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

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Conflicts of Interest: Nil
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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)phosphoryl]methoxy)propyl]adeninefumarate[1:1]. [7]

Molecular formula is C₅₃H₄₅N₉O₁₄P 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]

Emtricitabine is a 1'-4-amino-5-fluoro-1-[(2R,5S)-2-[(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1H)-pyrimidinone. [7] The molecular formula is C₅₃H₄₅F₁₀N₉O₇S and molecular weight is 247.3 gm/mol.[8, 9] It is a synthetic fluoro 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]

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

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drug</th>
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<th>Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>Tenofovir Disoproxil Fumarate in tablet dosage form</td>
<td>UV spectrophotometric Method</td>
<td>Detection wavelength: 260nm Solvent: Methanol Linearity range: 10-100µg/ml Correlation coefficient: 0.9905 % Recovery: 99.50%</td>
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<td>2.</td>
<td>Tenofovir Disoproxil Fumarate in API and tablet dosage form</td>
<td>UV spectrophotometric method</td>
<td>Detection wavelength: 261nm Solvent: Triple distilled water Linearity range: 5-90µg/ml Correlation coefficient: 0.9981 % Recovery: 100.062%</td>
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<td>3.</td>
<td>Tenofovir Disoproxil Fumarate in bulk and pharmaceutical formulation</td>
<td>RP-HPLC method</td>
<td>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: 99.988 LOD: 0.28 µg/ml LOQ: 0.85 µg/ml</td>
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<td>Tenofovir Disoproxil Fumarate in pharmaceutical formulation and spiked human plasma</td>
<td>RP-HPLC method</td>
<td>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 Correlation coefficient: 0.9991 LOD: 0.059 µg/ml LOQ: 0.199 µg/ml</td>
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<td></td>
<td>Description</td>
<td>Method</td>
<td>Parameters</td>
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<td>Tenofovir Disoproxil in bulk and pharmaceutical formulation</td>
<td>RP-HPLC Method</td>
<td>Detection wavelength: 260nm Mobile phase: Acetonitrile: 0.05mM Phosphate buffer pH 6.0 (50:50 % v/v) Column: Reverse 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</td>
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<td>6</td>
<td>Tenofovir Disoproxil in bulk and pharmaceutical formulation</td>
<td>RP-HPTLC method</td>
<td>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</td>
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<tr>
<td>7</td>
<td>Emtricitabine in tablet dosage form</td>
<td>UV spectrophotometric method</td>
<td>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</td>
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<td>Emtricitabine in synthetic mixture</td>
<td>Emtricitabine and related substance (drug substance)</td>
<td>Emtricitabine from drug substance matrix</td>
<td>Emtricitabine in bulk and pharmaceutical dosage form</td>
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</table>
| 9 | 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 | 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 | 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 |
| 10 | LC 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 | 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 | 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 |
| 11 | 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 | 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 | 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 |
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<tr>
<td>1.</td>
<td>Tenofovir disoproxil fumarate and Emtricitabine in combined tablet dosage form</td>
<td>UV spectrophotometric method</td>
<td>Detection wavelength: Tenofovir DF – 261 nm Emtricitabine - 281 nm 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</td>
<td>22</td>
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<tr>
<td>2.</td>
<td>Tenofovir disoproxil fumarate and Emtricitabine in pure and fixed dose combination</td>
<td>UV spectrophotometric method</td>
<td>Detection wavelength: Tenofovir DF – 210 nm Emtricitabine - 281 nm 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</td>
<td>23</td>
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<td></td>
<td>Tenofovir disoproxil fumarate and Emtricitabine in truvada</td>
<td>Stability indicating UV spectrophotometric method</td>
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<td>3.</td>
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<td>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</td>
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<td>24</td>
</tr>
<tr>
<td>4.</td>
<td>Tenofovir disoproxil fumarate and Emtricitabine in pharmaceutical dosage form</td>
<td>UV spectrophotometric method</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Description</td>
<td>Method</td>
<td>Detection wavelength:</td>
<td>Linearity range:</td>
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<tr>
<td>5.</td>
<td>Tenofovir disoproxil fumarate and Emtricitabine in bulk and tablet dosage form</td>
<td>UV Spectrophotometric Method</td>
<td>Tenofovir DF – 260.5nm</td>
<td>Tenofovir DF -5-25µg/ml</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Emtricitabine - 281nm</td>
<td>Emtricitabine – 10-50 µg/ml</td>
</tr>
<tr>
<td>6.</td>
<td>Tenofovir disoproxil fumarate and Emtricitabine in bulk and pharmaceutical dosage form</td>
<td>Stability indicating RP-HPLC method</td>
<td>261nm</td>
<td>Mobile phase: Methanol: phosphate buffer (30:70% v/v)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Column: C18(Agilent TC C18(2), 5µm,4.6x250mm)</td>
<td>Flow rate: 1.0 ml/min</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Injection volume: 20μl</td>
<td>% Recovery:</td>
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<td></td>
<td></td>
<td></td>
<td>Retention time: Tenofovir DF – 2.8 min</td>
<td>Emtricitabine – 4.7 min</td>
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<tr>
<td></td>
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<td></td>
<td>LOD: Tenofovir DF – 1.9 µg/ml</td>
<td>Emtricitabine – 0.0112 µg/ml</td>
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<td></td>
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<td></td>
<td>LOQ: Tenofovir DF – 6.2 µg/ml</td>
<td>Emtricitabine – 11.5 µg/ml</td>
</tr>
</tbody>
</table>
| 7. | Tenofovir disoproxil fumarate and Emtricitabine in tablet dosage form | **RP-HPLC method** | **Detection wavelength:** 260nm  
**Mobile phase:** Acetonitrile: KH₂PO₄(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 |
### 8. Tenofovir disoproxil fumarate and Emtricitabine in tablet dosage form

**RP-HPLC method**

- **Detection wavelength:** 260nm
- **Mobile phase:** 10mM Phosphate buffer (pH 6.8): Acetonitrile (40:60% v/v)
- **Column:** Phenomenex Luna C18, (25 cm × 4.6 mm, 5 µm)
- **Flow rate:** 1.0 ml/min
- **Injection volume:** 20 µl
- **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

### 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
| 10. | Tenofvir and Emtricitabine in tablet dosage form | HPTLC Method | Detection wavelength: 270nm  
Mobile phase: Toluene: 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 |
| 11. | Tenofvir 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 |

**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.

REFERENCE


