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A REVIEW ON ESTIMATION OF EMTRICITABINE AND TENOFOVIR DISOPROXIL FUMARATE IN BULK AND IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Antiretroviral therapy has ability to reduce HIV infection by increasing CD4+ (cluster of differentiation) count. Emtricitabine belong to class nucleoside reverse transcriptase inhibitor and Tenofovir Disoproxil fumarate belong to nucleotide reverse transcriptase inhibitor but same use (HIV1 and hepatitis B) and same route of transmission. The literature has revealed that number of method have been published for estimation of Emtricitabine and Tenofovir Disoproxil fumarate like RP-HPLC, Spectrophotometric (UV), UPLC, etc. These methods were reported for analysis. The review gives information of Development and validation of Emtricitabine and Tenofovir Disoproxil fumarate in bulk and pharmaceutical dosage form and validation as per ICH Guideline. This is a Fixed drug combination available in this era. These drugs are also compatible with other antiretroviral drugs having greater or equal potency and less side effect than traditional antiretroviral drugs.

KEYWORDS: Emtricitabine, Tenofovir Disoproxil fumarate, Analytical methods, Antiretroviral.

INTRODUCTION

The Human Immunodeficiency Virus (HIV) is a retrovirus that infects & causes reduction in CD4+ (helper & inducer). CD4+ count in normal human being is 500-1500 cells/µl. AIDS is specific opportunistic infection where CD4+ Count is <200 cells/µl. HIV types HIV-1 &

HIV-2 but HIV-1 is more virulent and causative than HIV-2. HIV infection has a very complex pathogenesis and varies substantially in different patients. Therefore, it can easily be considered as a very host-specific infection.[2] The specificity of pathogenesis often complicates treatment options that are currently available for HIV infection. Effective management of HIV infection is possible using different combinations of available drugs. This method of treatment is collectively known as antiretroviral therapy. Effective antiretroviral therapy often helps control the multiplication of HIV in infected patients and increases the count of CD4+ cells, thus, prolonging the asymptomatic phase of infection, slowing the progression of the disease, and also helps in reducing the risk of transmission.

Route of transmission of HIV is Sexual Transmission, Transmission via Blood & blood products, Maternofetal transmission, Occupational Transmission, Transmission by other body fluids.[3]

Diagnosis by It is confirmed by demonstrating certain serological tests ELISA, Western blot test, Test for defects in immunity – CD4+ T cell count.[7] Tenofovir Disoproxil fumarate is formulated in binary mixture with the reverse transcriptase inhibitor Emtricitabine (EMT) to prevent HIV from altering the genetic material of healthy T cells. Combining the two drugs in one tablet helps in reduction of the pill burden and increases the compliance with antiretroviral therapy.[9]

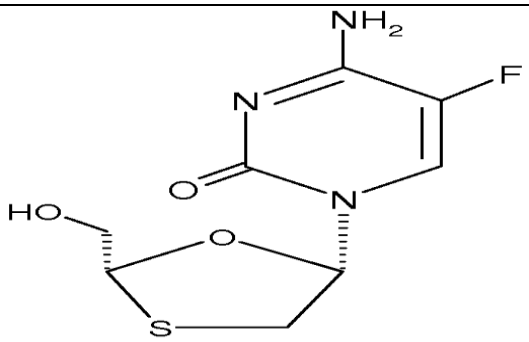
Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI). Chemically, it is 5-fluoro-1-(2R,5S)-[2-(hydroxyethyl)1,3-oxathiolan-5-yl] cytosine. EMT is the (–) enantiomer of the thio analog of cytidine, which differs from other cytidine analogs in that it has fluorine in the fifth position. EMT is an antiviral agent used for the prevention of perinatal HIV-1 reverse transcriptase. It is also active against the hepatitis B virus. EMT is phosphorylated by cellular enzymes to form EMT 5-triphosphate, which competes with deoxycytidine 5triphosphate and terminates the amino acid chain of newly forming viral DNA.[12] Tenofovir Disoproxil fumarate salt of bis (isopropyloxycarbonyl oxy methyl ester of (R)-9-(2-phosphono methoxy Spropyl) adenine with fumaric acid is a nucleotide analogue reverse transcriptase inhibitor, which blocks the reverse transcriptase, in HIV infected people.[15]

Tenofovir Disoproxil fumarate is a fumarate salt prepared from equimolar amount of Tenofovir Disoproxil and fumaric acid.[18]

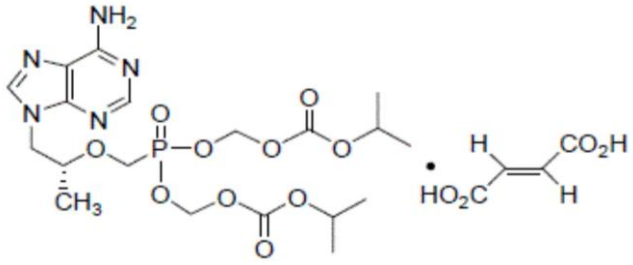
A combination dosage form of Emtricitabine and Tenofovir Disoproxil fumarate is available for treatment of HIV infection. combination of Emtricitabine and Tenofovir Disoproxil fumarate is marketed as a Tablet TENVIR-EM (TDF 300mg +EMT200 mg) and Truvada (EMT 167mg +TDF 250 mg).

