A Systematic and Concise Review on the Development of Analytical and Bioanalytical Methods for the Simultaneous Estimation of Abacavir Sulfate and Lamivudine

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ABSTRACT

One of the biggest problems facing worldwide public health today is the human immunodeficiency virus (HIV). In 2007 UNAIDS estimated that 33.2 million people were living with HIV. Treatment of HIV infection has typically been carried out using nucleoside analogs and a protease inhibitor. Such regimens can be complex and have high pill burdens. Using alternative regimens, such as double nucleoside-based regimens, can improve adherence and decrease toxicities associated with protease inhibitor therapy. A formulation of abacavir sulfate and lamivudine shows a good pharmacological effect against HIV. The present review assesses the various approaches for the simultaneous estimation of abacavir and lamivudine in bulk drugs and various formulations. A concise review represents the compilation and discussion of nearly 100 analytical methods, including UV-Spectroscopy, HPLC, HPTLC, UPLC, HPTLC, and LC-MS methods implemented for investigating abacavir and lamivudine in biological matrices, bulk samples, and in the different dosage formulations. This detailed review will greatly help the researcher in working on the simultaneous estimation of abacavir and lamivudine.

KEYWORDS

Abacavir, Analytical techniques, Bioanalytical techniques, Lamivudine, Method development.

INTRODUCTION

Pharmaceutical analysis is crucial in quality assurance, quality control, and analysis of bulk drugs and pharmaceutical formulations. The demand for novel analytical techniques in the pharmaceutical industries has risen due to the rapid expansion of pharmaceutical industries and the manufacture of pharmaceuticals in different parts of the world. [1] Numerous tools, various analytical method approaches, and newly developed hyphenated techniques help in more accurate quantification and qualification of the drug moieties quickly. [2] ICH and USFDA guidelines demonstrate the crucial validation step for various developed methodologies. [3] Degradation studies of new drug substances and products are necessary to show the specificity of stability-indicating methodologies, give insight into the drug substance's degradation pathways and degradation products, and aid in elucidating the structure of the degradation products. [4] In order to provide trustworthy data that can be adequately interpreted, it is crucial to use well-characterized and fully validated bioanalytical procedures. Bioanalytical methods are of the utmost importance during drug research and development, culminating in marketing clearance. [5]

The discovery of HIV-1 as the causative agent of AIDS in 1981 has been estimated that the HIV-1 pandemic main group has spread the infection to over 60 million people globally within the last 30 years, mainly by sexual, percutaneous, and perinatal routes. [6] Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) were the first antiretroviral drugs approved by the FDA. [7] Abacavir is a carbocyclic 2'-deoxyguanosine nucleoside reverse transcriptase inhibitor widely used to treat human immunodeficiency virus (HIV) infection. [8] Abacavir Sulfate, {(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] cyclopent-2-en-1-yl} helps to halt the inroads of the human immunodeficiency virus (HIV), used either as a 600-mg once-daily or 300-mg twice-daily regimen exclusively in the treatment of human immunodeficiency virus (HIV) infection. [9] Abacavir is rapidly absorbed after oral administration, with peak concentrations occurring 0.63-1 hour after dosing. The absolute bioavailability of abacavir is approximately 83%. Abacavir pharmacokinetics are linear and dose-proportional over 300-1200 mg/day. [10] All subjects were well-tolerated abacavir; mild to moderate asthenia, abdominal pain, headache, diarrhoea, and dyspepsia were the most frequently reported adverse effect. [11] Lamivudine is a deoxycytidine analog that is active against the hepatitis B virus (HBV), inhibits HBV replication, reduces hepatic necro-inflammatory activity and the progression of fibrosis in patients with chronic hepatitis B, ongoing viral replication and compensated liver disease, including HBeAg-negative patients. [12] Lamivudine (2′-deoxy-3′-thiacytidine, 3TC) is a first-generation nucleoside reverse

transcriptase inhibitor (NRTI) that was approved for the treatment of HIV-1 infection in 1995 and hepatitis B virus (HBV) infection in 1998. [13] Lamivudine is a highly effective agent that can be dosed once or twice daily due to its long intracellular half-life. It also has one of the best tolerability and long-term safety profiles among all antiretroviral agents and continues to be preferred as part of initial or subsequent combination therapy in HIV-infected patients. [14] Figures 1 show the structures of abacavir and lamivudine, respectively. In 2008, the International AIDS Society (IAS) and the British HIV Association (BHIVA) first recommended the use of abacavir and lamivudine fixed-dose combinations (FDC) for the treatment of HIV. [15] Available commercial abacavir and lamivudine marketed formulations brands are Abamune L (600 mg of abacavir and 300 mg of lamivudine), Epzicom (600 mg of abacavir and 300 mg of lamivudine), albavir (600 mg of abacavir, and 300 mg of lamivudine), etc. No clinically significant drug interactions have been observed between recommended doses of abacavir, lamivudine, or other drugs or drug substances. [16]

