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A REVIEW ON DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF LAMIVUDINE, TENOFOVIR DISPROXIL AND DOLUTEGRAVIR IN BULK AND COMBINED DOSAGE

Nachiket Dighe, Prachi P. Kanawade* and Ganesh S. Shinde

Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravanagar, Tal- Rahata, Dist. - Ahmednagar.

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***Corresponding Author**
Prachi P. Kanawade
Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravanagar, Tal- Rahata, Dist. - Ahmednagar.

ABSTRACT

Lamivudine is an antiretroviral medication used to prevent and treat HIV or AIDS. It is also used in the treatment of hepatitis B when other options are not possible. The drug lamivudine was patented in 1995 and was approved for use in the U.S. in 1995. It shows its action by blocking the enzyme HIV reverse transcriptase. Lamivudine comes under the category of nucleoside reverse transcriptase inhibitor. Tenofovir disproxil is an agent which can be used with other antiretroviral agents, shows its action as a nucleotide reverse transcriptase inhibitor and lowers the ability of the virus to replicate. It is used when there is high risk before exposure for treatment of HIV or AIDS. Dolutegravir is an antiretroviral agent which is also used for prevention and in the treatment of HIV or AIDS. It is a HIV integrase strand transfer inhibitor. It works by blocking the functions of HIV integrase which is important for replication of virus. Dolutegravir and Abacavir and Lamivudine combination is widely used to treat HIV. The objective of this review is, to describe and develop simple, accurate and selective method for estimation of Lamivudine, Tenofovir Disproxil and Dolutegravir in the bulk and tablet dosage form by RP-HPLC technique. The RP-HPLC technique can be validated by various parameters like system suitability, method precision, accuracy, linearity, limit of detection and limit of quantitation, robustness, etc.

KEYWORDS: Lamivudine, TenofovirDisproxil, Dolutegravir, HIV, Hepatitis B, RP-HPLC.

INTRODUCTION

Human immunodeficiency virus (HIV) is that the virus that causes acquired immune deficiency syndrome (AIDS) and is transmitted through contact with infected blood and bodily fluids. Such contact can occur through unprotected sex, through sharing of needles or other drug injection equipment, through mother-to-child transmission during pregnancy or breast-feeding.

HIV infects immune cells in the body called CD4 positive (CD4+) T cells, which are essential for fighting infections. HIV converts these cells into "factories" that produce more of the HIV virus to infect other healthy cells, eventually destroying the CD4+ cells.

HAART may be a customized combination of various classes of medicines that a physician prescribes supported such factors because the patient's viral load, the particular strain of the virus, the CD4+ cell count, and other considerations (e.g., disease symptoms). Because HAART cannot rid the body of HIV, it must be taken a day for all times. HAART can control viral load, delaying or preventing the onset of symptoms or progression to AIDS, thereby prolonging survival in people infected with HIV.[16]

Component I: Lamivudine

Drug profile

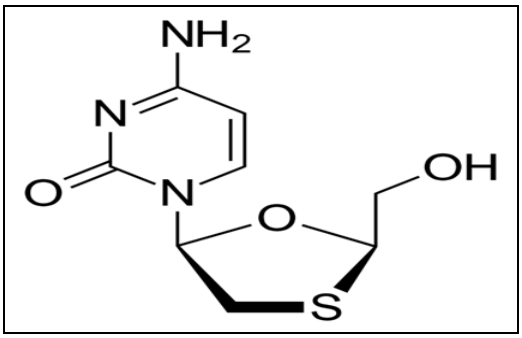

Fig No.01 Chemical structure of Lamivudine.

The IUPAC name for Lamivudine is: 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. comes under the trade name Epivir.[14]

Lamivudine is a synthetic nucleoside analogue with activity against hepatitis B virus (HBV) and HIV. Intracellularly, lamivudine is phosphorylated to its active metabolites, lamiduvine triphosphate (L-TP) and lamiduvine monophosphate (L-MP). In HIV, L-TP

inhibits HIV-1 polymerase (RT) via DNA chain termination after incorporation of the nucleoside analogue into viral DNA. In HBV, incorporation of L-MP into viral DNA by HBV polymerase leads to DNA chain termination. L-TP may be a weak inhibitor of mammalian DNA polymerases alpha and beta, and mitochondrial DNA polymerase.

