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A REVIEW ON ANALYTICAL METHODS FOR ESTIMATION OF TENOFOVIR DISOPROXIL FUMARATE AND EMTRICITABINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

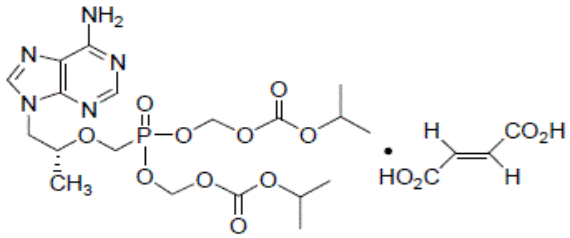
Tenofovir Disoproxil Fumarate and Emtricitabine are very effectively used in the prevention of HIV-1 infections. They are generally administered as tablets. These are Nucleotide Reverse Transcriptase Inhibitors (NtRTIs), an acyclic nucleoside phosphonate (nucleotide) analog of adenosine 5'-monophosphate. Emtricitabine and Tenofovir disoproxil fumarate reveals equally prevention of the enzyme that is HIV-1 reverse transcriptase. For determination of Tenofovir disoproxil fumarate and Emtricitabine in bulk and pharmaceutical dosage form, several analytical methods including UV, HPLC, UPLC and HPTLC techniques are reported in literature. For qualitative and quantitative estimation of Tenofovir disoproxil fumarate and Emtricitabine these analytical methods can be used and also for the related degradants in bulk formulations and biological fluid. The present paper illustrates the review on analytical methods which involves the estimation of the antiviral drugs.

Keywords: Emtricitabine, Tenofovir disoproxil fumarate, UV Spectroscopy, RP-HPLC, UPLC, HPTLC.

INTRODUCTION

The human immunodeficiency viruses (HIV) is grouped to the genus Lentivirus within the family of Retroviridae, initiates the HIV infection and the over time Acquired Immunodeficiency Syndrome (AIDS). The HIV has been categorized as the HIV type-1 and HIV type-2. HIV type-1 is more virulent and more infective than HIV type-2. In the majority cases, HIV is a sexually transmitted infection and arises by contact with or transfer of blood, pre-ejaculate, semen, and vaginal fluids.[1,2] Non-sexual transmission can take place from an infected mother to her infant during pregnancy, childbirth via her blood or vaginal fluid, and breast milk.[3] HIV infects vital cells in the human immune system, for example helper T cells (particularly CD4+ T cells), macrophages, and dendritic cells.[4] HIV infection leads to low levels of $CD4^+$ T cells, whilst $CD4^+$ T cell numbers turn down below a critical level, the cell mediated immunity is lost, and the body is turn out to be gradually more liable to infections, primary to the development of AIDS.[5,6]

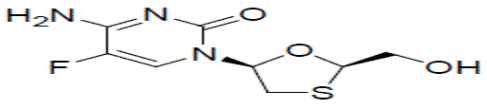
Tenofovir disoproxil fumarate is a prodrug, fumaric acid salt form of a Tenofovir. It is a **9-((R)-2((Bis(((isopropoxycarbonyl)oxy)methoxy)phosphinyl)methoxy)propyl)adeninefumarate**(1:1).[7]
Molecular formula is $C_{23}H_{34}N_5O_{14}P$ and the molecular weight is 635.52gm/mol. It is a nucleotide reverse transcriptase inhibitor (NtRTIs), selectively inhibits the viral reverse transcriptase enzyme crucial for the viral production of Human Immunodeficiency Virus (HIV) infected individuals. This drug prevents viral DNA chain elongation through inhibition of enzymes necessary for host cell infection viral replication in HIV-1 and Hepatitis B infections. [8, 9]



Tenofovir disoproxil fumarate

Emtricitabine is a -4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1H)-pyrimidinone.[7] The molecular formula is $C_8H_{10}FN_3O_3S$ and molecular weight is 247.3 gm/mol.[8, 9] It is a synthetic flouro derivative of thiacytidine with effective antiviral activity. Emtricitabine is phosphorylated to form a emtricitabine 5'- triphosphate within the cell. This metabolite inhibits the activity of HIV reverse transcriptase both by contending with natural

substrate deoxycytidine 5'- phosphate by incorporating into viral DNA causing DNA chain elongation. [9]