SAMPLE PREPARATION

Solubility

Fundamentally, a substance's solubility is influenced by the solvent employed, temperature, and pressure. [17] Abacavir sulfate and lamivudine are biopharmaceutics classification system (BCS) class III categories of drugs, so it’s low solubility and low permeability. The API is thus highly soluble over the pH range of 1 to 6.8. [18] Solubility improved by approaching some techniques like polymorphs, amorphous form, crystallization, Change of pH, use of buffers, derivatization, complexation, salt formation, Supercritical fluid process, use of adjuvant like surfactant, solubilizers, cosolvency, hydrotrophy, and novel excipients, etc. [19]

Sample preparation strategies

Sample preparation is essential for nearly every type of chemical and biochemical analysis in use today. [20] Various diluents are used for the sample preparation of abacavir and lamivudine. Mostly, methanol was used as a diluent. The sample preparation techniques for the extraction of PIO from biological matrices (plasma, serum, and urine) include protein precipitation with acetonitrile, liquid–liquid extraction using diethyl ether, dichloromethane, ethyl acetate, methyl t-butyl ether, and n-butyl ether; hollow fiber liquid phase micro-extraction using di n-hexyl ether; and solid phase extraction.

ANALYTICAL TECHNIQUES

Numerous analytical methods have been created to evaluate the potency, bioavailability, and stability of drugs as well as their purity and physical characteristics. The most common analytical techniques are gas chromatography-mass spectrometry (GC-MS) and liquid

chromatography-mass spectrometry (LC-MS), which are hyphenated techniques. The major analytical techniques include the UV-visible spectrophotometric method, IR spectroscopy, spectrofluorimetric method, and chromatographic methods like high-performance liquid chromatography (HPLC), gas chromatography (GC), and spectrofluorimetric technique. [21-22] Based on the literature survey observed, reported methods are developed by UV-visible spectrophotometric method, High-performance liquid chromatography (HPLC), Ultra-performance liquid chromatography (UPLC), HPTLC, and LC-MS.

UV-visible spectrophotometric method

A spectrophotometer is a measuring tool for quantitative analysis frequently used to characterise chemical substances by calculating the volume of light that the analyte has partially absorbed in the solution. [23] The ultraviolet-visible (UV-Vis) spectroscopy, a simple, cost-effective, and non-destructive technique, has applications in environmental, pharmaceutical, and other related fields. For example, the British and United States Pharmacopoeias employ UV-Vis-based methods to assay some pharmaceutical products and an adjunct method for identifying certain active pharmaceutical ingredients. [24] Table 1 represents the developed reported analytical methods for quantifying and qualification the combination drug abacavir and lamivudine by UV-visible spectrophotometric method.

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) analysis of any drug is to confirm the identity of a drug and provide quantitative results and monitor the progress of the therapy of the disease. [25] A high-performance liquid chromatography method has been developed and validated for the simultaneous separation and determination of various types of analytes in chemical and biochemical substances. [26] Table 2 represents the developed reported analytical methods for quantifying and qualification of the combination drug abacavir and lamivudine by High-performance liquid chromatography (HPLC).

Ultra-performance liquid chromatography (UPLC)

The commercialization of ultra-performance liquid chromatography (UPLC) has allowed more researchers to take advantage of the benefits of this work. Many researchers are exploring this technique to reduce analytical throughput and increase resolution. [27] Ultra-performance liquid chromatography (UPLC) utilizes sub-2-micron particles with high linear solvent velocities to effect dramatic increases in resolution, sensitivity, and speed of analysis. The reduction in particle size to below 2 microns requires instrumentation that can operate at pressures in the 6000-15,000 psi range. The typical peak widths generated by the UPLC system are 1-2 s for a 10-minute separation. [28] Table 3 represents the developed reported analytical

methods for quantifying and qualification the combination drug abacavir and lamivudine by Ultra-performance liquid chromatography (UPLC).

Hyphenated techniques

The hyphenated technique is developed by coupling a separation technique and an online spectroscopic detection technology. The impressive advancements in hyphenated analytical techniques over the past two decades have greatly expanded their range of applications in the study of biomaterials, particularly natural goods. Recent developments in the applications of various hyphenated techniques, such as GC-MS, LC-MS, LC-FTIR, LC-NMR, CE-MS, etc., are discussed in this article. [29] In pharmaceutical analysis, the analytical development, and quality control of drug substances and dosage forms, mass spectrometry (MS) combined with chromatographic separation is the most powerful technique for the monitoring, characterization, and identification of impurities. [30] Table 3 and 4 represents the reported analytical methods for quantifying and qualifying the combination drug abacavir and lamivudine by Hyphenated techniques.