Drug Profile

Emtricitabine

Structure	 **Figure 1 – Structure of Emtricitabine.**
IUPAC Name	4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl] pyrimidin-2-one
Molecular formula	$C_8H_{10}FN_3O_3S$
Molecular weight	247.24 g/mol
Appearance	White to off white powder
Category	NRTIs (nucleoside reverse transcriptase inhibitor)
Melting Point	136-140 °C
Solubility	Soluble in Acetonitrile & methanol and slightly soluble in water

Tenofovir disoproxil fumarate

Structure	 **Figure 2 – Structure of Tenofovir Disoproxil Fumarate.**
IUPAC Name	(2E)-but-2-enedioic acid; bis({[(propan-2-yloxy) carbonyl] oxy} methyl) {[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methane phosphonate
Molecular formula	$C_{23}H_{34}N_5O_{14}P$
Molecular weight	635.52g / mol
Appearance	White to off-white crystalline powder
Category	nucleotide reverse transcriptase inhibitor, analogue of adenosine
Melting Point	279°C
Solubility	Soluble in methanol, slightly soluble in water, very slightly soluble in dichloromethane & sparingly soluble in acetone

Mechanism of Action

Emtricitabine works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Emtricitabine is a synthetic nucleoside analogue of cytidine. It is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate, which is responsible for the inhibition of HIV-1 reverse transcriptase. It competes with the natural substrate deoxycytidine 5'-triphosphate and incorporates into nascent viral DNA, resulting in early chain termination. Therefore, emtricitabine inhibits the activity of HIV-1 reverse transcriptase (RT) both by competing with the natural substrate deoxycytidine 5'-triphosphate and by its incorporation into viral DNA. By inhibiting HIV-1 reverse transcriptase, emtricitabine can help to lower the amount of HIV, or "viral load", in a patient's body and can indirectly increase the number of immune system cells (called T cells or CD4+ T-cells). Both of these changes are associated with healthier immune systems and decreased likelihood of serious illness. NRTIs and NTRTIs (nucleoside/tide reverse transcriptase inhibitors) lack a 3'-hydroxyl group on the deoxyribose moiety. As a result, following incorporation of an NRTI or an NtRTI, the next incoming deoxynucleotide cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain. Thus, when an NRTI or NtRTI is incorporated, viral DNA synthesis is halted, a process known as chain termination. All NRTIs and NtRTIs are classified as competitive substrate inhibitors.[5]

Pharmacokinetic data

1. Route of Administration: By Oral (Tablets)
2. Bioavailability: 93%
3. Protein binding: Very low (less than 4%)
4. Metabolism: Hepatic oxidation and glucuronidation. CYP system not involved
5. Elimination half-life: 10 hours
6. Excretion: Renal (86%) and fecal (14%)

Reported Analytical Methods

Title	Method	Description
[6] A Validated RP-HPLC Method for Simultaneous Estimation of Tenofovir Disoproxil Fumarate and Emtricitabine in a Tablet dosage form	RP-HPLC	**Column**-C18 **Mobile phase** –acetonitrile: potassium dihydrogen phosphate buffer: trimethylamine(70:30:0.5v/v) **Flow rate** - 1.5 ml /min **Retention time** EMT 1.78 Min TDF 2.27 Min. **Run time** -4 Min.
[7] Method development and validation for simultaneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in pure and Tablet dosage form by using RP-HPLC	RP-HPLC	**Column**-C18 **Mobile phase** –Methanol: phosphate buffer (70:30v/v) **Flow rate** - 1 ml /min **Retention time** EMT 2.605Min TDF3.781 Min. **Run time** -4 Min.
[8] A Validated RP-HPLC Method for Simultaneous Estimation of Emtricitabine & Tenofovir Disoproxil Fumarate in Pure and in Tablet Dosage Form	RP-HPLC	**Column**-C18 **Mobile phase** – Acetonitrile: Methanol: Water (30:50:20 v/v) **Flow rate** – 0.6 ml /min **Retention time** EMT 2.77 Min TDF 3.49 Min.
[9] Development and Validation of RP-HPLC Method for the Simultaneous Determination of Emtricitabine and Tenofovir Disoproxil Fumarate in Pharmaceutical Dosage Form	RP-HPLC	**Column**- Inert Shell ODS **Mobile phase** – Methanol: Water (80:20 v/v) **Flow rate** – 1.0 ml /min **Retention time** EMT-2.893 Min TDF -3.932 Min.
[10] Development and Validation of different spectrophotometric method for estimation of Tenofovir Disoproxil Fumarate from bulk drug and Tablets	UV-spectrophotometric method	**Solvent** –Phosphate buffer **ʎ max** - UV at 259nm **Linearity** - 5-45µg/ml. **LOD** - 1.37 µg/ml. **LOQ** - 4.17
[11] Development and Validation of spectrophotometric method for Estimation of Emtricitabine in Tablet Dosage Form	UV-spectrophotometric Method(derivative spectroscopy)	**Method A** EMT ʎ max-241.1 nm LOD-0.0684µg/ml. LOQ-0.207 µg/ml. **Method B** EMT ʎ max-232.7nm LOD-0.185µg/ml. LOQ-0.555µg/ml.
[12] Spectrophotometric Determination of Tenofovir Disoproxil Fumarate	UV Spectrophotometric Method	**Method A** ʎ max-500.2nm Range -2-10µg/ml.