Emtricitabine

Combination of the Tenofovir disoproxil fumarate and Emtricitabine is marketed as a tablet (TENVIR-EM) and it constitutes the 300 mg of Tenofovir disoproxil fumarate and 200 mg of Emtricitabine. It is an intended for the treatment of the HIV-1 virus and Hepatitis B infections. Reported methods are categorized depending on the following conditions-

Table 1: Methods for determination of Tenofovir disoproxil fumarate and Emtricitabine single by UV Spectroscopy, Chromatography and other techniques

Sr. No	Drug	Method	Description	Ref No.
1.	Tenofovir Disoproxil Fumarate in tablet dosage form	UV spectrophotometric Method	**Detection wavelength:** 260nm **Solvent:** Methanol **Linearity range:** 10-100µg/ml **Correlation coefficient:**0.9905 **% Recovery:** 99.50%	**10**
2.	Tenofovir Disoproxil Fumarate in API and tablet dosage form	UV spectrophotometric method	**Detection wavelength:** 261nm **Solvent:** Triple distilled water **Linearity range:** 5-90µg/ml **Correlation coefficient:**0.9981 **% Recovery:** 100.062%	**11**
3.	Tenofovir Disoproxil Fumarate in bulk and pharmaceutical formulation	RP-HPLC method	**Detection wavelength:** 260nm **Mobile phase:** Sodium dihydrogen orthophosphate buffer:Methanol (49:51%v/v) **Column:**C18(150mm×2.1mmi.d,5µm) **Flow rate:** 1.0 ml/min **Injection volume:** 20 µl **Linearity range:** 50-300µg/ml **Correlation coefficient:**0.999 **% Recovery:** 99.98% **LOD:** 0.28 µg/ml **LOQ:** 0.85 µg/ml	**12**
4.	Tenofovir Disoproxil Fumarate in pharmaceutical formulation and spiked human plasma	RP-HPLC method	**Detection wavelength:** 259nm **Mobile phase:** Acetonitrile:Water(75:25%v/v) **Column:** CLC C18(25cm×4.6mm i.d., 5µm) **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Linearity range:** 0.2-10µg/ml **Correlationcoefficient:**0.9991 **LOD:** 0.059 µg/ml **LOQ:** 0.199 µg/ml	**13**

5.	Tenofovir Disoproxil in bulk and pharmaceutical formulation	RP-HPLC Method	**Detection wavelength:** 260nm **Mobile phase:** Acetonitrile: 0.05mM Phosphate buffer pH 6.0 (50:50 % v/v) **Column:** Revese Phase Insertsil ODS-3(150×4.6mm), 5µm **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Linearity range:** µg/ml **Correlation coefficient:**0.9954 **% Recovery:** 100.50% **Retention time:**4.45min **LOD:** 0.15 µg/ml **LOQ:** 0.60 µg/ml	**14**
6.	Tenofovir Disoproxil in bulk and pharmaceutical formulation	RP-HPTLC method	**Detection wavelength:** 260nm **Mobile phase:** Chloroform: Methanol(9:1 % v/v) **Flow rate:** 1.0 ml/min **Linearity range:** 300-1500 ng/spot **Correlation coefficient:**0.9994 **% Recovery:** 99.25% **Rf value:** 0.49	**15**
7.	Emtricitabine in tablet dosage form	UV spectrophotometric method	**Detection wavelength:** 241.1nm **Solvent:** **Linearity range:** 5-30µg/ml **Correlation coefficient:**0.9996 **% Recovery:** 99.20% **LOD:** 0.068 µg/ml **LOQ:** 0.207µg/ml	**16**
8.	Emtricitabine in bulk and capsules	Stability indicating RP-HPLC method	**Detection wavelength:** 280nm **Mobile phase:** Buffer: Acetonitrile (85:15 % v/v) **Column:**Phenomenex Luna RP C18(2), 250×4.6mm, 5µm) **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Linearity range:** 20-600µg/ml **% Recovery:** 99.46% **Retention time:** 9.341 min **LOD:** 5.53 µg/ml **LOQ:** 16.78 µg/ml	**17**