Bioanalytical Techniques

Bioanalysis is an essential part of drug discovery and development. Bioanalysis generally describes the quantitative measurement of a compound (drug) or its metabolite in biological fluids, primarily blood, plasma, serum, urine, or tissue extracts. A bioanalytical method consists of two main components: Sample preparation and Detection of the compound. [31] For accurate results that can be correctly understood, it is crucial to use bioanalytical methodologies developed that have been thoroughly defined and confirmed. It is acknowledged that bioanalytical methods and procedures are frequently on the cutting edge of technology and constantly improving. [32]

CONCLUSION

Various analytical techniques used to estimate lamivudine and abacavir are illustrated in the current review. The simultaneous assessment of abacavir and lamivudine in bulk, as well as its combined pharmaceutical formulations and biological components, had been the subject of numerous investigations, including bio-analytical, stability indicating, HPLC, HPTLC, UV-Visible Spectroscopy, LC-MS, etc. The most researched method for simultaneously estimating lamivudine and abacavir in bulk and pharmaceutical dosage forms is liquid chromatography with UV detection. At the same time, hyphenated such as LC-MS methods are reported to determine abacavir and lamivudine and their metabolite in plasma and other biological fluids. The proposed review is very much beneficial to the quantitative and qualitative determination of abacavir and lamivudine.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

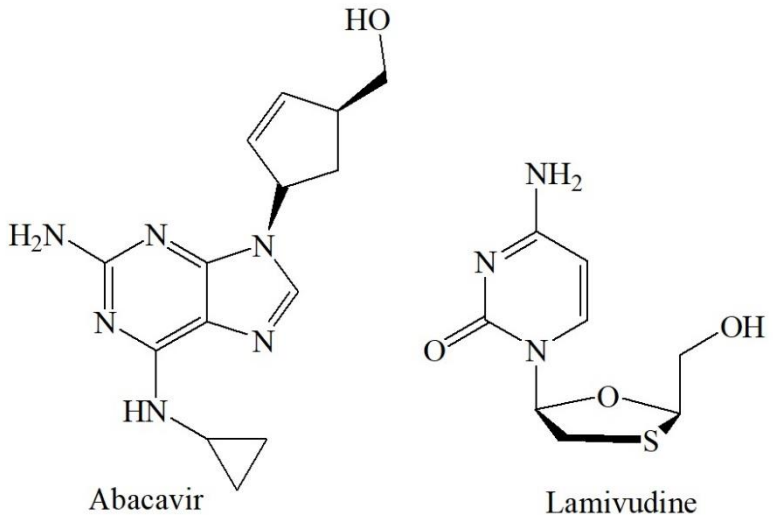

Figure 1: Structure of Abacavir and Lamivudine

Table 1: UV- Spectrophotometry Methods for determination of Abacavir and Lamivudine

Techniques/ Methods	Drug & Matrix	Solvent	Detection	Linearity, LOD, and LOQ	Ref.
Spectrophotometric Method	Abacavir and Lamivudine in combined tablet dosage form	Distilled water	284 nm (Abacavir) 270 nm (Lamivudine)	Linearity: 5-30 µg/ml	34
Simultaneous spectrophotometric method	Abacavir and Lamivudine in tablet dosage form	Distilled water	Simultaneous equation method: 284 nm (Abacavir) and270 nm (Lamivudine), Q- analysis method: 284 nm (Abacavir) and 270 nm (Lamivudine)	Linearity: 5-30 mcg/ml	35
Simultaneous spectrophotometric method	Abacavir sulfate and Lamivudine in their binary mixture	Methanol	First derivative zero crossing technique: 234.2 nm (Lamivudine) and 287.3 nm (Abacavir), Ratio spectra first derivative spectrophotometric method: 233.0 nm (Abacavir) and 236.4 nm (Lamivudine)	Linearity: 5-30 µg/ml (Abacavir) and ,2.5-15 µg/ml (Lamivudine)	36
UV- Spectrophotometric method	Abacavir and Lamivudine in combined tablet dosage form	Acetonitrile and methanol (3:2)	260 nm (Abacavir) and 271 nm (Lamivudine)	Linearity: 5-25 µg/ ml	37
UV-Spectroscopy	Abacavir sulphate and Lamivudine in tablet dosage form	Methanol and water (15:85)	228 nm	Linearity: 10-50 µg/ml (Abacavir) and 5-25 µg/ml (Lamivudine), LOD: 0.598 µg/ml and 0.598 µg /ml (Abacavir and Lamivudine), LOQ: 8.163 µg /ml (Abacavir) and 1.814 µg /ml (Lamivudine)	38
Simultaneous spectrophotometric method	Lamivudine and Abacavir sulphate in bulk and in tablet dosage form	0.1 N HCl	270 nm (Lamivudine) and 289 nm (Abacavir)	Linearity: 5 to 30 µg/ml	39
Spectrophotometric method	Lamivudine and Abacavir	1 M HCl	280 nm (Lamivudine) and 297 nm (Abacavir)	Linearity: 2-12 µg/ml	40
UV-Spectrophotometric method	Abacavir sulfate and Lamivudine in tablet dosage form	Acetonitrile and methanol	224, 241, 257, 280, and 296 nm	-	41

