Review Article

Review on Analytical Methods for Determination of Lamivudine, Dolutegravir and Tenofovir Disoproxil Fumarate in Fixed Dose Combination

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ABSTRACT

Pharmaceutical medicines play an important role in human life that helps to cure different diseases. For the chemical and pharmaceutical analysis of the drug effective quality control and pharmacodynamic and pharmacokinetic studies are needed. Several methods have been developed and validated for its pharmaceutical and biological materials since it was introduced as an important antiretroviral agent. The literature survey reveals that only four RP HPLC method and one HPTLC was developed for the simultaneous estimation of Dolutegravir, Lamivudine and Tenofovir disoproxil fumarate in tablet dosage form. These three drugs are used as antiretroviral medicines which are used for HIV or AIDS prevention and treatment. The goal of this review is to define and establish a simple, precise and selective method for estimating the dosage of Lamivudine, Tenofovir Disoproxil fumarate and Dolutegravir in biological and pharmaceutical dosage form using the HPLC, HPTLC, UPLC, UV Visible spectroscopy, LC/MS, Infrared spectroscopy, NMR spectroscopy, Microbiological assay, Electrochemical studies and Capillary electrophoresis. UV-detector HPLC is commonly used in pharmaceuticals and LC-MS are widely used for biological materials with mass and tandem mass spectrometer detector systems. Various parameters such as device suitability, process accuracy, precision, linearity, detection limit will validate the UV Visible spectroscopy and RP-HPLC technique.

Keywords: Lamivudine, Dolutegravir, Tenofovir disoproxil fumarate, analytical techniques, Spectroscopy, HPLC, LC-MS, Pharmaceutical dosage form.

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INTRODUCTION

The human immunodeficiency virus (HIV) is a virus that causes the syndrome of acquired immune deficiency (AIDS) and is transmitted by contact with blood and body fluids that are infected. Unprotected sex, needle sharing or other equipment for drug injection, mother-to-child transmission during pregnancy, or breast-feeding, may result in such contact. HIV infects the body's immune cells, which are important for battling infections, called CD4 positive (CD4+) T cells. HIV turns these cells into factories that create more HIV viruses to infect other healthy cells, thereby killing the CD4+ cells.[1]

Lamivudine is an antiretroviral drug which is used for HIV or AIDS prevention and treatment. In the treatment of hepatitis B, it is often used when other options are not possible. Tenofovir disoproxil fumarate is an agent that can be used with other antiretroviral agents, demonstrates its activity as an inhibitor of reverse transcriptase nucleotides, and decreases the virus' ability to replicate. It is used for HIV or AIDS care when there is a high risk prior to exposure. Dolutegravir is an antiretroviral agent that is also used for HIV or AIDS prevention and treatment.

Component I: Lamivudine Drug profile

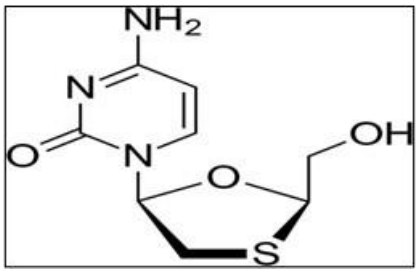

Figure 1: Chemical structure of Lamivudine.

The IUPAC: 2',3'-dihyroxy-3'thiacytidine 4-Amino-1-[(2R,5S)-2- (hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidinone. Molecular formula is C8H11N3O3S, molecular weight 229.29 gm/mol.[2]

Lamivudine is a synthetic nucleoside analog with anti-hepatitis B (HBV) and HIV activity. Lamivudine is phosphorylated intracellularly into its active metabolites, lamivudine triphosphate (L-TP) and lamivudine monophosphate (L-MP). In HIV, after incorporation of the nucleoside analogue into viral DNA, L-TP inhibits HIV-1 polymerase (RT) via DNA chain termination. In HBV, the incorporation of L-MP by HBV polymerase into viral DNA leads to termination of the DNA chain. L-TP can be a weak inhibitor of alpha and

beta mammalian DNA polymerases and mitochondrial polymerase DNA.

