A Precise Review on Tenofovir Disoproxil Fumarate: An Analytical Profile

Vinod A Chaure¹, Saurabh B Ganorkar¹, Ganesh U Chaturbhuj², Sanjay J Surana¹ and Atul A Shirkhedkar¹

¹R. C. Patel, Institute of Pharmaceutical Education and Research, Shirpur; Dist: Dhule (MS) India 425 405
²Institute of Chemical Technology, ICT, Matunga, Mumbai 400019

*Email: shirkhedkar@gmail.com

ARTICLE INFORMATION

Received: July 11, 2018
Revised: Sep. 17, 2018
Accepted: Nov. 01, 2018
Published online: Nov 02, 2018

Keywords:
Tenofovir Disoproxil Fumarate; Review Article; Validation; HPLC; HPTLC

ABSTRACT

Tenofovir Disoproxil Fumarate (TDF) is antiretroviral medicine used treat AIDS as well as chronic Hepatitis-B. TDF is a prodrug of tenofovir and exists as dominant form due to lesser oral bioavailability of parent drug. TDF is now available in a fixed-dose combination with various antiretrovirals like Cobicistat, Efavirenz, Elvitegravir, Emtricitabine, Lamivudine, Rilpivirine, and Nevirapine. Hence, pharmaceutical analysis of TDF and applicability of different analytical methods have gained crucial importance. The present review article assesses the published analytical methods and a variety of approach for investigation of TDF in bulk drug as well as pharmaceutical formulations including combinations. This detailed review includes examination of around eighty analytical methods published during 2008 to 2016 using various techniques which include HPLC, HPTLC, and UV/Visible-Spectrophotometry. The review also illustrates the scope and limitations of many published analytical methods for analysis of TDF. Such detailed review will be of great help to the researcher who is working on TDF. Miscellaneous methods of rare but unique pharmaceutical distinction have also been given due consideration. The diagrammatic illustrations provide the statistical overview about the various methods referred for analysis of TDF.

1. Introduction

Tenofovir Disoproxil Fumarate (TDF) Fig. 1 is an antiretroviral medicine used to treat HIV/AIDS and chronic hepatitis B (Goicoechea et al., 2008). TDF is a prodrug of Tenofovir (TNF) and exists as dominant form due to low oral bioavailability of parent drug. TDF is now available in a fixed-dose combination with various antiretrovirals like Cobicistat, Efavirenz, Elvitegravir, Emtricitabine, Lamivudine, Rilpivirine, and Nevirapine. Hence, pharmaceutical analysis of TDF and applicability of different analytical methods have gained crucial importance. The present review article assesses the published analytical methods and a variety of approach for investigation of TDF in bulk drug as well as pharmaceutical formulations including combinations. This detailed review includes examination of around eighty analytical methods published during 2008 to 2016 using various techniques which include HPLC, HPTLC, and UV/Visible-Spectrophotometry. The review also illustrates the scope and limitations of many published analytical methods for analysis of TDF. Such detailed review will be of great help to the researcher who is working on TDF. Miscellaneous methods of rare but unique pharmaceutical distinction have also been given due consideration. The diagrammatic illustrations provide the statistical overview about the various methods referred for analysis of TDF.

The present review offers a critical account on analytical methods published during 2008 to 2016 for determination of TDF.

1.1 Chemistry of Tenofovir Disoproxil Fumarate

(TDF) is chemically 9-(R)-2-[bis (isopropoxycarbonyl) oxy] methyl] phosphinyl] methoxy) propyl adenine Fumarate. The molecular formula is C₂₃H₂₅N₄O₁₀P and molecular weight is 521.46. Melting point is 279 °C, drug is faintly soluble in water, soluble in methanol, very slightly soluble in dichloromethane [5].
TDF is absorbed in the gut and quickly converted into TNF. After administration of TDF in the body, ester hydrolysis takes place and in the blood dissociation of two ester linkages occurs. The first cleavage of the ester group offers monoester of TDF then second group of ester gets TNF metabolized intracellularly and followed by its conversion into an anabolite; tenofovir diphosphate which inhibits HIV-1 reverse-transcriptase competitively and stops the DNA synthesis (Kearney et al., 2006).