9.	Emtricitabine in synthetic mixture	HPLC method	**Detection wavelength:** 280nm **Mobile phase:** Sodium dihydrogen orthophosphate (0.02M): Methanol (50:50% v/v) **Column:** Phenomenex C18, 250×4.6 mm, 5µm) **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Linearity range:** 80-240µg/ml **% Recovery:** 99.53% **Retention time:** 9.341 min **LOD:** 0.0112 µg/ml **LOQ:** 0.0375 µg/ml	**18**
10.	Emtricitabine and related substance (drug substance)	LC method	**Detection wavelength:** 280nm **Mobile phase:** Phosphate buffer (pH 4.4):Water (5:95 % v/v) **Column:** Hypersil BDS C18 25×4.6mm i.d.) **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Linearity range:** 0.1-0.625µg/ml **Retention time:** 9.0 min	**19**

1.	Emtricitabine from drug substance matrix	UPLC method	**Detection wavelength:** 284nm **Mobile phase:** Potassium dihydrogen phosphate buffer (0.015M) pH 2.2 : Acetonitrile (75:25 % v/v) **Column:** Waters ACQUITY BEH C18, 50×2.1 mm, 1.7µm) **Flow rate:** 0.25 ml/min **Injection volume:** 1.0µl **Linearity range:** 50.38-151.13µg/ml **% Recovery:** 100.43% **Retention time:** 1.2 min **LOD:** 0.503 µg/ml **LOQ:** 1.511 µg/ml	**20**
12.	Emtricitabine in bulk and pharmaceutical dosage form	HPTLC method	**Detection wavelength:** 284nm **Mobile phase:** Toulene:Ethyl acetate: Methanol(2:8:1 % v/v) **Linearity range:** 30-110 ng/spot **Correlation coefficient:**0.9997 **% Recovery:** 100.88% **Rf value:** 0.26 **LOD:** 10 ng/spot **LOQ:** 30 ng/spot	**21**

Table 2: Methods for determination of Tenofovir disoproxil fumarate and Emtricitabine in combination by UV Spectroscopy, Chromatography and other techniques

Sr. No	Drug	Method	Description	Ref No.
1.	Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form	UV spectrophotometric method	**Detection wavelength:** Tenofovir DF – 261 nm Emtricitabine - 281nm **Linearity range:** 5-25µg/ml **Correlation coefficient:** Tenofovir DF - 0.999 Emtricitabine - 0.999 **% Recovery:** Tenofovir DF - 100.2% Emtricitabine - 99.6% **LOD:** Tenofovir DF – 0.609 µg/ml Emtricitabine – 0.201 µg/ml **LOQ:** Tenofovir DF – 0.792 µg/ml Emtricitabine – 0.261 µg/ml	**22**
2.	Tenofovir disoproxil fumarate and Emtricitabine in pure and fixed dose combination	UV spectrophotometric method	**Detection wavelength:** Tenofovir DF – 210 nm Emtricitabine - 281nm **Linearity range:** 4 -24µg/ml **Correlation coefficient:** Tenofovir DF - 0.9997 Emtricitabine - 0.9999 **% Recovery:** Tenofovir DF – 99.11% Emtricitabine - 99.15% **LOD:** Tenofovir DF – 0.773 µg/ml Emtricitabine – 0.136 µg/ml **LOQ:** Tenofovir DF – 2.344 µg/ml Emtricitabine – 0.413 µg/ml	**23**