Table 2: HPLC method for Abacavir and Lamivudine

Drug/Matrix	Methods	Stationary phase	Mobile phase	Detection	Linearity, LOD, and LOQ	Flow rate	Retention time	Ref.
Abacavir sulfate and Lamivudine	Gradient RP-HPLC	Zorbax C18 (4.6 nm×150 mm, 3.5 µm)	Phosphate buffer and methanol	270 nm	Linearity: Abacavir: 88–266 µg/ml and Lamivudine: 38–116 µg/ml	1.5 ml/min.	Abacavir: 3.66 min and Lamivudine: 10.71 min	42
Lamivudine and Abacavir in bulk samples as well as in tablet dosage forms	RP-HPLC method	Dionex C18 column	Methanol (40% volume) and potassium dihydrogen phosphate (60% volume) with 0.1 M strength and pH 3.5	232 nm	Linearity:150 to 450 µg/ml (Abacavir), 75 to 225 µg/ml (Lamivudine)	1 ml/min	2.397 min (Abacavir), 3.296 min(Lamivudine)	43
Lamivudine and Abacavir sulphate in tablets	Reverse phase liquid chromatographic method	Phenomenex C18 (250 x 4.6 mm, 5 µm particle size) column	Phosphate buffer (pH 7.8) and methanol (50:50 % v/v)	216 nm	Linearity: 80-280 µg/ml (Lamivudine) and 75-450 µg/ml (Abacavir)	1.0 ml/min	3.147 min (Lamivudine) and 6.367 min (Abacavir)	44
Abacavir sulphate and Lamivudine in tablet dosage form	RP-HPLC method	Grace C_{18} column (150x4.6 mm)	Methanol: water (15:85 v/v %)	228 nm	Linearity: 20-100 µg/ml, LOD AND LOQ: 0.598 and 1.97 µg/ml	1.0 ml/min	7.38 min(Abacavir) and 3.15 min (Lamivudine)	45
Abacavir and Lamivudine in bulk and tablet dosage forms	RP-HPLC method	Symmetry Premsil C18 (250 mm × 4.6 mm, 5 µm)	Methanol: water (0.05% orthophosphoric acid with pH 3) 83:17 v/v	245 nm	Linearity: 20-100 µg/ml (Abacavir) and 10-50 µg/ml (Lamivudine)	1 ml/min	3.5 min (Abacavir) and 7.4 min (Lamivudine)	46
Abacavir and Lamivudine in pure form and marketed formulation	RP-HPLC method	Develosil ODS HG-5 RP C18 (5 µm, 15 cmx4.6 mm)	Methanol: phosphate buffer (0.2 M, pH 3) (75:25)	259 nm	Linearity: 5–30 µg/ml (Abacavir), 10–60 µg/ml (Lamivudine) LOD and LOQ: 0.05 µg/ml and 0.15 µg/ml (Abacavir), 0.09 µg/ml and 0.27 µg/ml (Lamivudine)	1.0 ml/min	8.0 mins	47
Lamivudine and Abacavir as tablet dosage form	RP-HPLC Method	THERMOSIL C18 (150X4.6 mm,5 µm)	Water: acetonitrile: buffer 2.5 (45:15:40)	232 nm	Linearity: 300-700 ppm (Lamivudine) 600-1400 ppm (Abacavir) LOD and LOQ:	1 ml/min	2.087 min (Lamivudine) and 6.067 min (Abacavir)	48