	method A (1,10-phenanthroline) Method B(2,2'-Bipyridyl) Method C(MBTH)	**Method B** ʎ max-511.2nm Range- 5-25 µg/ml. **Method C** ʎ max-640nm Range -5-25µg/ml.
[13] spectrophotometric method Development and Validation for Simultaneous estimation of Tenofovir Disoproxil Fumarate and Emtricitabine in bulk drug and Tablet Dosage Form	**Method A** simultaneous equation **Method B** Absorption ratio	**Method A (simultaneous equation)** ʎ max-259nm ʎ max-286nm **Method B (Absorption ratio)** ʎ max-286nm ʎ max-247.6nm
[14] Simple spectrophotometric method for Determination of Tenofovir Fumarate and Emtricitabine in bulk power in Tablets	UV Spectrophotometric Method **Method -A** **Method -B**	**Method –A** ʎ max-298.5nm **Method -B** ʎ max-251.5nm
[15] development and validation of a Novel Colorimetric Method for Estimation of Emtricitabine in Bulk and Tablet Formulation	Colorimetric Method	ʎ max-559nm linearity -100-500 µg/ml.
[16] Simultaneous spectrophotometric method for Determination of Emtricitabine and Tenofovir Disoproxil Fumarate in three-component Tablet Formulation Containing Rilpivirine Hydrochloride	Simultaneous spectrophotometric method	EMT ʎ max-240.8 nm TDF ʎ max-257.6nm **Range** **Calibration curve -4-12** µg/ml. EMT-4-12 µg/ml. TDF -6-18 µg/ml.
[17] Stability indicating RP-UPLC Method for Assay of Emtricitabine and Tenofovir Disoproxil Fumarate in Bulk and Dosage Form	RP-UPLC	**Column-C18** **Mobile Phase** Potassium Dihydrogen Orthophosphate Buffer: Methanol (45:55) **Flow Rate** -1.2ml/Min **Temperature**-Ambient **ʎ Max**-261nm **Tailing Factor** EMT -2427&3685 FDF-1.16&1.23 **Resolution time** EMT-3.12min TDF-2.23min **%Recovery** EMT-101.48 TDF-103.22
[18] HPLC Method for the Determination of Emtricitabine and Related Degradation Substances	HPLC	**Column**-C18 **Mobile Phase**- ammonium formate: methanol **ʎ Max** -280 nm. **LOD**-0.02 µg/ml. **LOQ**-0.05 µg/ml.

[19] Development and validation of RP-HPLC method for the estimation of antiretroviral drugs and their pharmaceutical formulations	RP-HPLC	**Column**-C18 **Mobile Phase** -Methanol: Phosphate cushion [68:32 % v/v]. **Flow Rate** -1.0 ml/min **Resolution time** EMT-5.54min TDF-9.48min
[20] RP-HPLC method Development and Validation of Emtricitabine in synthetic mixture	RP-HPLC	**Column** -C18 (250mm x 4.6mm,5µm) **ʎ Max** -280 nm **Temperature-** ambient **mobile phase**- sodium dihydrogen orthophosphate: methanol (50:50v/v) **flow rate**- 1ml/minute **Retention time** – EMT-3.5minute **LOD**- 0.0112 µg/ml. **LOQ**- 0.0375µg/ml. **Accuracy** -99.53%
[21] A Simple RP-HPLC Method For Simultaneous Estimation of Tenofovir Disoproxil Fumarate and Emtricitabine in Tablet Dosage Form	RP-HPLC	**Column** -C18 **ʎ Max** -270 nm **Mobile phase**- Acetonitrile: Water (70:30 v/v) **Flow rate**- 1.5 ml/min **Retention time** – EMT- 2.82 min TDF – 4.38 mins

CONCLUSION

This review study encircles basic approach for various analytical methods of analysis of Emtricitabine and Tenofovir Disoproxil Fumarate in bulk and pharmaceutical dosage form, such as RP-HPLC, UPLC, UV-Spectrophotometric, etc. the different chromatographic parameters are used in respective methods, which results in differences in resolution time. This help in determining the selection of optimum chromatographic parameters such as cheaper mobile phase with lesser resolution time. Emtricitabine and Tenofovir Disoproxil Fumarate are available in combination as well as single drugs with less side-effects. As recent literature reviews, suggest that there are only few methods reported for simultaneous estimation of Tenofovir Disoproxil Fumarate and Emtricitabine by RP-HPLC method was optimized and validated as per ICH guidelines.

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