Component II

Tenofovir Disoproxil fumarate Drug profile

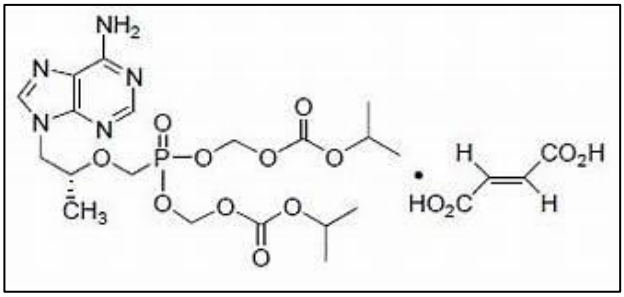

Figure 2: Chemical structure of Tenofovir Disoproxil Fumarate

The Tenofovir disoproxil fumarate IUPAC name is [(2*R*)-1-(6-aminopurin-9-yl) propan-2-yl] oxymethyl-(propan-2-yloxycarbonyloxymethoxy) phosphoryl] oxymethyl propan-2-yl carbonate; but-2-enedioic acidhaving molecular formula $C_{23}H_{34}N_5O_{14}P$. Tenofovir Disoproxil fumarate molecular weight is 635.5gm/mol. It is a crystalline white powder with a melting point of 229°C. It is an inhibitor of nucleotide reverse transcriptase. It inhibits reverse transcriptase, a major HIV enzyme. Tenofovir Disoproxil fumarate also inhibits DNA polymerase-like human enzymes. It is an adenosine analogue.[3]

Component III: Dolutegravir Drug profile

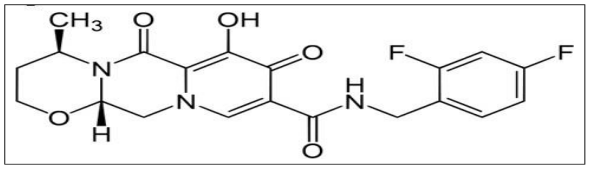

Figure 3: Chemical structure of Dolutegravir.

It is (4*R*,12a*S*)-*N*-(2,4- difluoro benzyl)-7-hydroxy-4-methyl-6,8-dioxo-3,4,6,8,12,12a- hexahydro-2H-pyrido [1',2':4,5] pyrazino [2,1-b] [1,3] oxazine-9- carboxamide. The molecular formula of Dolutegravir is: C20H19F2N3O5 with a molecular weight of 419.38gm/mol. It indicates the 190-193oC melting point. Dolutegravir is an orally bioavailable strand-transfer integrase (INSTI) inhibitor with activity against infection with the human immunodeficiency virus type 1 (HIV-1). Dolutegravir binds to the integrase site, an HIV enzyme, upon oral administration, which catalyzes the conversion of viral genetic material into human chromosomes. This forestalls integrase from authoritative to retroviral desoxyribonucleic corrosive (DNA), and squares the strand move step, which is significant for the HIV replication cycle. This forestalls HIV-1 replication.[4]

Quantitative Analytical Techniques for 3TC, DTG and TDF

Quantitative analysis techniques help to determine precisely the concentration of each variable present in the sample.

HPLC

For the determination of the active pharmaceutical ingredients and related substance using a variety of colonnade, solvents and detectors in the same phase HPLC offers a consistent quantitative accuracy and precision and can be performed on fully automated instruments with HPLC System. HPLC offers excellent replicability and is applied through careful choice of HPLC column chemistry to a wide variety of different compound forms. Chiral molecules are also possible to be isolated by HPLC into their respective enantiomers. HPLC is the best way to satisfy most of the requirements for quantitative analysis for a number of medicines. HPLC, especially reversed HPLC process, today. It is essentially a fluid chromatographic process involving the isolation and quantification of complex mixtures of resolved materials. Depending on the form of stationary phase packed in the column, the separation may be by adsorption, division, exclusion and ion-exchange.[5]

Table 1: Performance Characteristics of HPLC

Sl. No	Drug	Stationary Phase: Mobile Phase	Linearity Range (µg/ml)	Application
1	Lamivudine and tenofovir disoproxil fumarate[6]	Phenomenax Luna C18 (150 mm x 4.6 mm i.d., particle size 5µm) column acetonitrile: methanol: water (30:50:20 (v/v))	20-60ppm mL-1	Bulk drug and tablet
2	Lamivudine, tenofovir disoproxil and dolutegravir[7]	column (Luna C8 150*4.6mm) 0.1%v/v TFA in water and Acetonitrile	75.0 – 225.0µg/ml, 75.0 – 225.0µg/ml and 12.5 – 37.50µg/ml.	Bulk and pharmaceutical dosage form
3	Lamivudine and tenofovir disoproxil fumarate[8]	Phenomenex Luna C18 (150 mm x 4.6 mm i.d., particle size 5µm) column acetonitrile: methanol: water (30:50:20 (v/v))	2 –12 µg mL-1	Bulk and pharmaceutical dosage form