3.	Tenofovir disoproxil fumarate and Emtricitabine in truvada	Stability indicating UV spectrophotometric method	**Detection wavelength:** Tenofovir DF – 258.7 nm Emtricitabine – 282.2nm **Linearity range:** Tenofovir DF - 6-30 µg/ml Emtricitabine - 4-24 µg/ml **Correlation coefficient:** Tenofovir DF - 0.998 Emtricitabine - 0.999 **% Recovery:** Tenofovir DF –100.76% Emtricitabine – 100.58% **LOD:** Tenofovir DF – 0.332 µg/ml Emtricitabine – 0.755 µg/ml **LOQ:** Tenofovir DF – 1.108 µg/ml Emtricitabine – 2.518 µg/ml	**24**
4.	Tenofovir disoproxil fumarate and Emtricitabine in pharmaceutical dosage form	UV spectrophotometric method	**Detection wavelength:** Tenofovir DF – 261 nm Emtricitabine – 289.9 nm **Linearity range:** Tenofovir DF – 4-24µg/ml Emtricitabine - 6-30 µg/ml **Correlation coefficient:** Tenofovir DF - 0.997 Emtricitabine - 0.999 **% Recovery:** Tenofovir DF –99.45% Emtricitabine –101.4% **LOD:** Tenofovir DF – 1.706 µg/ml Emtricitabine – 0.561 µg/ml **LOQ:** Tenofovir DF – 5.170 µg/ml Emtricitabine – 1.702 µg/ml	**25**

5.	Tenofovir disoproxil fumarate and Emtricitabine in bulk and tablet dosage form	UV Spectrophotometric Method	**Detection wavelength:** Tenofovir DF – 260.5nm Emtricitabine - 281nm **Linearity range:** Tenofovir DF -5-25µg/ml Emtricitabine – 10-50 µg/ml **Correlation coefficient:** Tenofovir DF - 0.9972 Emtricitabine - 0.9996 **% Recovery:** Tenofovir DF - 100.2% Emtricitabine - 99.6% **LOD:** Tenofovir DF – 1.706 µg/ml Emtricitabine – 0.561 µg/ml **LOQ:** Tenofovir DF – 5.170 µg/ml Emtricitabine – 1.702 µg/ml	**26**
6.	Tenofovir disoproxil fumarate and Emtricitabine in bulk and pharmaceutical dosage form	Stabiltiy indicating RP-HPLC method	**Detection wavelength:** 261nm **Mobile phase:** Methanol: phosphate buffer (30:70% v/v) **Column:** C18(Agilent TC- C18(2), 5µm,4.6×250mm) **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Correlation coefficient:** Tenofovir DF - 0.999 Emtricitabine - 0.999 **Linearity range:** 40-80µg/ml **% Recovery:** Tenofovir DF – 97.75% Emtricitabine – 97.70% **Retention time:** Tenofovir DF – 2.8 min Emtricitabine – 4.7 min **LOD:** Tenofovir DF – 1.9 µg/ml Emtricitabine – 0.0112 µg/ml **LOQ:** Tenofovir DF – 6.2 µg/ml Emtricitabine – 11.5 µg/ml	**27**

7.	Tenofovir disoproxil fumarate and Emtricitabine in tablet dosage form	RP-HPLC method	**Detection wavelength:** 260nm **Mobile phase:** Acetonitrile: KH_2PO_4(pH3.0):Triethylamine (70:30:0.5% v/v) **Column:** LunaC18,25×4.6mm **Flow rate:** 1.5 ml/min **Injection volume:** 20µl **Correlation coefficient:** Tenofovir DF - 0.9986 Emtricitabine - 0.9995 **Linearity range:** 5-50µg/ml **% Recovery:** Tenofovir DF – 100.08% Emtricitabine – 100.04% **Retention time:** Tenofovir DF – 2.27 min Emtricitabine – 1.78 min **LOD:** Tenofovir DF – 0.039 µg/ml Emtricitabine – 0.015 µg/ml **LOQ:** Tenofovir DF – 0.117 µg/ml Emtricitabine – 0.045 µg/ml	**28**

