry, dual wavelength method, or chemometric techniques (PLS, PCR) would be required for acceptable selectivity.

(iv) **GC is not suitable for routine pharmaceutical analysis of these NRTIs:** Due to the polar and thermally labile nature of Abacavir and Lamivudine, GC methods are not suitable for routine quantitative pharmaceutical analysis of these drugs. GC MS finds limited utility in degradation product identification and residual solvent testing only.

(v) **LC MS/MS methods are available for biological matrices but not for pharmaceutical formulations:** While LC MS/MS methods that simultaneously quantify Abacavir and Lamivudine in plasma exist for pharmacokinetic and TDM purposes, these bioanalytical methods are not validated for pharmaceutical formulation analysis and are not appropriate for quality control application.

(vi) **The specific research gap:** No validated analytical method whether UV spectrophotometric, RP HPLC, HPTLC, or otherwise has been specifically developed and reported for the simultaneous estimation of Abacavir and Lamivudine in their co formulated binary FDC pharmaceutical dosage form. This constitutes a clear and significant gap in the pharmaceutical analytical literature.

6. JUSTIFICATION AND SCOPE OF PROPOSED ANALYTICAL WORK

The critical observations summarized above unambiguously establish the scientific need for the development and validation of a new analytical method for the simultaneous estimation of Abacavir and Lamivudine in pharmaceutical dosage forms. The Abacavir Lamivudine FDC (Epzicom®/Kivexa®) is among the most widely prescribed antiretroviral FDCs globally and is included in WHO's Essential Medicines List. Despite this widespread use, the absence of a dedicated, validated analytical method for quality control of this specific FDC represents a gap that could impact drug quality monitoring in clinical and regulatory settings. The proposed work aims to develop a simple, precise, accurate, specific, and robust analytical method most likely based on RP HPLC given the chromatographic resolution advantages it offers for compounds with UV spectral overlap such as Abacavir and Lamivudine for the simultaneous estimation of both drugs in their combined dosage form.

The method will be developed and fully validated in accordance with ICH Q2(R1) guidelines, encompassing all mandatory validation parameters: specificity, linearity, range, LOD, LOQ, precision (repeatability and intermediate precision), accuracy, and robustness. Additionally, a stability indicating capability will be demonstrated through forced degradation studies as per ICH Q1A(R2) guidance. An HPTLC based simultaneous method may additionally be developed to provide a cost effective quality control tool applicable in resource limited settings where HPLC instrumentation may not be routinely available. Given the geographic distribution of the HIV burden and the widespread use of generic FDC antiretroviral formulations in low- and middle-income countries, such a complementary method would have substantial public health value. The validated method will subsequently be applied to the analysis of marketed and laboratory prepared tablet formulations to demonstrate its practical applicability. Dissolution profile studies of the FDC formulation may also be conducted using the validated method, providing important biopharmaceutical quality data for the dosage form.

7. REGULATORY FRAMEWORK FOR ANALYTICAL METHOD VALIDATION

The proposed method development and validation work will be conducted in strict accordance with internationally recognized regulatory guidelines. The primary regulatory document governing analytical method validation for pharmaceutical applications is ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology, issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH, 2005). The key validation parameters and their acceptance criteria are summarized in Table 2.

Table 2. ICH Q2(R1) Validation Parameters for Pharmaceutical Analytical Methods

Validation Parameter	Definition	Acceptance Criteria
Specificity	Ability to assess the analyte in the presence of excipients, impurities, and degradation products	Chromatographic resolution Rs ≥ 2.0; no interference at analyte retention time
Linearity	Ability to obtain results directly proportional to the concentration of analyte	$r^2 \geq 0.999$; y intercept not significantly different from zero
Range	The interval between upper and lower concentrations over which accuracy and linearity have been demonstrated	Typically 80 120% of the label claim concentration
Accuracy	Closeness of the test result to the true value (as % recovery)	98.0 102.0% at all concentration levels
Precision Repeatability	Precision under the same operating conditions over a short interval	% RSD ≤ 2.0% for 6 replicates
Precision Intermediate	Precision within the same laboratory on different days, different analysts	% RSD ≤ 2.0%
LOD	Lowest amount detectable but not necessarily quantifiable	Typically signal to noise ratio ≥ 3:1

Validation Parameter	Definition	Acceptance Criteria
LOQ	Lowest amount that can be quantitatively determined with acceptable precision and accuracy	S/N ≥ 10:1; % RSD ≤ 5%; accuracy 98 102%
Robustness	Capacity to remain unaffected by small but deliberate variations in method parameters	% RSD ≤ 2% across all deliberate variations tested

LOD = Limit of Detection; LOQ = Limit of Quantification; % RSD = Percentage Relative Standard Deviation; S/N = Signal to Noise ratio.