4	Lamivudine, Tenofovir Alafenamide and Dolutegravir[9]	Agilent C18 (250 × 4.6 mm, i.d., 5 µm) column 0.05M phosphate buffer pH 6.2 (solvent A) and acetonitrile (solvent B) 60:40 v/v	10-80 µg/ml	Bulk and pharmaceutical dosage form
5	Tenofovir[10]	Column (hyper ODS2 C18): methanol and phosphate buffer (90:10v/v)	20 – 110 mcg/ml	Bulk and pharmaceutical dosage form
6	Dolutegravir[11]	C8 column (150 × 4.6 mm) 0.1% trifluoroacetic acid in water: methanol	-	Impurity detection
7	Dolutegravir and lamivudine[12]	Xbridge Phenyl (250 × 4.6 mm, 5 µ,100 A0) column methanol: buffer (0.1% v/v trifluoroacetic acid in water) (85:15 v/v)	5–15 µg/mL and 30–90 µg/mL	Bulk drug and tablet, SIAM
8	Lamivudine and tenofovir disoproxil fumarate[13]	Thermosil C18 (150mm x 4.6mm, 3.5µm particle size) column KH2PO4 buffer (pH 6.0 with dilute orthophosphoric acid): Methanol: Water (33:65:2%v/v/v)	50 -150 µg/mL	Bulk and pharmaceutical dosage form
9	Lamivudine and tenofovir disoproxil fumarate[14]	Inertsil ODS3V column (4.6*250mm) Phosphate buffer and acetonitrile 55:45(v/v)	70-170µg/ml for Lamivudine and 60-140 µg/ml	Bulk drug and tablet
10	Dolutegravir, lamivudine, tenofovir disoproxil fumarate[15]	core-shell bi-phenyl, 250x4.6mm, 5µm column Sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of octane sulfonic sodium salt monohydrate adjusted pH 2.5 with orthophosphoric acid: was sodium dihydrogen phosphate monohydrate (10mM) with 0.5g/L of 1-octane sulfonic sodium salt monohydrate adjusted pH 2.5, acetonitrile: Methanol in the ratio of 20:60:20%v/v/v.	-	Forced degradation study of pharmaceutical dosage form
11	Lamivudine and dolutegravir[16]	Waters C18 column (150×4.6mm, particle size 5µ) buffer (pH adjusted to 3.0 using orthophosphoric acid), acetonitrile and methanol (55:35:10 v/v).	18-90µg/ml and 3-15µg/ml	Bulk and pharmaceutical dosage form
12	Lamivudine, tenofovir and dolutegravir[17]	C18 column (250 _ 4.6 mm, 5micron 0.05 M Phosphate buffer pH 6.2 ± 0.05 adjusted with dilute potassium hydroxide solution, Acetonitrile	27-162 mg/mL, 27-162 mg/mL and 4.5-28 mg/mL	Bulk and pharmaceutical dosage form
13	Lamivudine and Dolutegravir[18]	Inertsil ODS 3V (250 × 4.6 mm, 5 µm) column phosphate buffer, pH 3.0: acetonitrile: methanol (50:20:30% v/v/v)	14.98 to 91.25 µg/mL and 2.54 to 15.35 µg/ mL	Bulk and pharmaceutical dosage form
14	Lamivudine and tenofovir[19]	Inertsil C18 column (15 cm x 4.6 mm, 5 µm) phosphate buffer (6.5 mM) adjusted to pH 2.5 with orthophosphoric acid and acetonitrile (50:50 v/v).	60-140 µg/ml and 180-420 µg/ml	Bulk and pharmaceutical dosage form

15	Lamivudine and tenofovir disproxil fumarate[20]	Thermo scientific TM hypersil TM BDS 5µ C18 120 A Acetonitrile and phosphate buffer pH3.5 (80:20%v/v)	15-35 µg / ml	Bulk and tablet
16	Lamivudine, abacavir and dolutegravir[21]	non polar column-Kromasil 250 mm × 4.5 mm, 5 µm, Buffer: acetonitrile (65:35v/v)	15 to 90 ppm, 30 to 180 ppm and 2.5 to 15 ppm	Bulk and pharmaceutical dosage form
17	Lamivudine and tenofovir disoproxil fumarate[22]	Agilent eclipse XDB-C18 (5 µm, 4.6 mm × 150 mm) column methanol and potassium di-hydrogen orthophosphate (0.02 M) in the ratio 60:40 v/v	5-50 µg / ml	Bulk and pharmaceutical dosage form
18	Dolutegravir Sodium, Lamivudine and Tenofovir Disoproxil Fumarate[23]	*C18 symmetry C-18 column (250 × 4.6 mm i.d., 5.0 µm)* sodium dihydrogen phosphate with SDS (PH: 2.0) and ACN (20:80%v/v)	0.05-7.5 µg/ml	Bulk and tablet dosage form
19	Dolutegravir Sodium[24]	Acetonitrile: Water (Ph 7.5) (80:20 V/V)	5–35 mg/ml	Bulk and Pharmaceutical Dosage Form