8.	Tenofovir disoproxil fumarate and Emtricitabine in tablet dosage form	RP-HPLC method	**Detection wavelength:** 260nm **Mobile phase:** 10mMPhosphate buffer(pH 6.8): Acetonitrile (40:60% v/v) **Column:** Phenomenex Luna C18, (25 cm×4.6mm,5µm) **Flow rate:** 1.0 ml/min **Injection volume:** 20µl **Correlation coefficient:** Tenofovir DF - 0.999 Emtricitabine - 0.993 **Linearity range:** Tenofovir DF – 60-360 µg/ml Emtricitabine – 40-240 µg/ml **% Recovery:** Tenofovir DF – 100.08% Emtricitabine – 100.04% **Retention time:** Tenofovir DF – 7.42 min Emtricitabine – 2.81 min. **LOD:** Tenofovir DF – 4.60 µg/ml Emtricitabine – 1.54 µg/ml **LOQ** Tenofovir DF – 11.65 µg/ml Emtricitabine – 4.45 µg/ml	**29**
9.	Tenofovir disoproxil fumarate and Emtricitabine in human plasma	HPTLC Method	**Detection wavelength:** 276nm **Mobile phase:**Toluene: Ethyl acetate: Methanol: Acetic acid (6:4:3:0.4 %v/v/v) **Linearity range:** Tenofovir DF- 15-1500ng/spot Emtricitabine-100-1000ng/spot **Rf value:** Tenofovir DF- 0.41 Emtricitabine- 0.68 **Correlation coefficient:** Tenofovir DF - 0.9998 Emtricitabine - 0.9996 **% Recovery:** Tenofovir DF – 0.50 Emtricitabine – 1.32 **LOD:** Tenofovir DF – 13.99 ng/spot Emtricitabine – 7.37 ng/spot **LOQ:** Tenofovir DF – 42.40 ng/spot Emtricitabine – 22.32 ng/spot	**30**

10.	Tenofovir and Emtricitabine in tablet dosage form	HPTLC Method	**Detection wavelength:** 270nm **Mobile phase:**Toulene: Methanol: Ethyl acetate: Acetic acid (4:2:5:0.1 %v/v/v/v) **Linearity range:** Tenofovir DF- 120-600ng/spot Emtricitabine- 80-560 ng/spot **Rf value:** Tenofovir DF- 0.52 Emtricitabine- 0.40 **Correlation coefficient:** Tenofovir DF - 0.9996 Emtricitabine - 0.9996 **LOD:** Tenofovir DF – 40 ng/spot Emtricitabine – 30 ng/spot **LOQ:** Tenofovir DF - 100 ng/spot Emtricitabine – 60 ng/spot	**31**
11.	Tenofovir and Emtricitabine in tablet dosage form	HPTLC Method	**Detection wavelength:**265nm **Mobile phase:** Chloroform: Ethanol: (9:1 %v/v) **Linearity range:** 200-1000 ng/spot **Rf value:** Tenofovir DF- 0.47 Emtricitabine- 0.18 **Correlation coefficient:** Tenofovir DF - 0.9996 Emtricitabine - 0.9995 **% Recovery:** Tenofovir DF – 99.69% Emtricitabine – 99.54% **LOD:** Tenofovir DF –50 ng/spot Emtricitabine – 100 ng/spot **LOQ:** Tenofovir DF –190 ng/spot Emtricitabine – 160 ng/spot	**32**

CONCLUSION

This review portrays that the accounted Spectroscopic and Chromatographic methods developed and validated for estimation of Tenofovir disoproxil fumarate and Emtricitabine. Different Spectroscopic and Chromatographic methods are accessible for single and combination. Also it was found that the mobile phase comprise Phosphate buffer, Methanol, Toulene, Acetonitrile were common for most of the chromatographic methods to give more resolution. It was observed that most common combination of Tenofovir disoproxil fumarate were with Emtricitabine. For the chromatographic method, flow rate is observed in the range of 1.0 to 1.5 ml/min to obtain good resolution time. For most of the spectroscopic methods common solvent is Methanol. These all methods are claimed to be simple, accurate, economic, precise and reproducible in nature.

Majority of methods were of RP-HPLC, HPTLC and UV absorbance detection because these methods confer with best available reliability, repeatability, analysis time and sensitivity.

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