2. Pharmacological Profile

2.1 Mechanism of Action

This drug inhibits HIV-1 transcriptase and HBV retro-transcriptase. Tenofovir diphosphate is a weak inhibitor of DNA polymerases α and β (mammalian) and DNA polymerase γ (mitochondrial) (James et al., 2004).

2.2 Dosage Forms and Recommended Dose

TNF is available only in oral tablet dosage forms having 245 mg of TNF corresponding to 300 mg of TDF. The recommended dose for the drug is a tablet per day (James et al., 2004).

2.3 Pharmacodynamics and Pharmacokinetics

The range for oral bioavailability is in-between 25 % and 40 % (depending on the amount of fat), and can be administered safely to patients having liver function impaired (Birkus et al., 2002; Cihlar et al., 2002).

2.4 Adverse Effects and Contraindications

Obesity and prolonged nucleoside therapy may be predisposing factors (Porche, 2002). Slight rise in serum creatinine and decrease in serum phosphate levels occurring 4–12 months after starting TDF is characterized by nephrotoxicity (Fontana, 2009).

3. Various Approaches for Pharmaceutical Analysis

The use of analytical methods for pharmaceutical analysis is an essential part of drug development and validation procedure. Analytical techniques like UV/Visible-Spectrophotometry, Atomic Absorption, Capillary-Electrophoresis, Liquid -Chromatography either alone or coupled with Mass Spectroscopy, Measurement of Luminescence, Voltammetry and Polarography have been explored for analysis of drugs in bulk as well as in various formulations and biologicals (Rajput et al., 2015). Amongst all these methods, Chromatographic methods HPLC, HPTLC and UV/Visible-Spectrophotometry have generally been studied and preferred over other methods due to sensitivity and simplicity of analysis (Siddiqui et al., 2013).

TDF is available as individual or in combination with other drugs such Emtricitabine, Rilpivirine, Lamivudine, Nevirapine, Efavirenz, Elvitegravir and Cobicistat in various dosage forms.

Thorough literature survey revealed various analytical methods viz HPLC, HPTLC (simple and stability-
indicating), UV-Spectrophotometry, bio-analytical methods for analysis in human plasma and biological fluids used for analysis of TDF alone or in combination with other drugs. Few pharmaceutical analytical methods such as Capillary-Electrophoresis and specified Electro-Analytical determinations are also critically been reviewed.

### 3.1 Chromatographic Analyses

#### 3.1.1 HPLC analysis

Total twenty seven methods have been reported for determination for TDF alone and or in combined dosage form. Reversed-Phase HPLC analyses for TDF (Sarala et al., 2014; SM and Nandedkar, 2009; Sundar and Edla, 2011; Sharma et al., 2012; Balaji et al., 2012; Kandagad et al., 2008; Sharma et al., 2010) Seven RP-HPLC procedures have been reported in literature for determination of TDF using isocratic mode. The identification and separation of the TDF was achieved using C18 column with dimension 250/150 x 4.6 mm; 5 µm and variable combination of mobile phases containing acetonitrile, Methanol, and Water. Whereas, in three developed methods combination of Acetonitrile/Methanol and Sodium Hydrogen phosphate, pH adjusted to 2.3 was chosen as mobile phase. UV/Visible detector / Photo-Diode-Array detectors were used to perform the detection. The retention time for all these methods reported to be below 10 min. Small values of LOD and LOD in the reported methods indicate that these methods are sensitive. All these established methods are claimed to be simple, economical and less time consuming. In many studied analytical methods, the percentage amount of TDF estimated in pharmaceutical formulation was reported to be in the range of 97 - 101, indicating superior conformity with the label claimed.