HPTLC

High performance chromatography with a thin layer (HPTLC) was developed as an important medication analysis method as the advances in the technology. HPTLC is a fast, flexible separation process for analysis of a large number of samples. This method is in many ways beneficial because it is easily managed and requires a short time for analysis of the raw sample clean-up complex. HPTLC assesses all chromatograms without time constraints with a number of parameters. In addition, multiple samples and standards on each plate are being produced concurrently but separately, resulting in greater reliability of performance. HPTLC is used for quantitative use of medications, such as ethinyl estradiol, cyposterone, alfuzosin and pentazocin.[25]

Table 2: Performance Characteristics of HPTLC

Sl. No	Drug	Mobile Phase	Linearity Range (µg/ml)	Application
1	Lamivudine and tenofovir disoproxil fumarate[26]	Silica gel 60 F254 plates, Chloroform: Methanol (9:1v/v)	100-700ng/ band	Bulk drug and tablets; SIAM
2	Lamivudine and tenofovir[27]	20mL of toluene and 10mL of methanol	2.000-3.100 µg/ml	Pharmaceutical dosage form
3	Lamivudine, Tenofovir Disoproxil Fumarate and Efavirenz[28]	Chloroform–Methanol–Toluene (9:1.2:0.3, V/V)	400–800 Ng Spot–1, 400–800 Ng Spot–1 And 800–1600 Ng Spot–1 For	Tablet Dosage Form
4	Lamivudine and Tenofovir Disoproxil Fumarate[29]	Chloroform: Methanol: Toluene (8: 2: 2, V/V/V)	60-210 Ng Spot-1	Pharmaceutical Dosage Form
5	Dolutegravir Sodium[30]	Acetonitrile: Water (Ph 7.5) (80:20 V/V)	5–35 mg/ml	Bulk and Pharmaceutical Dosage Form

UPLC

Ultra-Performing liquid chromatography for particles under 2µm of diameter to achieve better resolution, velocity and sensitivity than liquid chromatography of high-performance (HPLC). In 20 first century pharmaceutical markets, new approaches are being explored and drug production time are being shortened. UPLC analysis in the meantime offers improved product consistency, and this growth is no exception in analytical laboratories. The UPLC is isolated and quantified under very high pressure (up to 100M Pa).[31]

Table 3: Performance Characteristics of UPLC

Sl. No	Drug	Mobile Phase	Linearity Range (µg/ml)	Application
1	Dolutegravir[32]	50 mmol/L formic acid and 50 mmol/L ammonium acetate in water (mobile phase A), and 100% acetonitrile (mobile phase B)	0.25–10 mcg/mL	Plasma
2	lamivudine, Abacavir and Dolutegravir[33]	phosphate buffer (pH 3.0) and methanol (30:70 %v/v)	15-75, 30-150 and 2.5 -12.5	Bulk and pharmaceutical dosage form
3	lamivudine and tenofovir disoproxil fumarate[34]	carbon di-oxide /methanol (containing 0.5 % v/v n-butylamine)	1.5 -7.5 µg/mL and 7.5 - 37.5 µg/mL	Bulk and pharmaceutical dosage form
4	Dolutegravir[35]	10 mM acetate buffer (pH 4.0) and methanol	-	Degradation study
5	Lamivudine and Dolutegravir[36]	Potassium dihydrogen orthophosphate pH 3 adjusted with orthophosphoric acid: methanol (30:70% v/v)	105 to 315 µg/ml and 17.5 to 52.5 µg/ml	Bulk and tablet dosage form
6	Dolutegravir And Rilpivirine[37]	0.1% ortho phosphoric acid and acetonitrile in the ratio 55:45%v/v	12.5 – 75.0 µg/mL and 6.25 – 37.5 µg/mL	Bulk and pharmaceutical dosage form

UV Visible spectroscopy

Spectrophotometric methods based on UV absorption and chemical reactions are a tool that is important in pharmacopeia. The quantitative analysis of a material's reflection or transmission properties as a function of the wavelength is spectrophotometry. Low time and labor consumption are the advantage of these approaches. These techniques are also very accurate and accurate. In the past couple of years, it has been rapidly increasing the use of UV-vis spectrophotometry especially in the method of developing the pharmaceutical dose. We know much of the atomic and molecular structure by observing the interaction of atoms and molecules with light (EMR). As a result of such interactions, EMR spectrum regions provide various types of information.[38]