Component II: Tenofovir Disproxil

Drug profile

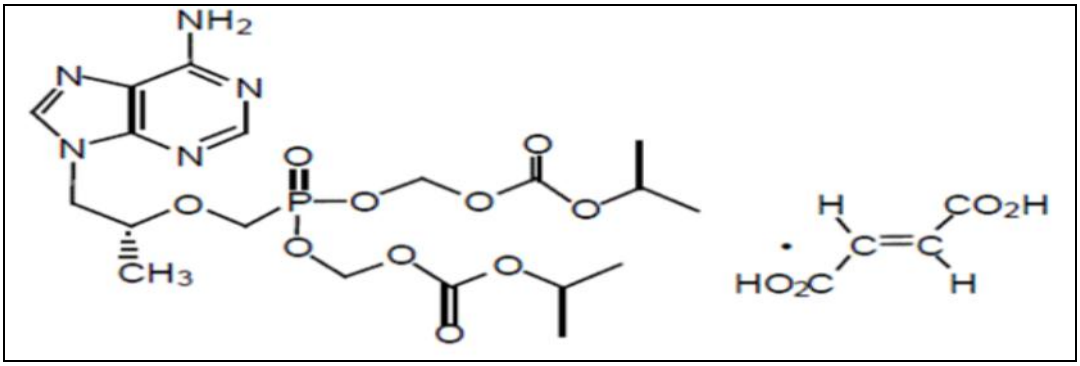

Fig No.02 Chemical structure of Tenofovir Disproxil.

The IUPAC name of Tenofovir disproxil is Bis{[(isopropoxycarbonyl)oxy]methyl} ({[(2R)-1-(6-amino-9H-purin-9-yl)-2-propanyl]oxy}methyl)phosphonate having molecular formula C19H30N5010P. The molecular weight of Tenofovir Disproxil is 519.443gm/mol. It is white crystalline powder having melting point 229^{o}C. It is a nucleotide reverse transcriptase inhibitor. It inhibits reverse transcriptase, important enzyme in HIV. Tenofovir Disproxil also inhibits human enzyme like DNA polymerase. It is a analogue of adenosine.[15]

Component III: Dolutegravir

Drug profile

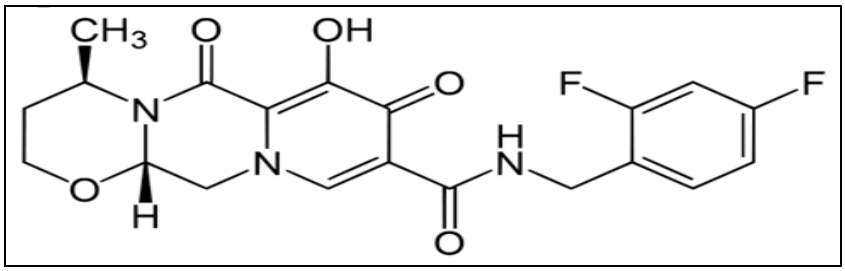

Fig.No.03 Chemical structure of Dolutegravir.

It is (4*R*,12a*S*)-*N*-(2,4- difluorobenzyl)-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 Dolutegravir having molecular formula: C20H19F2N3O5 with a molecular weight

419.38gm/mol. It shows melting point 190-193^{o}C. Dolutegravir is an orally bioavailable integrase strand-transfer inhibitor (INSTI), with activity against human immunodeficiency virus type 1 (HIV-1) infection. Upon oral administration, dolutegravir binds to the site of integrase, an HIV enzyme that catalyzes the transfer of viral genetic material into human chromosomes. This prevents integrase from binding to retroviral desoxyribonucleic acid (DNA), and blocks the strand transfer step, which is important for the HIV replication cycle. This prevents HIV-1 replication.[14]

Reported Analytical Methods

Chromatographic Methods

Table No.01.

Title	Method	Description	Detection mode
1. Development and validation for the simultaneous estimation of Lamivudine, Tenofovir Disproxil and Dolutegravir in drug product by RP-HPLC.[1]	RP-HPLC	Mobile phase- 0.1%TFA in water : Acetonirile Column- Luna C8 column (150*4.6mm) Flow rate- 1.0ml/min Retension time- Lamivudine – 2.03 min Tenofovir Disproxil- 5.330 min Dolutegravir – 7.673 min	UV at 260nm
2. Development and validation of RP-HPLC method for the Simultaneous estimation of Lamivudine, Tenofovir Alafenamide and Dolutegravir bulk and their combined Dosage Form.[2]	RP-HPLC	Mobile phase- 0.05M phosphate buffer(pH-6.2): acetonitrile(60:40v/v) Column- Agilent C18(250x 4.6 mm) Flow rate -1ml/min. Retension time- Lamivudine-3.09 min Tenofovir alafenamide-6.19 min Dolutegravir- 9.61 min	PDA detector at 260 nm.
3. A validated stability indicating RP-HPLC method for simultaneous determination of Abcavir, Lamivudine, and Dolutegravir in bulk and pharmaceutical dosage form.[3]	RP-HPLC	Mobile phase- phosphate buffer (pH-3.0): Acetonirile: Methanol (50:20:30%v/v) Column- Inertsil ODS 250 x 4.6mm,5µm particle size. Retension time- Lamivudine –2.169 Abacavir- 2.676 Dolutegravir-6.367	PDA detector at 257nm
4.Development and validation of stability- indicating HPLC method for simultaneous estimation of Lamivudine, Tenofovir and Dolutegravir in bulk and their tablet dosage form.[4]	HPLC	Mobile phase- 0.05 M phosphate buffer(pH- 6.2±0.05): Acetonirile Column – reverse phase C18 column(250x 4.6 mm,5µ) Flow rate-1 ml/min Retension time- Lamivudine-2.8 min	UV at 260nm