Reversed-Phase HPLC analyses for TDF Combinations (Sharma et al., 2010; Abdelhay et al., 2013; Lavanya et al., 2012; Karunakaran et al., 2010; Srinivasan et al., 2014; Venkatesh et al., 2013; Viswanath et al., 2013; Yenumula et al., 2015; Sharma and Gupta, 2009; Vanitha et al., 2014; Devanaboyina et al., 2012; Kumar et al., 2012; Gorja and Bandla, 2011; Mali et al., 2015; Khan et al., 2014; Jayapalu et al., 2014; Bhavsar et al., 2012; Raju et al., 2008; Ramaswamy Dhas, 2014; Prathap and Rao, 2014; Bhargavi et al., 2012). Total twenty RP-HPLC methods have been reported for analysis of TDF in combination with other drugs. RP-HPLC methods using Isocratic modes have been explored in around sixteen methods; whereas, only four methods were studied using gradient mode of HPLC. In most of these analytical methods C18 column was exclusively applied for separation of TDF and other drugs in its combination. The mobile phase chosen for analysis contains various proportions of Acetonitrile/Methanol and Sodium Hydrogen Phosphate buffer/ water and the pH adjusted below 7.0. The retention time below 11 min was reported for TDF and its combinations with other drugs. The coefficient correction value for linearity of both these drugs were reported to be greater than 0.99.

The detailed account of mobile phases used for determination, sample matrix, wavelength maximum, linearity, retention time and the correlation coefficient is described in Table 1. Whereas, the comparison of the column(s) with the specification(s) for HPLC analysis along with the conditions of flow rate, temperature, detector and type of analysis are quoted in Table 2.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug(s)</th>
<th>Mobile Phase (v/v)</th>
<th>Detection λ nm</th>
<th>Linearity µg/mL</th>
<th>tR (min)</th>
<th>Correlation Coefficient (r^2)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TDF</td>
<td>H₂O; MeOH(45:55)</td>
<td>230</td>
<td>0-120</td>
<td>5.69</td>
<td>0.996</td>
<td>[15]</td>
</tr>
<tr>
<td>2</td>
<td>TDF</td>
<td>HCCOH:ACN(50:50)</td>
<td>305</td>
<td>5-30</td>
<td>6.718</td>
<td>0.9993</td>
<td>[16]</td>
</tr>
<tr>
<td>3</td>
<td>TDF</td>
<td>MeOH: ACN: OPA (85:10:05)</td>
<td>260</td>
<td>5-35</td>
<td>2.3</td>
<td>0.999</td>
<td>[17]</td>
</tr>
<tr>
<td>4</td>
<td>TDF</td>
<td>NaH₂PO₄ buffer (pH2.3): MeOH (49:51)</td>
<td>260</td>
<td>50-300</td>
<td>9.437</td>
<td>0.9995</td>
<td>[18]</td>
</tr>
<tr>
<td>5</td>
<td>TDF</td>
<td>Acetate Buffer pH 2.8 : ACN : MeOH(40: 40:20)</td>
<td>260</td>
<td>20 - 60</td>
<td>2.47</td>
<td>0.9992</td>
<td>[19]</td>
</tr>
<tr>
<td>6</td>
<td>TDF</td>
<td>ACN: H₂O (75:25)</td>
<td>259</td>
<td>0.2–10</td>
<td>1.92</td>
<td>0.9966</td>
<td>[20]</td>
</tr>
<tr>
<td>7</td>
<td>TDF</td>
<td>NaH₂PO₄ buffer (pH 2.3): MeOH (49:51)</td>
<td>260</td>
<td>50-300</td>
<td>9.437</td>
<td>0.9995</td>
<td>[21]</td>
</tr>
<tr>
<td>8</td>
<td>TDF + EMT</td>
<td>ACN:50 mM Na₂HPO₄ (pH 6.0): TEA (50:50:0.1)</td>
<td>TDF</td>
<td>TDF - TDF - EMT</td>
<td>4.98</td>
<td>0.99996</td>
<td>[22]</td>
</tr>
</tbody>
</table>