Table 4: Performance Characteristics of UV Visible spectroscopy

Sl. No	Drug	Diluent and colouring reagent	λ_{max}(nm)	Linear Range (µg/ml)	Application
1	Tenofovir disoproxil fumarate[39]	Distilled water	220	4 to 14	Forced degradation study
2	.amivudine and tenofovir disoproxil fumarate[40]	Water: water (50:50v/v)	268 and 258	*2-10*	Bulk a pharmaceutical dosage form
3	Lamivudine[41]	Water (MBTH, IDBA and resorcinol)	590 and 540	10- 60	Pure and tablet dosage form
4	Lamivudine[42]	Water (2,4-dinitrophenylhydrazine solution)	438	5-35	Pure and tablet dosage form
5	Lamivudine[43]	Water (nitrous acid and beta naphthol)	553	5-20	Tablet
6	Dolutegravir[44]	Methanol	259.80	5- 40	tablet

7	Dolutegravir[45]	Phosphate buffer	255	-	In vitro dissolution study
8	Lamivudine and tenofovir[46]	Water	271	5 - 40	Bulk and combined dosage form
9	Tenofovir disoproxil fumarate[47]	Water (Nitrous acid, Phloroglucinol and Resorcinol)	520 and 600	2- 10	Bulk and pharmaceutical dosage form
10	Efavirenz, Tenofovir disoproxil fumarate and Lamivudine[48]	methanol: water (50:50)	247, 259 and 272	.0-60, 5-30 and 5-30	Multicomponent analysis

LC -MS Techniques

LC/MS is increasingly evolving and is a favourite method for liquid chromatographs. LC/MS is the preferred chromatographic tool. Liquid spectrometry-chromatographic mass (LC-MS/MS) is a method utilizing mass spectrometry fluid chromatography (HPLC). Analytical chemistry combines the ability to isolate liquid chromatography (or HPLC) mechanically with the ability of mass spectrometry for mass analysing. In laboratory research for drug ingredients, medical products and biological samples (LC-MS/MS) is used widely in quality and quantity analysis. It has persistently been used in the development of drugs at several different levels, including metabolic stability screening, metabolite detection, live drug screening, impurity identification, peptide mapping, glycoprotein mapping. In several fields, LC-MS has successfully been implemented, including therapeutic medicinal monitoring (TDM) as well as clinical and forensic toxicology and control of doping. This development in the LC-MS was originally and is still motivated by the need for stronger analytical and bio-analytical techniques which are sensitive and selective in accurately and accurately discriminating target analytes from high complexity mixtures. The use of liquid (LC) and mass spectrometric (MS) chromatography has become a potent technique with two-dimensional hyphenated (2D) developments in instrumentation.[49]

Table 5: Performance Characteristics of LC-MS Techniques

Sl. No	Drug	Detection	MP / Reagent	Application
1	Lamivudine[50]	LC–MS/TOF and MS*n*	water, 0.1N HCl, 0.1N NaOH and 3% H2O2	SIAM, Nature of degradation products, pathways of mass fragmentation of the drug and degradation products
2	Tenofovir Prodrug[51]	LC-MS/MS	DMSO (TAF) or water (TFV)	Plasma
3	Dolutegravir[52]	electrospray ionization in positive ion mode on an AB Sciex API-5000 triple quadrupole MS.	Methanol: acetonitrile (0:50 v/v) with 2% formic acid	Hair
4	Tenofovir Disoproxil fumarate[53]	positive ionization mode on the triple quadrupole MS	water (containing 0.1% formic acid) and acetonitrile (90:10, v/v).	Human Plasma and toxicity Monitoring
5	Lamivudine, emtricitabine and tenofovir[54]	TSQ Quantum Ultra triple quadrupole MS	0.1% formic acid in water and 0.1% formic acid in acetonitrile.	Dried blood and Dried breast milk
6	Tenofovir, Lamivudine and Nevirapine[55]	triple quadrupole instrument operated in the negative ionization mode.	1 mM ammonium acetate in water (pH 6.5 ± 0.3): acetonitrile (50:50, v/v)	Human plasma
7	Tenofovir, Emtricitabine, and Lamivudine[56]	electrospray ionization in the positive mode on an AB Sciex API-5000 triple quadrupole MS	750mM ammonium acetate (A) and 75:25 5mM ammonium acetate: acetonitrile, pH 10.1 (B)	Dried blood
8	Lamivudine and abacavir triphosphate[57]	electrospray ionization (ESI)	40% ACN, 0.06% acetic acid, and 10 mM ammonium formate in water, and	Metabolite in mouse blood and Tissue