		Tenofovir- 5.2 min Dolutegravir- 11.5 min	
5.Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine and Tenofovir Disproxil Fumerate in Combined Dosage Form.[5]	RP-HPLC	Mobile phase- HPLC Methanol : Water(pH-3.2),70:30 v/v Column- COLUMN-ENABLE C18 G 5 µM, 250×4.6mm Flow rate- 1mL/min Retension time- Lamivudine - 3.048min Tenofovir Disproxil fumerate-5.354min	UV at 260nm
6.Development and validation for the simultaneous estimation of Dolutegravir and Lamivudine in drug product by RP-HPLC.[6]	RP-HPLC	Mobile phase- 0.1%v/v TFA in water: ACN (30:70). Column- C18 column (Inertsil ODS 3V 250*4.6mm) Flow rate- 0.8ml/ minute Retension time- lamivudine- 2.373min Dolutegravir- 4.558min	UV at 260nm
7. Development and validation of analytical method for the estimation of Lamivudine and Dolutegravir sodium in dosage form.[7]	HPLC	Mobile phase-Buffer (pH-3): acetonitrile : methanol(55:35:10 v/v) Column-C18 column(150×4.6mm, particle size 5µ) Flow rate-1ml/min Linearity – Lamivudine-18-90µg/ml Dolutegravir sodium-3-15µg/ml	UV at 260nm
8. Simultaneous stability indicating method for the determination of Abacavir, Dolutegravir, Lamivudine by RP-HPLC.[8]	RP-HPLC	Mobile phase- acetonitrile: water Column-Kinetex 5 µ C18 100 A (250 mm x 4.6 mm). Flow rate-1ml/min Retention time- Abacavir-5.2 min Dolutegravir-8.4 min Lamivudine-3.1min	DAD detector at 258 nm.
9.Development and Validation of Analytical Method for Determination of Dolutegravir Sodium, Lamivudine and Tenofovir Disoproxil Fumarate Using Reverse Phase High Performance Liquid Chromatography.[9]	RP-HPLC	Mobile phase-acetonitrile and methanol Column-C18 symmetry C-18 column (250 × 4.6 mm i.d., 5.0 µm) Flow rate-1.0 ml/min Retension time- Dolutegravir Sodium-11.0 min Lamivudine-5.2 min TenofovirDisoproxilFumarate-13.0 min	UV detector at 260nm
10.RP-HPLC Analytical Method Development And Validation For Lamivudine And Zidovudine In	RP-HPLC	Mobile phase- Methanol: Phosphate buffer (55:45 % v/ v) Column: C18 column (150mm x 4.6 mm)	UV at 271 nm

Pharmaceutical Dosage Forms.[10]		Flow Rate : 0.5ml/min Linearity – Lamivudine-10-50 µg/ml Zidovudine-10-50 µg/ml	
11.Method Development and Validation for Simultaneous Estimation of Lamivudine and Zidovudine by RP-HPLC.[11]	RP-HPLC	Mobile phase: phosphate buffer (pH 3) and ACN(70:30, v/v) Column: Shiseido C18 (250 mm x 4.6 mm, 5 µm particle size) Flow rate: 1.0 ml/min Retention time- Lamivudine- 2.512 min Zidovudine-3.721 min	UV at 228 nm
12. Simultaneous estimation of lamivudine, Abacavir and Dolutegravir by UPLC method.[12]	UPLC	Mobile phase:phosphate buffer (pH 3.0) and methanol (30:70 %v/v) Column:zodiac sil RP C18 (4.6 mm Ã— 250 mm, 3.0 Âµm) Flow rate:0.25 ml/min Retension time- Lamivudine-1.763 min Abacavir-2.247min Dolutegravir-3.175 min	UV at 260 nm
13.Development and validation for the simultaneous estimation of Lamivudine and dolutegravir in drug product by RP-HPLC.[13]	RP-HPLC	Mobile phase- 0.1%v/v Trifluoro acetic acid in water: Methanol (300:700) Column: C18 column (Eclipse XDB-Phenyl 250*4.6mm) Flow rate: 1.0ml/ min Retension time- Lamivudine-2.412 min Dolutegravir-3.263 min	UV at 260 nm

CONCLUSION

The above study gives the analytical methods for analysis of Lamivudine, Tenofovir disproxil and Dolutegravir in their bulk and combined dosage form by using RP-HPLC methods and UV spectroscopic methods. The validation parameters such as precision, accuracy, linearity, analysis time are performed. Literature survey reveals that various methods are reported for the development and validation of various drugs. These methods are reported and published for various parameters as ICH guidelines. These methods give ideas for development and validation of new analytical methods.

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