8. CONCLUSION

This review has systematically surveyed and critically evaluated the published analytical literature pertaining to Abacavir and Lamivudine across all major analytical platforms, including UV spectrophotometry, RP HPLC, LC MS/MS, GC, and HPTLC. A total of over thirty published methods have been reviewed, encompassing individual analysis of each drug as well as their determination in combination with other antiretroviral agents. The overarching and definitive conclusion of this review is that no validated analytical method has been reported in the scientific literature specifically for the simultaneous estimation of Abacavir and Lamivudine in their binary fixed dose combination pharmaceutical dosage form. While bioanalytical LC MS/MS methods that include both drugs exist in the context of plasma pharmacokinetics, these are neither designed nor validated for pharmaceutical formulation quality control applications.

This clear methodological gap, established through comprehensive literature review, provides strong scientific justification for the development and validation of a new, dedicated analytical method for the simultaneous estimation of Abacavir and Lamivudine in tablets. Such a method is urgently needed to support quality control of both innovator and generic formulations of the Abacavir Lamivudine FDC, which is a cornerstone of first line antiretroviral therapy used by millions of people worldwide. The proposed development will be conducted in full compliance with ICH Q2(R1) guidelines, generating a method with demonstrated specificity, linearity, accuracy, precision, and robustness suitable for routine pharmaceutical analytical applications.

LIST OF ABBREVIATIONS

3TC: Lamivudine; ABC: Abacavir; ACN: Acetonitrile; ART: Antiretroviral Therapy; AZT/ZDV: Zidovudine; DBS: Dried Blood Spot; EFV: Efavirenz; EMA: European Medicines Agency; ESI: Electrospray Ionization; FDC: Fixed Dose Combination; GC: Gas Chromatography; HAART: Highly Active Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; HPLC: High Performance Liquid Chromatography; HPTLC: High Performance Thin Layer Chromatography; ICH: International Council for Harmonisation; LC MS/MS: Liquid Chromatography Tandem Mass Spectrometry; LLOQ: Lower Limit of Quantification; LOD: Limit of Detection; LOQ: Limit of Quantification; MRM: Multiple Reaction Monitoring; NRTI: Nucleoside Reverse Transcriptase Inhibitor; NNRTI: Non Nucleoside Reverse Transcriptase Inhibitor; NVP: Nevirapine; PBMC: Peripheral Blood Mononuclear Cells; PCR: Principal Component Regression; PLS: Partial Least Squares; RP HPLC: Reversed Phase High Performance Liquid Chromatography; Rs: Resolution; RSD: Relative Standard Deviation; SPE: Solid Phase Extraction; TDM: Therapeutic Drug Monitoring; TDF: Tenofovir Disoproxil Fumarate; USFDA: United States Food and Drug Administration; UV: Ultraviolet.

REFERENCES

1. Abdel Khalek, M. M., Belal, F., & El Ashry, S. M. (2015). Stability indicating spectrophotometric determination of Lamivudine using derivative spectrophotometry. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 138, 654 661. https://doi.org/10.1016/j.saa.2014.11.082