			30%ACN, 0.3% ammonium hydroxide, and 1 mM ammonium formate, in deionized water.	
9	Tenofovir, Emtricitabine, and Lamivudine[58]	multiple reaction monitoring (MRM) and positive ion mode	0.5% formic acid in water and acetonitrile (55:45, v/v)	Human plasma with bioequivalence study
10	Tenofovir[59]	Mass Lynx version 4.0 software	0.3% trifluoroacetic acid, 100% acetonitrile and 100mM ammonium acetate (95: 0: 5 v/v/v)	Plasma
11	Tenofovir and Lamivudine[60]	electrospray ionization in multiple-reaction monitoring mode	0.1% formic acid in water and acetonitrile (90:10 v/v)	Plasma and pharmacokinetic study
12	Lamivudine, Stavudine and Nevirapine[61]	positive ionization mode with an electrospray ionization	methanol–water (20:80, v/v)	Plasma and pharmacokinetic study
13	Lamivudine, Stavudine and Nevirapine[62]	multiple reaction monitoring mode (MRM)	0.5% glacial acetic acid in water: acetonitrile (20:80, v/v)	Plasma
14	Dolutegravir[63]	ESI positive ionization tandem MS	acetonitrile/water (60:40) with 0.1% formic acid.	Human plasma
15	Tenofovir, emtricitabine, and dolutegravir[64]	positive electrospray ionization mode with multiple reaction monitoring (MRM)	water and 0.1% formic acid in acetonitrile	Human brain microvascular endothelial cell
16	Tenofovir[65]	ESI with positive ion mode	3% acetonitrile/1% acetic acid	Plasma
17	Lamivudine and Zidovudine[66]	TurboIon Spray Interface	acetonitrile–water (55:45, v/v)	Human seminal plasma

Capillary electrophoresis

In advancement of the life sciences, capillary electrophoresis (CE) played a major role. This method is now used to analyze large and small molecules in applications in which it works better than fluid chromatography or is complementary to them. In that study, the concepts of different techniques of electromigration are defined and the instrumentation with emphasis on the coupling of mass spepectrometry and microchip (CE) has been developed with a focus on capillary isoelectric focus (CIEF), capillary gel (CGE), and capillary zone electrophoresis (CZE). Routine CE analyzes and latest advances in metabolomic methods are explored for profiling small molecules in biological samples.[67]

Table 6: Performance Characteristics of Capillary electrophoresis

Sl. No	Drug	Buffer	Detection (nm)	Linearity range (µg/ml)	Application
1	Lamivudine, Didanosine And Saquinavir[68]	100nM N, N-dimethyl octylamine in 80mM phosphate buffer (pH 2.5).	UV detector at 210 nm.	0.4–37.8µg/ml 1.4–34 µg/ml and 0.5–24.4 µg/ml.	Human Serum
2	Lamivudine, Stavudine, and Nevirapine[69]	10 mM sodium tetraborate (pH 9.8), 100 mM sodium dodecyl sulfate (SDS) and 15% (v/v) 2-propanol	UV detector of 200 nm.	20–200µg/ml, 5–50µg/ml and 25–250µg/ml.	Pharmaceutical Dosage Form
3	Lamivudine and zidovudine[70]	12.5mM sodium tetraborate decahydrate and 15mM boric acid adjusted at pH 10.8, containing 90mM SDS and 5% (v/v) acetonitrile (ACN)	210 nm.	10–80 µg/ml and 10–100 µg/ml.	Pharmaceutical Dosage Form

Electrochemical method

The analysis of the chemical reaction of electrochemical an electric stimulation system. The scientist The Studying electrical failure electrochemistry (oxidation) or electron gain (reduction) of the material During the stimulation of electricity. This decrease and Reactions to oxidation are commonly referred to as redox reactions and details can be given Mechanisms of reaction, concentration, kinetics, chemical Species in solution status and other behaviour. Apparent Electrode knowledge can be obtained surface area.[71]