ISSN No.: 2321-2217(Print) ISSN No.: 2321-2225(Online); Registration No. : CHAENG/2013/50088
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Mobile Phase</th>
<th>Wavelength (nm)</th>
<th>TDF Concentration</th>
<th>EMB Concentration</th>
<th>TDF RSD</th>
<th>EMB RSD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>TDF + EMT</td>
<td>MeOH: Phosphate buffer (65:35)</td>
<td>260</td>
<td>TDF 10-50</td>
<td>EMB 10-50</td>
<td>6.231</td>
<td>2.461</td>
<td>[23]</td>
</tr>
<tr>
<td>10</td>
<td>TDF + EMT</td>
<td>ACN: MeOH: H₂O (30:50:20)</td>
<td>258</td>
<td>TDF 1-6</td>
<td>EMB 2-12</td>
<td>2.77</td>
<td>3.49</td>
<td>0.9998 [24]</td>
</tr>
<tr>
<td>11</td>
<td>TDF + EMT</td>
<td>ACN: Phosphate buffer (pH 3.5) (60:40)</td>
<td>270</td>
<td>TDF 3-15</td>
<td>EMB 2-10</td>
<td>2.85</td>
<td>3.55</td>
<td>0.999 [25]</td>
</tr>
<tr>
<td>12</td>
<td>TDF + EMT</td>
<td>Phosphate Buffer pH 3.0: ACN (60:40)</td>
<td>260</td>
<td>TDF 75-450</td>
<td>EMB 50-300</td>
<td>4.5</td>
<td>3.3</td>
<td>0.999 [26]</td>
</tr>
<tr>
<td>13</td>
<td>TDF + EMT</td>
<td>ACN: Phosphate (pH 3.5) buffers (60:40)</td>
<td>270</td>
<td>TDF 3-15</td>
<td>EMT 2-10</td>
<td>2.84</td>
<td>3.55</td>
<td>0.999 [27]</td>
</tr>
<tr>
<td>14</td>
<td>TDF + EMT</td>
<td>ACN: 10 mM Phosphate buffer (pH 6.8) (60:40)</td>
<td>260</td>
<td>TDF 60-360</td>
<td>EMT 40-240</td>
<td>7.42</td>
<td>3.89</td>
<td>0.999 [28]</td>
</tr>
<tr>
<td>15</td>
<td>TDF + EMT</td>
<td>ACN: KH₂PO₄ buffer (pH 3.0): TEA (70:30:0.5)</td>
<td>260</td>
<td>TDF-5-50</td>
<td>EMT-5-50</td>
<td>2.27</td>
<td>1.78</td>
<td>0.9986 [29]</td>
</tr>
<tr>
<td>16</td>
<td>TDF + EMT</td>
<td>ACN: Phosphate buffer (pH 6.8) (60:40)</td>
<td>260</td>
<td>TDF-6-36</td>
<td>EMT-4-24</td>
<td>3.89</td>
<td>2.883</td>
<td>0.999 [30]</td>
</tr>
<tr>
<td>17</td>
<td>TDF + EMT</td>
<td>MeOH: ACN: TEA (46:50:04)</td>
<td>259</td>
<td>TDF-40-100</td>
<td>EMT-40-100</td>
<td>1.5</td>
<td>3.5</td>
<td>0.9999 [31]</td>
</tr>
<tr>
<td>18</td>
<td>TDF + LMV</td>
<td>TEA buffer (pH 5.0): ACN: MeOH (30:40:30)</td>
<td>260</td>
<td>TDF-50-300</td>
<td>LMV-50-300</td>
<td>9.437</td>
<td>6.966</td>
<td>0.9999 [32]</td>
</tr>
<tr>
<td>19</td>
<td>TDF + LMV</td>
<td>KH₂PO₄ buffer (pH 6.0): MeOH:H₂O (33:65:2)</td>
<td>260</td>
<td>TDF-50-150</td>
<td>LMV-50-150</td>
<td>3.4</td>
<td>2.3</td>
<td>1.000 [33]</td>
</tr>
<tr>
<td>20</td>
<td>TDF + LMV</td>
<td>ACN: H₂O (80:20)</td>
<td>260</td>
<td>5-25</td>
<td>2.933</td>
<td>2.966</td>
<td>0.9987 [34]</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>TDF + EMT + RPV</td>
<td>MeOH : H₂O (85:15) ACN : Buffer (pH 3.5) (70:30)</td>
<td>265</td>
<td>TDF-120-1440</td>
<td>EMT-80-960</td>
<td>8.386</td>
<td>6.250</td>
<td>0.999 [35]</td>
</tr>
</tbody>
</table>
Table 2: HPLC chromatographic columns and optimized analytical parameters.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drugs</th>
<th>Column</th>
<th>Dimensions (mm), Particles(µm)</th>
<th>Detector</th>
<th>Flow rate mL/min</th>
<th>Mode of analysis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TDF</td>
<td>Develosil ODS HG -5 RP</td>
<td>150 × 4.6, 5</td>
<td>UV</td>
<td>1.3</td>
<td>Isocratic</td>
<td>[15]</td>
</tr>
<tr>
<td>2</td>
<td>TDF</td>
<td>Luna C18</td>
<td>250 × 4.6, 5</td>
<td>UV</td>
<td>0.8</td>
<td>Isocratic</td>
<td>[16]</td>
</tr>
<tr>
<td>3</td>
<td>TDF</td>
<td>Inertsil ODS C18</td>