					2.97 ppm and 9.98 ppm (Lamivudine), 3.04 ppm and 9.94 ppm (Abacavir)			
Lamivudine and Abacavir combined tablet	RP-HPLC method	Waters symmetry C 18 (100 X 4.6 mm, 3.5 µm)	Phosphate buffer (pH 3.0): Methanol (80:20 v/v)	276 nm	Linearity: 50-150 µg/ml (Lamivudine) 100-300 µg/ml (Abacavir)	0.5 ml/min	2.122 min (Lamivudine) and 3.194 min (Abacavir)	49
Abacavir sulphate and Lamivudine in tablet dosage forms	RP-HPLC method	Inertsil ODS (150×4.6 mm, 5 µm)	Phosphate buffer (pH 4.0) and Acetonitrile	254 nm	Linearity: 20-120 µg/ml (Abacavir), 10-60 µg/ml (Lamivudine) LOD and LOQ: 0.0049 µg/ml and 0.0184 µg/ml (Abacavir) 0.0268 µg/ml and 0.0150 µg/ml (Lamivudine)	1 ml/min	2.487 min (Abacavir) and 4.107 min (Lamivudine)	50
Abacavir and Lamivudine	RP-HPLC method	Gemini C18 column (250 mm X 4.6 mm, 5 µm)	MeOH: phosphate buffer (74.3:25.7%v/v) (pH 6.85, buffer strength 0.05M)	260 nm	Linearity: 2-12 µg/ml (Abacavir and Lamivudine) LOD and LOQ: 0.0589 µg/ml and 0.0204 µg/ml (Lamivudine), 0.0012 µg/ml and 0.0115 µg/ml (Abacavir)	1.2 ml/min	3.55 min	51
Abacavir, Dolutegravir and Lamivudine bulk drug and in the formulation	RP-HPLC	Kinetex 5 µ C18 100 A (250 mm x 4.6 mm)	Acetonitrile	258 nm	Linearity: Abacavir 20– 100 µg/ml, Dolutegravir 2-16 µg/ml, Lamivudine 10-80 µg/ml. LOD and LOQ: 2.05 µg/ml and 6.73 µg/ml (Abacavir), 0.28 µg/ml and 0.94 µg/ml (Dolutegravir), 2.32 µg/ml and 7.72 µg/ ml (Lamivudine)	0.9 ml/min	Abacavir: 5.2min, Dolutegravir: 8.4 min, Lamivudine: 3.1 min	52
Abacavir, Lamivudine and Zidovudine in tablet dosage forms	RP-HPLC Method	Luna C18 column	Acetonitrile, Methanol and 0.05M ammonium dihydrogen phosphate (70:20:10 v/v/v)	266 nm	Linearity: 50-150 µg/ml (Abacavir), 20-60 µg/ml (Lamivudine), 30-90 µg/ml (Zidovudine)	1.2 ml/min	2.6 min (Abacavir), 6.1 min (Lamivudine) , 8.9 min (Zidovudine)	53

Lamivudine, Zidovudine and Abacavir in tablet dosage forms	RP HPLC method	HiQ Sil C 18 V column	0.01 M potassium dihydrogen ortho-phosphate (pH 3.0) and methanol (55:45 v/v)	272 nm	Linearity: 5-250 µg/ml (Abacavir and Zidovudine) 5-140 µg/ml (Lamivudine)	0.8 ml/min	3.8 min (Lamivudine), 6.3 min (Abacavir) and 8.1 min (Zidovudine)	54
Lamivudine, Abacavir and Dolutegravir in combined dosage form	Simultaneous HPLC method	Kromasil 250 mm x4.5 mm,5 µm	Buffer: acetonitrile (65:35)	257 nm	Linearity: 15-90 ppm (Lamivudine), 30-180 ppm (Abacavir), 2.5-15 ppm (Dolutegravir) LOD and LOQ: 0.08 µg/ml and 0.2 µg/ml (Lamivudine), 0.06 µg/ml and 0.19 µg/ml (Abacavir), 0.03 µg/ml and 0.10 µg/ml (Dolutegravir)	1 ml/min	2.25 min (Lamivudine), 2.734 min (Abacavir), 9.633 min (Dolutegravir)	55
Abacavir, Lamivudine and Dolutegravir in bulk and pharmaceutical dosage form	RP-HPLC method	Inertsil ODS 250×4.6 mm, 5 µm	Phosphate buffer (pH 3.0): acetonitrile: methanol (50:20:30 v/v/v%)	257 nm	Linearity: 15 -90 µg/ml (Lamivudine), 30-180 µg/ml (Abacavir), 2.5-15 µg/ml (Dolutegravir)	1.0 ml/min	2.169 min (Lamivudine) 2.676 min (Abacavir) 6.367 min (Dolutegravir)	56
Abacavir, Lamivudine, and Zidovudine combination tablet	RP-HPLC method	C18, 250X4.6 mm 5 µm HPLC column	Acetate buffer (pH 3.0)	310 nm	Linearity: LOQ level to 250%, LOD: 0.002 and 0.009 (%w/w) and LOQ: 0.0006–0.0029 (%w/w)	1.0 ml/min to 0.9 mL/min and 1.1 mL/min	14 min (Lamivudine), 32 min (Zidovudine), and 38 min (Abacavir)	57
Abacavir, Lamivudine, Nevirapine, and Zidovudine in pharmaceutical dosage form	RP-HPLC method	Inertsil ODS 250×4.6 mm, 5 µm	Buffer: methanol: acetonitrile (650:200:150)	270 nm	-	1.0 ml/min	2.4 min (Lamivudine), 3.49 min (Zidovudine), 4.78 min (Abacavir) and 7.7 min (Nevirapine)	58
Lamivudine, Abacavir sulphate and Dolutegravir in combined dosage form	RP-HPLC method	Inertsil ODS column (4.6×150 mm, 5 µm)	Phosphate buffer (pH 3.5): acetonitrile: water (60:30:10 v/v/v %)	245 nm	Linearity: 15-90 µg/ml (Lamivudine), 30-180 µg/ml (Abacavir) and 2.5-15 µg/ml (Dolutegravir)	1.0 ml/min	1.692 min (Lamivudine), 2.210 min (Abacavir) and 4.155 min (Dolutegravir)	59