Table 7: Performance Characteristics of Electrochemical method

Sl. No	Drug	Methods	Linearity Range	Application
1	Lamivudine[72]	hanging mercury drop electrode (HMDE)	2×10^{-6} to $\times10^{-4}$ M	Human Serum and pharmaceutical formulation
2	Lamivudine[73]	Ti/SnO2-Sb/Ce-PbO2 anode	-	Electrochemical Degradation Study
3	Lamivudine[74]	voltametric detection	-	Hepatitis B Virus Genotype
4	Lamivudine and Tenofovir Disoproxil fumarate[75]	cyclic voltammetry (CV), linear sweep voltammetry (LSV), chronoamperometry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS).	5-18 μM	Pharmaceutical formulation
5	Tenofovir[76]	Voltammetry Techniques: cyclic, differential pulse, square wave and adsorptive stripping	6.0x10-7– $6.0x10^{-5}$	Pharmaceutical formulation
6	Tenofovir[77]	boron-doped diamond electrode (BDDE) -square-wave voltammetry (SWV)	5.0 × 10−6 to 1.0 × 10−4 mol L−1	Pharmaceutical formulation
7	Tenofovir[78]	adsorptive stripping differential pulse and adsorptive stripping square wave voltammetry.	6.0×10^{-8}–1.0×10^{-6}M	Tablet
8	Lamivudine[79]	Ion Selective Electrode	10-6 to 10-2 mol L-1	Bulk, Pharmaceutical formulation, Plasma and Impurity study
9	Lamivudine and Dothiepin[80]	platinum indicator electrode	-	Pharmaceutical formulation

Infrared spectroscopy

The measurement by absorption, emission or reflection of the interaction of infrared irradiation with material is infrared spectroscopy (IR Spectroscopy or Vibration Spectroscopy). It is employed in the analysis and recognition of solid, liquid or gaseous chemicals or functional groups. The infrared spectroscopy process or technique is done with an instrument called an infrared spectrometer, which produces an infrared spectrum. The infrared component of the electro-magnetic spectrum is commonly divided into three areas, naming their relationship to the visible spectrum: the near-, mid- and far-infrared. Higher energy close to the IR will excite overtone or molecular vibration combinations of about 14,000–4,000 cm^{-1} (0.7–2.5 µm wavelength). Midinfrared is used for studying fundamental vibrations and related rotational vibrational structures approximately 4,000 to 400 cm^{-1} (2.5–25 microns). Approximately 400-10 cm^{-1} (25–1,000 µm) far-infra range is low in energy, ideal for use with low frequency and rotational spectroscopy.[81]

Table 8: Performance Characteristics of Infrared spectroscopy

Sl. No	Drug	Study	Form	Application
1	Lamivudine and Theophylline[82]	FT-IR spectroscopy and MCR-ALS	polymorphs I and II of new pharmaceutical co-crystals	API
2	Lamivudine[83]	Vibrational spectroscopic study-FTIR	polymorphism and polymorphic transformation	Pharmaceutical dosage form
3	Lamivudine[84]	FTIR, DSC, scanning electron microscopy (SEM)	Cellulose polymers	Microcapsule and in vitro dissolution studies
4	Lamivudine[85]	FTIR	Polymethacrylic Acid	Drug delivery nanoparticle
5	Zidovudine and Lamivudine[86]	NIR (PLS and MLR)	-	Chemical image and quality control study of tablet
6	Lamivudine[87]	SCXRD, PXRD and FTIR spectroscopy.	Cytosine salt	Co-crystal screening
7	Zidovudine and Lamivudine[88]	Thermal analysis and infrared spectroscopy	hydroxypropyl methylcellulose films	Pharmaceutical dosage form
8	Tenofovir disoproxil fumarate[89]	optical microscopy, single crystal X-ray crystallography, powder X-ray diffraction, **Fourier transform infrared spectroscopy**, Raman, solid-state **NMR**, differential scanning calorimetry, thermogravimetric analysis, and organic vapor sorption isotherms	Polymorphs	Characterization and Anisotropic Lattice Expansion/Contraction of API

NMR Spectroscopy

The NMR Spectroscopy is a spectroscopic technique that monitors local magnet fields around atomic nuclei, most commonly known as NMR or Magnetic Resonance Spectroscopy (MRS). The sample is put in a magnetic field and the NMR signal is generated by a nuclear-resonant arousal of the nuclei sample with radio waves which is sensitive radio receivers detect. The intra-molecular magnetic field around an atom in a molecule changes the frequency of the resonance, thus providing information on the electronic structure and functional groups of a molecule.[90]

Table 9: Performance characteristics of NMR Spectroscopy

Sl. No	Drug	m/z value	Application
1	Dolutegravir[91]	182.44 (DP)	Degradation Study
2	Tenofovir disoproxil fumarate[92]	288 (TDF) 302(DP-1) 316(DP-2)	Characterisation of Degradant
3	enofovir, tenofovir disoproxil fumarate and emtricitabine[93]	248(emicitrabine)	Fragmentation pattern, stability and Sequestration of sodium cation in TDF purine moiety
4	Lamivudine and tenofovir[94]	471(isomeric product)	Structures of two isomeric interaction products