<td>250 × 4.6, 5</td>
<td>UV/visible</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[17]</td>
</tr>
<tr>
<td>4</td>
<td>TDF</td>
<td>Inertsil ODS-3</td>
<td>150 × 4.6, 5</td>
<td>PDA</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[18]</td>
</tr>
<tr>
<td>5</td>
<td>TDF</td>
<td>Symmetry C18</td>
<td>150 × 4.6, 5</td>
<td>UV/Visible</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[19]</td>
</tr>
<tr>
<td>6</td>
<td>TDF</td>
<td>CLC C18</td>
<td>25 × 4.6, 5</td>
<td>SPD10AVP</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[20]</td>
</tr>
<tr>
<td>7</td>
<td>TDF</td>
<td>Develosil ODS HG -5 RP</td>
<td>150 × 4.6, 5</td>
<td>UV/Visible</td>
<td>1.3</td>
<td>Isocratic</td>
<td>[21]</td>
</tr>
<tr>
<td>8</td>
<td>TDF + EMT</td>
<td>Inertsil ODS-3</td>
<td>150 × 4.6, 5</td>
<td>UV</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[22]</td>
</tr>
<tr>
<td>9</td>
<td>TDF + EMT</td>
<td>Zorbax SB-C8</td>
<td>250 × 4.6, 5</td>
<td>UV</td>
<td>0.4</td>
<td>Isocratic</td>
<td>[23]</td>
</tr>
<tr>
<td>10</td>
<td>TDF + EMT</td>
<td>Phenomenax C-18</td>
<td>250 × 4.6, 5</td>
<td>UV/Visible</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[24]</td>
</tr>
<tr>
<td>11</td>
<td>TDF + EMT</td>
<td>Phenomenax Luna C18</td>
<td>150 × 4.6, 5</td>
<td>UV/visible</td>
<td>0.6</td>
<td>Isocratic</td>
<td>[25]</td>
</tr>
<tr>
<td>12</td>
<td>TDF + EMT</td>
<td>Hypersil C18</td>
<td>250 × 4.6, 5</td>
<td>PDA</td>
<td>1.0</td>
<td>Isocratic</td>
<td>[26]</td>
</tr>
</tbody>
</table>
Table 3: Pharmaceutical Analysis of TDF/TNF in biological fluids.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drug(s)</th>
<th>Sample matrix</th>
<th>Method</th>
<th>Column Dimensions (mm), particle (µm)</th>
<th>Detector</th>
<th>Internal standard</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TNF</td>
<td>Human plasma</td>
<td>LC/MS</td>
<td>RP, Chromolith, C18,100 × 4.6,5</td>
<td>UV, fluorescence, and LC–tandem MS</td>
<td>Adefovir</td>
<td>[42]</td>
</tr>
<tr>
<td>2</td>
<td>TNF</td>
<td>Human plasma</td>
<td>LC/MS/MS</td>
<td>Kromasil100, C18, 150 × 4.6,5</td>
<td>HPLC/UV</td>
<td>Fluconazole</td>
<td>[43]</td>
</tr>
<tr>
<td>3</td>
<td>TNF</td>
<td>Human plasma</td>
<td>LC-ESI MS-MS</td>
<td>Supelco C8, 150 × 4.6,5</td>
<td>UV</td>
<td>Tenofovir and Tenofovir D6</td>
<td>[44]</td>
</tr>
<tr>
<td>4</td>
<td>TNF</td>
<td>Human plasma</td>
<td>LC–MS/MS</td>
<td>Zorbax 5 micron SCX HPLC column, 3.0 × 50</td>
<td>UV, fluorescence and MS</td>
<td>Tenofovir and Tenofovir D6</td>
<td>[45]</td>
</tr>
<tr>
<td>5</td>
<td>TNF+ EMT</td>
<td>Human plasma</td>
<td>LC-MS/MS</td>
<td>Synergi Polar-RP, 150 × 2.0 mm,</td>
<td>MS/MS, ESI</td>
<td>Iso-TVF and Iso-FTC</td>
<td>[46]</td>
</tr>
<tr>
<td>6</td>
<td>TNF+ EMT</td>
<td>Human plasma</td>
<td>LC-MS/MS</td>
<td>Synergi Polar RP HPLC Column pore size, 80°A, 150 × 2.0 , 4</td>
<td>HPLC/UV</td>
<td>TDF- adefovir and dideoxyuridine EMT-deoxyfluorocytidine</td>
<td>[47]</td>
</tr>
<tr>
<td>7</td>
<td>TNF+ LMV</td>
<td>Human plasma</td>
<td>LC-MS/MS</td>
<td>Chromolith ROD speed C18 250 × 4.6,5</td>
<td>HPLC/MS</td>
<td>Abacavir</td>
<td>[48]</td>
</tr>
<tr>
<td>8</td>
<td>TNF+ EMT</td>
<td>Human plasma</td>
<td>LC-MS/MS</td>
<td>HiQ Sil C18HS 250 × 4.6,5</td>
<td>UV</td>
<td>Clonazepam</td>
<td>[49]</td>
</tr>
</tbody>
</table>
3.1.2 Bioanalytical methods of analysis