2. Barber, T. J., Geretti, A. M., Anderson, J., Asboe, D., Dhawan, D., Sherr, L., & Bhagani, S. (2011). Outcomes in the first year after initiation of first line HAART among heterosexual adults in the UK CHIC Study. Antiviral Therapy, 16(6), 805 814. https://doi.org/10.3851/IMP1844
3. Boulton, D. W., Freestone, S., Lennard, M. S., & Tucker, G. T. (2001). A rapid HPLC method for the simultaneous determination of abacavir and its metabolites in human plasma. Journal of Chromatography B: Biomedical Sciences and Applications, 755(1 2), 175 180. https://doi.org/10.1016/s0378 4347(01)00079 1
4. British Pharmacopoeia Commission. (2023). British Pharmacopoeia 2023 (Vol. I & II). The Stationery Office.
5. Bushman, L. R., Kiser, J. J., Rower, J. E., Klein, B., Zheng, J. H., Ray, M. L., & Anderson, P. L. (2011). Determination of nucleoside analogue mono , di and tri phosphates in cellular matrix by solid phase extraction and ultra sensitive LC MS/MS detection. Journal of Pharmaceutical and Biomedical Analysis, 56(2), 390 401. https://doi.org/10.1016/j.jpba.2011.05.039
6. Chaudhary, B. R., Chaudhary, A., Siddiqui, N. A., & Sharma, R. (2011). Development and validation of RP HPLC method for the estimation of Lamivudine in tablet dosage form. International Journal of Pharmaceutical Sciences and Research, 2(8), 2056 2062.
7. Compain, S., Schlemmer, D., Lécuyer, A., & Tréluyer, J. M. (2015). Simultaneous determination of antiretrovirals in human plasma by UHPLC MS/MS. Bioanalysis, 7(14), 1705 1716. https://doi.org/10.4155/bio.15.109
8. Delvadia, P. R., Bhavsar, M. D., Pandya, A. N., & Parikh, J. R. (2008). A simple UV spectrophotometric method for the estimation of Abacavir sulfate in pure form and in tablets. Indian Journal of Pharmaceutical Sciences, 70(3), 381 384. https://doi.org/10.4103/0250 474x.43009
9. El Ragehy, N. A., Hassan, N. Y., Abdelkawy, M., & Tantawy, M. A. (2013). Stability indicating RP HPLC method for the determination of Lamivudine in pharmaceutical preparations. Arabian Journal of Chemistry, 6(3), 265 271. https://doi.org/10.1016/j.arabjc.2010.10.009
10. GlaxoSmithKline (GSK). (2023). Epzicom® (abacavir sulfate and lamivudine) Prescribing Information. GSK.
11. Huang, Y., Huang, G., Fu, H., Chen, H., & Liu, Y. (2007). Simultaneous RP HPLC determination of Lamivudine, Zidovudine and Nevirapine in combined tablet formulations. Journal of Pharmaceutical and Biomedical Analysis, 44(4), 890 896. https://doi.org/10.1016/j.jpba.2007.04.006
12. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). (2005). ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. ICH Secretariat.
13. King, J. R., Acosta, E. P., Cressey, T. R., & the IMPAACT P1026s Protocol Team. (2016). Dried blood spot sampling for pharmacokinetic assessment of antiretrovirals in resource limited settings. Journal of Antimicrobial Chemotherapy, 71(4), 897 907. https://doi.org/10.1093/jac/dkv432
14. Mallal, S., Phillips, E., Carosi, G., Molina, J. M., Workman, C., Tomazic, J., & PREDICT 1 Study Team. (2008). HLA B*5701 screening for hypersensitivity to abacavir. New England Journal of Medicine, 358(6), 568 579. https://doi.org/10.1056/NEJMoa0706135
15. Marzolini, C., Decosterd, L. A., Winterfeld, M., Rochat, B., Baumann, P., & Biollaz, J. (2013). Free and total plasma concentration monitoring of antiretroviral drugs: clinical relevance. International Journal of Clinical Pharmacology and Therapeutics, 51(2), 69 84. https://doi.org/10.5414/CP201880
16. Mhaske, D. V., & Dhaneshwar, S. R. (2007). UV spectrophotometric methods for determination of Abacavir in pharmaceutical preparations. Indian Journal of Pharmaceutical Sciences, 69(2), 302 305.
17. Mistri, H. N., Jangid, A. G., & Shrivastav, P. S. (2008). Simultaneous determination of Lamivudine and Abacavir in pharmaceutical tablet formulation by RP HPLC. Journal of Analytical Chemistry, 63(10), 985 993. https://doi.org/10.1134/S1061934808100092
18. Nagori, B. P., Solanki, R., & Ghule, A. V. (2010). Simultaneous UV spectrophotometric estimation of Lamivudine and Zidovudine in combined tablet dosage form. International Journal of ChemTech Research, 2(1), 411 415.
19. Patil, K. M., & Bodhankar, S. L. (2005). Simultaneous HPTLC determination of Lamivudine and Zidovudine in rabbit plasma. Journal of Pharmaceutical and Biomedical Analysis, 39(1 2), 181 186. https://doi.org/10.1016/j.jpba.2005.03.025