Abacavir, Lamivudine and Dolutegravir tablet dosage form	RP-HPLC method	STD Agilent C18 (150 x 4.6 mm, 5 µm)	Water: acetonitrile (60:40 v/v %)	284 nm	LOD and LOQ: 0.04 µg/ml and 0.132 µg/ml (Abacavir), 0.11 µg/ml (Lamivudine) and 0.08 µg/ml (Dolutegravir)	0.8 ml/min	2.233 min (Abacavir, 2.700 min (Lamivudine) and 3.426 min (Dolutegravir)	60
Lamivudine, Abacavir and Dolutegravir in bulk and their combined dosage form	Gradient RP-HPLC method	Luna Phenyl Hexyl, (250 mm x 4.6 mm, 5 µm)	Acetonitrile and 0.1 M Ortho phosphoric acid buffer	258 nm	Linearity: 3-45 µg/ml (Lamivudine) 6-90 µg/ml (Abacavir) 0.5-7.5 µg/ml (Dolutegravir) LOD and LOQ: 0.036 µg/ml and 0.112 µg/ml (Lamivudine), 0.065 µg/ml and 0.198 µg/ml (Abacavir), 0.021 µg/ml and 0.071 µg/ml (Dolutegravir)	1 ml/min	3.3 min (Lamivudine, 4.5 min (Abacavir) and 6.3 min (Dolutegravir)	61
Lamivudine, Abacavir, Dolutegravir in Pharmaceutical dosage forms	RP-HPLC method	Inersil ODS (4.6 × 250 mm x 5 µm)	Phosphate buffer (pH-7) and acetonitrile	254 nm	Linearity: 0–150 µg/ml (Lamivudine), 0-300 µg/ml (Abacavir) and 0-25 µg/ml (Dolutegravir) LOD and LOQ: 0.31 µg/ml and 0.94 µg/ml (Lamivudine), 0.21 µg/ ml and 0.65 µg/ ml (Abacavir) and 0.48 µg/ ml and 1.46 µg/ ml (Dolutegravir).	1.2 ml/min	2.555 min (Lamivudine), 4.282 min (Abacavir) and 7.101 min (Dolutegravir)	62
Lamivudine, Zidovudine and Abacavir in tablet dosage forms	Ultra-performance liquid chromatographic method	Inertsil ODS-3V (250 x 4.6 mm, 5.0 µm) column	Mobile phase A: ammonium dihydrogen phosphate and diammonium hydrogen phosphate buffers pH 3.9 and mobile phase B: methanol	270 nm	-	1 ml/min	-	63
Abacavir, Zidovudine and Lamivudine in tablet dosage forms	RP-HPLC method	X terra symmetry C18 (4.6 x 150 mm, 3.5 µm)	Methanol: buffer (pH 3.5)	258 nm	Linearity: 7.5-45 µg/ml (Abacavir), 11.25-67.5 µg/ml (Zidovudine), 22.5-135 µg/ml (Lamivudine),	1 ml/min	15 min	64