Total ten bioanalytical methods are reported so far for determination of TDF in biological samples either individual or in combination with other antiretroviral drugs viz EMT, LMV and NVP (Yadav et al., 2009; Rao et al., 2013; Palival et al., 2014; Podany et al., 2015; Delahunty et al., 2009; Zheng et al., 2014; Matta et al., 2012; Patel et al., 2015; Gomes et al., 2008; Valluru et al., 2013). In most of these methods extraction of TDF and other drugs in its combination from human plasma have been performed using solid-phase extraction , liquid-liquid extraction, protein precipitation and solid-phase analytical derivatization were applied. Solvents used for extraction of TDF and other drugs from biological matrix include acetonitrile, methanol, water, trichloroacetic acid, and trifluoroacetic acid etc. The internal standards viz Adefovir, fluconazole, Tenoforiv, Tenoforiv D6, Abacavir, Clonazepam, didenosurydine and deoxy-fluorocytidine were used during analysis. The separation of the TNF and other drugs were performed on different analytical column such as Chromolith Chromolith ROD speed C18, Kromasil100, Synergi Polarr- RP Discovery Supelco C8, Phenomenex kinetex C18, HiQSil C18HS, Zorbax 5 micron SCX HPLC and Prontosil C18AQ with dimension (100/150/250 x 2/4.6 mm, 5 µm) under isocratic mode of analysis was used. Various proportion of mobile phase containing combination of acetonitrile, methanol and water was applied. Wherever necessary the pH of the water was adjusted using formic acid/ ammonium acetate/ammonium formate as modifier. In most of these analytical methods, the detection of TNF was performed by applying MS/ ESI -MS/MS/Tandem- MS/ Fluorescence/UV detectors. Details about, sample matrices, method type, λ-max, column, and use of internal standard if any are shown in Table 3.