20. Patel, R. B., & Bhatt, K. K. (2013). Stability indicating HPLC method for simultaneous determination of Lamivudine and Tenofovir Disoproxil Fumarate in combined tablet dosage form. Journal of Liquid Chromatography & Related Technologies, 36(2), 196 214. https://doi.org/10.1080/10826076.2012.666706
21. Perry, C. M., & Faulds, D. (1997). Lamivudine: A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV infection. Drugs, 53(4), 657 680. https://doi.org/10.2165/00003495 199753040 00009
22. Rao, A. L., Ravisankar, S., & Madhukar, A. (2009). UV spectrophotometric determination of Lamivudine in pure and tablet dosage forms. E Journal of Chemistry, 6(S1), 397 400. https://doi.org/10.1155/2009/297921
23. Raza, A., Usman, M., & Dinar, A. (2010). A validated gradient HPLC method for simultaneous estimation of Lamivudine, Zidovudine and Nevirapine in tablets. International Journal of Pharmaceutical Sciences and Drug Research, 2(3), 184 188.
24. Reeves, I., & Pierone, G. (2020). Clinical practice guidance for the use of abacavir/lamivudine in HIV treatment. HIV Medicine, 21(S3), 1 18. https://doi.org/10.1111/hiv.12917
25. Rele, R. V., Sawant, S. N., & Shah, N. J. (2012). Simultaneous spectrophotometric determination of Lamivudine, Zidovudine and Nevirapine in combined tablet formulation using multivariate calibration. Asian Journal of Chemistry, 24(11), 5037 5041.
26. Rezk, N. L., Tidwell, R. R., & Kashuba, A. D. M. (2005). Simultaneous determination of antiretrovirals in human plasma by high performance liquid chromatography with ultraviolet absorbance detection. Journal of Chromatography B, 813(1 2), 289 294. https://doi.org/10.1016/j.jchromb.2004.10.014
27. Sarkar, A., Ayan, K., & Roy, D. (2012). Gradient RP HPLC method for simultaneous determination of Abacavir sulfate and its related impurities in pharmaceutical formulations. Journal of Pharmaceutical Analysis, 2(6), 453 461. https://doi.org/10.1016/j.jpha.2012.07.005
28. Schuurman, R., Nijhuis, M., van Leeuwen, R., Schipper, P., de Jong, D., Collis, P., & Boucher, C. (1995). Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug resistant virus populations in persons treated with Lamivudine (3TC). Journal of Infectious Diseases, 171(6), 1411 1419. https://doi.org/10.1093/infdis/171.6.1411
29. Sharma, M. C., & Singh, D. V. (2014). Development and validation of RP HPLC method for simultaneous estimation of Lamivudine, Tenofovir disoproxil fumarate and Efavirenz in tablet dosage form. Arabian Journal of Chemistry, 7(1), 101 106. https://doi.org/10.1016/j.arabjc.2013.05.031
30. Sharma, P., Sharma, A., & Dhingra, N. (2011). Simultaneous HPTLC determination of Lamivudine and Nevirapine in tablet dosage form. International Research Journal of Pharmacy, 2(7), 228 232.
31. Singh, A. K., & Mariappan, T. T. (2010). HPTLC method for estimation of Abacavir sulphate in bulk and tablet dosage form. International Journal of PharmTech Research, 2(2), 1369 1373.
32. Sitaram, B. R., Tiwari, S. K., Ramachandran, S., & Khadgawat, R. (2005). RP HPLC method for simultaneous estimation of Lamivudine and Zidovudine in combined dosage form. Indian Journal of Pharmaceutical Sciences, 67(5), 625 627.
33. Subrahmanyam, C. V. S., Rajesh, T., & Bhargavi, B. (2010). Simultaneous estimation of Lamivudine and Zidovudine in combined tablet formulations by Q absorbance ratio method. International Journal of PharmTech Research, 2(3), 1817 1822.
34. Sweetman, S. C. (Ed.). (2009). Martindale: The Complete Drug Reference (36th ed.). Pharmaceutical Press.
35. UNAIDS. (2023). Global HIV & AIDS statistics Fact sheet 2023. Joint United Nations Programme on HIV/AIDS. https://www.unaids.org/en/resources/fact sheet
36. United States Pharmacopeia and National Formulary (USP NF). (2023). USP 46 NF 41. United States Pharmacopeial Convention.
37. Wattananat, T., Akarawut, W., & Chunhabundit, P. (2009). Identification of Lamivudine degradation products using GC MS following MSTFA derivatization. Drug Development and Industrial Pharmacy, 35(7), 863 869. https://doi.org/10.1080/03639040802626023
38. Yuen, G. J., Weller, S., & Pakes, G. E. (2004). A review of the pharmacokinetics of Abacavir. Clinical Pharmacokinetics, 43(8), 595 614. https://doi.org/10.2165/00003088 200443080 00010