					LOD and LOQ: 0.017 µg/ml and 0.061 µg/ml (Abacavir), 0.080 µg/ml and 0.243 µg/ml (Zidovudine), 0.04 µg/ml and 0.183 µg/ml (Lamivudine)			
Abacavir, Lamivudine, and Zidovudine in combined tablet dosage form	RP-HPLC method	Eurosphere 100-5 C_{18} column (250X4.6 mm)	Methanol: buffer (pH 3): acetonitrile: tetrahydrofuran (35:60:5:0.4 v/v/v/v)	271 nm	Linearity: 5-15 µg/ml (Lamivudine), 10-30 µg/ml (Abacavir and Zidovudine)	0.6 ml/min	6.61 min (Abacavir),4.87 min (Lamivudine) and 8.21 min (Zidovudine)	65
Abacavir, Lamivudine and Zidovudine in pharmaceutical dosage form	RP-HPLC method	Hypersil C18, (250 x 4.6 mm, 5 µm)	Methanol: water (50:50 v/v)	270 nm	Linearity: 0.5 to 400 µg/ml LOD and LOQ: 0.013 µg/ml and 0.044 µg/ml (Abacavir), 0.070 µg/ml and 0.230 µg/ml (Lamivudine), 0.030 µg/ml and 0.102 µg/ml (Zidovudine)	1 ml/min	6.3 min (Abacavir), 3.8 min (Lamivudine) and 8.6 min (Zidovudine)	66

Table 3: HPTLC method for Abacavir and Lamivudine

Drug/matrix	Mobile phase	Chamber saturation, TLC plate development time	Detection	Linearity, LOD and LOQ	Ref.
Lamivudine and Abacavir sulphate in tablet dosage form	Acetone: chloroform: methanol (4: 4: 2 v/v/v)	CSt and PDt= 30 min Rf value= Lamivudine: 0.09 Abacavir: 0.34	265 nm	Linearity: 5-30 µg/ml LOD and LOQ: 0.0083 µg/ml and 0.0254 µg/ml (Lamivudine), 0.0034 µg/ml and 0.0105 µg/ml (Abacavir)	67
Abacavir sulphate and Lamivudine in Tablet dosage form	Methanol: acetone: n-butyl acetate (1:1:2 v/v/v)	CSt=30min Rf Value= Abacavir: 0.58 Lamivudine: 0.35	284 nm	Linearity: 240-1200 ng/spot (Abacavir) 120-600 ng/spot (Lamivudine) LOD and LOQ: 0.691 ng/spot and 2.093 ng/spot(Abacavir), 1.114 ng/spot and 3.376 ng/spot(Lamivudine)	68
Abacavir, Lamivudine and Zidovudine in combined dosage form	Methanol: chloroform: acetonitrile (4: 8: 3 v/v/v)	-	275 nm	Linearity: 200-1450 ng/band LOD: 57.58 ng/band LOQ: 174.5 ng/band	69
Abacavir sulfate, Lamivudine hydrochloride, and Dolutegravir sodium in an in-house physical mixture	Ethyl acetate: ethanol: acetone: ammonia (4.478:0.740:0.50:0.15 v/v/v/v)	CSt=30min Rf value= Abacavir: 0.65 Lamivudine: 0.34 Dolutegravir: 0.26	267 nm	Linearity (µg/band): 4.8, 7.2, 9.6, 12.0, and 14.4 (Abacavir) and 2.4, 3.6, 4.8, 6.0, 7.2 (Lamivudine) and 0.4, 0.6, 0.8, 1.0, 1.2 and (Dolutegravir) LOD and LOQ (µg/band): 0.9972 and 3.0218 (Abacavir), 0.2544 and 0.7711(Lamivudine), 0.1004 and 0.3043(Dolutegravir)	70
Abacavir, Lamivudine, and Zidovudine in bulk and pharmaceutical dosage form	Toluene: ethyl acetate: methanol (8.0:1.0:1.0 v/v/v)	CSt=20min Rf value= Abacavir: 0.26 Lamivudine: 0.46 Zidovudine: 0.65	279 nm.	Linearity(ng/spot): 500-4000 (Abacavir) Lamivudine- 200-1200 Zidovudine- 300-1800 LOD and LOQ (ng/spot): 13.99 and 42.4 (Abacavir), 7.37 and 22.32(Lamivudine), 7.23 and 15.23 (Zidovudine)	71