3.1.3 HPTLC/TLC - densitometry determinations

Five HPTLC methods have been reported for TDF alone and for simultaneous estimation of TDF in combination with other drugs such as EMT and RPV (Pradeep et al., 2011; Mardia et al., 2012; Joshi et al., 2009; Rao et al., 2011; Saminathan and Vetrichelvan, 2016). The separation carried out on aluminium plates precoated with silica gel using various mobile phase composition such as methanol, chloroform, toluene and most of acidic modifier used for efficient separation such as Ethyl acetate, glacial acetic acid with various proportion. Detection carried out at different wavelength like 265 nm, 266 nm, 270 nm and 272 nm. The amount of the TDF and its combined drug estimated in pharmaceutical formulation was reported to be in superior conformity with label claimed. Details of mobile phase composition, detection of λmax, linearity, and retention factor and coefficient correlation are shown in Table 4.

### Table 4: HPTLC methods for determination of TDF/TNF.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of Drug</th>
<th>Mobile phase composition (v/v)</th>
<th>Detection (λ nm)</th>
<th>Linearity (ng/spot)</th>
<th>R²</th>
<th>r²</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TDF</td>
<td>CHCl₃: MeOH (8.5: 1.5)</td>
<td>270</td>
<td>200 - 1200</td>
<td>0.54</td>
<td>0.9994</td>
<td>[52]</td>
</tr>
<tr>
<td>2</td>
<td>TDF</td>
<td>EtOAc: MeOH: HCOOH (7:2:5:0.5)</td>
<td>266</td>
<td>125-750</td>
<td>0.78</td>
<td>0.991</td>
<td>[53]</td>
</tr>
<tr>
<td>3</td>
<td>TDF+EMT</td>
<td>CHCl₃: MeOH (9:1)</td>
<td>265</td>
<td>200 – 1000</td>
<td>0.47</td>
<td>0.9996</td>
<td>[54]</td>
</tr>
<tr>
<td>4</td>
<td>TDF+EMT</td>
<td>Toluene: MeOH : EtOAc: AcOH</td>
<td>270</td>
<td>120 – 600</td>
<td>0.52</td>
<td>0.999</td>
<td>[55]</td>
</tr>
<tr>
<td>5</td>
<td>TDF+EMT+RPV</td>
<td>CHCl₃: EtOAc: MeOH: Glacial Acetic acid (5:2:1:0.1)</td>
<td>272</td>
<td>80-560</td>
<td>0.40</td>
<td>0.9992</td>
<td>[56]</td>
</tr>
</tbody>
</table>

### 3.1.4 Stability-indicating chromatographic methods

Seven stability-indicating methods have been found so far for estimation of TDF in bulk and pharmaceuticals using different analytical techniques such as under different prescribed stress conditions (hydrolysis, oxidation, dry and wet heat and photolysis). Out of these, two methods are for reported for estimation of TNF alone and five describe stability-indicating methods for TDF in combination.
EMT, RPV, LMV, NVP, EFV, ELV and COBI (Havel and Dhaneshwar, 2012; Hussen et al., 2013; Agashe et al., 2015; Prasad et al., 2012; Sudha and Manjeera, 2012; Rao et al., 2016; Rao et al., 2014). The details about reported stability-indicating methods for TDF giving emphasis on sample matrix, mobile phase, $\lambda_{max}$, linearity, range, retention time and correlation coefficient is shown in Table 5.