Microbiological assay

Microbial or microbiological assays may be a kind of bioassays for the study of micro-organism compounds, or substances. They contribute to estimating antibiotic concentration and effectiveness. It is also important to decide the simplest antibiotic suitable for patient recovery. This is possible by immune testing for only a few illnesses.[95]

Table 10: Performance characteristics of Microbiological assay

Sl. No	Drug	Method	Stain/ Cell Line	Application
1	Dolutegravir[96]	MTT Assay and The Syncytia Inhibition Assay in C8166 -	*Portunus Sanguinolentus*	Chitosan Nanoparticles Loaded with Dolutegravir As Milk and Food Admixture
2	Lamivudine[97]	COBAS AMPLICOR Assay	Hepatitis B Virus	Drug Resistance
3	Lamivudine[98]	Line Probe Assay (INNO-Lipa HBV DR)	Hepatitis B Virus (HBV) Strains	Drug Resistance in Serum Sample
4	Lamivudine[99]	Real-Time PCR Assay	Hepatitis B Virus Strain	Drug Resistance in Biological Sample
5	Lamivudine[100]	-	*Mycobacterium Tuberculosis* Strain	AntiHIV And Anti Tubercular Activity
6	Lamivudine[101]	*In Vitro* Studies	COLO-205 Cell Lines	AntiColon Cancer Activity
7	Lamivudine and Adefovir[102]	Sensitive Line Probe Assay	Hepatitis B Virus	Detects Mutations Conveying Resistance to Lamivudine and Adefovir
8	Lamivudine[103]	*In Vitro* Studies	Lamivudine-Resistant Strains Of Hepatitis B Virus	Drug Resistance
9	Lamivudine and Emtricitabine[104]	Pyrimidine Assay	-	Biological matrix (urine)

CONCLUSION

Many of the medications now have been synthesized for a day also shows many subsequent effects of their products. For the chemical and pharmaceutical analysis of the drug effective quality control and pharmacodynamic and pharmacokinetic studies are needed. The study revealed that the determination of Lamivudine, Dolutegravir and tenofovir disoproxil fumarate fixed drug combination in pharmaceutical and biological materials is focused on HPLC, HPTLC, UPLC, spectrometric, electrochemical, IR, NMR microbiological assay, capillary electrophoresis and Electrochemical technology. However, UV-detector HPLC is commonly used in pharmaceuticals and LC-MS are widely used for biological materials with mass and tandem mass spectrometer detector systems. These techniques are applicable for detection API in bulk and pharmaceutical dosage form as well as on different biological matrix, in vitro dissolution studies, stability indicating study, structural isomerism, fragmentation pattern, biological activity studies…Invitro dissolution studies are useful for bioequivalence study in batch-to-batch manufacturing field as well for quality control testing.

RP-HPLC with UV-detection is, due to its high separation ability, selectivity, and sensitivity, the most commonly used chromatography tool for lamivudine, dolutegravir and tenofovir disoproxil fumarate combination determination in pharmaceutical. HPLC, however, is still to be identified with other detector systems, including fluorescence, electrochemical, capillary, and LC/MS. The need to develop a stability-specific evaluation system (SIAM) has become clearer with the advent of the guidelines of the International Conference on Harmonization (ICH). The Guidelines require specifically that forced decomposition studies be carried out in a variety of stress conditions such as pH, illumination, oxidation, dry heat, etc., and that drugs be isolated from degradation products.

Because of their sensitivity and selectivity, electrochemical methods were highly useful for drug testing. The methods also make electrodes well known. Redox's drug properties offer valuable information on your metabolic destiny or in vitro redox properties and drug behaviors. The complex production has been observed of CE methods for evaluating drugs but there are restricted uses for the Lamivudine, dolutegravir and tenofovir disoproxil fumarate testing.

Different microbiological assay was used for detection of antiviral activity of lamivudine, Dolutegravir and tenofovir disoproxil fumarate. In case of lamivudine resistance to hepatitis B viral activity also studied using microbiological assay. Using NMR and IR spectroscopic techniques, helpful to find out structural activity characterization of drug, fragmentation pattern, Co-crystal arrangement, screening of characteristic degradation product, and impurity detection. Finally, the testing of Terbinafine in pharmaceuticals and biological material was possible with a large range of techniques. The investigation study of the methods showed that for the determination of the drug in both pharmaceutical and biologic materials LC-MS was used extensively with mass and tandem mass spectrometer detector systems.

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