Table 4: UPLC method for Abacavir and Lamivudine

Drug	Method	Stationary phase	Mobile phase	Detection	Rt and flow rate	Linearity, LOD, LOQ	Ref.
Abacavir sulphate and Lamivudine	UPLC	Acquity BEH C8 (100 mm x 2.1 mm, 1.7 µm)	Triethylamine phosphate buffer (pH 2.5) and methanol (50:50% V/V)	230 nm	Abacavir sulphate: 0.83 and Lamivudine: 1.62 ,0.5 mL min-1	50 % to 150 % (assay)	72
Abacavir sulphate, Dolutegravir and Lamivudine	UPLC	Zodiac sil C18 column (4.6 mm × 250 mm, 3.0 µm)	Phosphate buffer (pH 3.0) and methanol (30:70 %v/v)	260 nm	Lamivudine 1.763 min, Abacavir 2.247 min, Dolutegravir, 3.175 min. Flow rate: 0.25 ml/min	Lamivudine15-75 µg/ml, Abacavir 30-150 µg/ml, Dolutegravir 2.5-12.5 µg/ml. LOD and LOQ: 0.021 and 0.056 µg/ml (Abacavir), 0.330 and 1.320 µg/ml (Dolutegravir), 0.038 and 0.095 µg/ml (Lamivudine)	73
Abacavir sulphate and Lamivudine	UPLC	Symmetry C18 (2.1 x 100 mm, 1.7 µm, Make: BEH)	Phosphate Buffer (pH3.0) Methanol (60:40 v/v%)	280 nm	Lamivudine-1.019 min, Abacavir-1.271 min, and Zidovudine-1.617 min. Flow rate 0.25 ml per min	Abacavir 20-60 ppm, Lamivudine 10-30 ppm and Zidovudine 20- 60 ppm LOD and LOQ: 0.002 µg/ml and 0.008 µg/ml (Abacavir), 0.003 µg/ml and 0.01 µg/ml(Lamivudine), 0.005 µg/ml and 0.02 µg/ml (Zidovudine)	74
Abacavir sulphate and Lamivudine	UPLC	HPLC C18 column, (250 × 4.6 mm, 5 µm)	Acetate buffer (pH 3.0), methanol, and acetonitrile	266 nm	Run time: 20 min. Flow rate: 0.35 ml/min	Linearity: LOQ level to about 250%. LOQ and LOD: 0.002 and 0.0006 % w/w (Abacavir), 0.009 and 0.0029 % (Lamivudine)	75

Table 5: Reported Bioanalytical Techniques for Abacavir and Lamivudine

Method	Drug	Biological fluid	Column	Mobile phase	Flow rate and retention time	Linearity, LOD, LOQ	Ref.
LC-MS/MS method	Lamivudine and Abacavir	Blood and tissues	CSH C18 (2.1×100 mm, 1.7 µm; Waters)	Mobile phase A: 40% ACN, 0.06 % acetic acid, and 10 mm ammonium formate in water, and mobile phase B 30% ACN, 0.3 % ammonium hydroxide, and 1 mm ammonium formate in deionized water	Flow rate: 0.45 ml/min and run time: 10 min	Lamivudine: 10 – 100,000 pg/ml and Abacavir: 4 – 40,000 pg/ml.	76
LC-MS–MS method	Abacavir and Lamivudine	Human plasma	Waters symmetry shield rp18 column (150 mm × 3.9 mm, 5 µm).	Acetonitrile and aqueous glacial acetic acid solution (0.05% v/v) 80:20 (v/v)	Flow rate: 0.8 mL min^{-1} and run time: 2.0 min.	Linearity: 100.0 – 7000.0 ng/mL (Abacavir) and 80.0– 5000.0 ng/mL (Lamivudine) LOQ and LOD: 100.0 and 1.0 ng/ml^{-1} (Abacavir), 80.0 and 1.2 ng mL^{-1} (Lamivudine)	77
liquid chromatography -electrospray ionization tandem mass spectrometry	Abacavir and Lamivudine	Human plasma	C18 column (5 µm, 150 × 4.6 mm)	Acetonitrile/ 10mm Ammonium Formate (80/20, V/V%)	Flow rate: 1.0 ml/min and run time: 3.0 minutes	Linearity: 5.005 – 4004.253 ng/ml(Abacavir) and 2.506-2005.148 ng/ml (Lamivudine)	78
High-performance liquid chromatography	Abacavir and Lamivudine	Rat plasma	HiQSil C8 column (250 mm × 4.6 mm, 5 µm)	0.01 mol/L potassium Dihydrogen orthophosphate buffer, acetonitrile and methanol (60:25:15)	Flow rate 1 ml/min and run time: abacavir and Lamivudine eluted at 5.608 and 3.458 min.	Linearity: 0.05-50 µg/ml	79
Micellar liquid chromatography	Abacavir, Lamivudine and Raltegravir	Plasma	C18 column (125 × 4.6 mm, 5 µm particle size).	0.05 M sodium dodecyl sulphate at pH 7	Running:1 ml/min^{-1} and retention time: <7.1%	Linearity: 0.25–25 µg/ml range LOD and LOQ: 0.090 and 0.250 µg/ml (Abacavir), 0.070 and 0.200 µg/ml (Lamivudine), 0.090 and 0.250 µg/ml(Raltegravir)	80

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