### Table 5: Stability-indicating methods for analysis of TDF/TNF

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of drug(s)</th>
<th>Mobile phase composition(v/v)</th>
<th>Detection ($\lambda$ nm)</th>
<th>Linearity ($\mu g/ml$)</th>
<th>$t_R$ (min)</th>
<th>$r^2$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TDF</td>
<td>MeOH: H$_2$O (60:40)</td>
<td>260</td>
<td>4–20</td>
<td>12.09</td>
<td>0.9999</td>
<td>[57]</td>
</tr>
<tr>
<td>2</td>
<td>TDF</td>
<td>ACN: 0.025M H$_2$PO$_4$ buffer (pH 3.0) (35:65)</td>
<td>260</td>
<td>0.1-50</td>
<td>7.5</td>
<td>0.999</td>
<td>[58]</td>
</tr>
<tr>
<td>3</td>
<td>TDF+EMT</td>
<td>NH$_4$Ac buffer (pH 5.5): MeOH (85:15)</td>
<td>260</td>
<td>TDF-10-60, EMT-10-60</td>
<td>TDF-31.83, EMT-11.6</td>
<td>TDF-0.9991, EMT-0.999</td>
<td>[59]</td>
</tr>
<tr>
<td>4</td>
<td>TDF+LMV+NVP</td>
<td>A.(NH$_4$)$_2$PO$_4$: dil. TFA, B. MeOH: ACN. (10:90)</td>
<td>260</td>
<td>0.05-0.50</td>
<td>7.5</td>
<td>0.999</td>
<td>[60]</td>
</tr>
<tr>
<td>5</td>
<td>TDF+LMV+EFV</td>
<td>TEA buffer: MeOH (35:65)</td>
<td>260</td>
<td>TDF-75-225, LMV-75-225</td>
<td>TDF-3.36, LMV-2.43</td>
<td>TDF-0.999, LMV-0.999</td>
<td>[61]</td>
</tr>
<tr>
<td>6</td>
<td>TDF+EMT+EFV</td>
<td>MeOH: TEA (70:30) (pH 7)</td>
<td>260</td>
<td>TDF-15-75, EMT-10-50, EFV-150-450</td>
<td>TDF-4.632, EMT-3.706, EFV-10.46</td>
<td>TDF-0.999, EMT-0.999, EFV-0.999</td>
<td>[62]</td>
</tr>
<tr>
<td>7</td>
<td>TDF+EMT+COBI+ELV</td>
<td>0.1%TFA: ACN</td>
<td>242</td>
<td>TDF-150-450, COBI-150-450</td>
<td>TDF-4.75, COBI-3.43</td>
<td>TDF-0.999, COBI-0.999</td>
<td>[63]</td>
</tr>
</tbody>
</table>

### 3.1.5 Spectrophotometric analysis

About sixteen UV-Spectrophotometry procedures have been studied for determination of TDF alone and in combined dosage forms. Table 6 depicts about spectrophotometry methods with respect to sample matrix, technique, solvent used, $\lambda_{max}$, linearity range, and correlation coefficient.

### Table 6: Spectrophotometric methods used for determination of TDF alone and in combined dosage form.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of drug</th>
<th>Method/ order</th>
<th>Detection ($\lambda$, nm)</th>
<th>Linearity ($\mu g/ml$)</th>
<th>$r^2$ Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TDF</td>
<td>I Zero</td>
<td>451</td>
<td>8-40</td>
<td>0.998</td>
<td>[64]</td>
</tr>
<tr>
<td>2</td>
<td>TDF</td>
<td>I Zero</td>
<td>261</td>
<td>5-90</td>
<td>0.9983</td>
<td>[65]</td>
</tr>
<tr>
<td>3</td>
<td>TDF</td>
<td>I Zero, II AUC</td>
<td>260, 250-270</td>
<td>5-30</td>
<td>0.999</td>
<td>[66]</td>
</tr>
<tr>
<td>4</td>
<td>TDF</td>
<td>I Zero</td>
<td>259</td>
<td>5-45</td>
<td>0.9995</td>
<td>[67]</td>
</tr>
<tr>
<td>5</td>
<td>TDF</td>
<td>I Zero, II AUC</td>
<td>260, 250-270</td>
<td>5-25</td>
<td>0.992</td>
<td>[68]</td>
</tr>
<tr>
<td>6</td>
<td>TDF</td>
<td>I Zero, II First</td>
<td>260, 273</td>
<td>4-24</td>
<td>0.999</td>
<td>[69]</td>
</tr>
<tr>
<td>7</td>
<td>TDF</td>
<td>I Zero</td>
<td>260</td>
<td>10-50</td>
<td>1.00004</td>
<td>[70]</td>
</tr>
<tr>
<td>8</td>
<td>TDF+EMT</td>
<td>I ACM</td>
<td>TDF-260, EMT-290</td>
<td>TDF- 5-25, EMT-7-35</td>
<td>0.999</td>
<td>[71]</td>
</tr>
<tr>
<td>9</td>
<td>TDF+EMT</td>
<td>I Zero</td>
<td>TDF-240, EMT-280</td>
<td>10-80</td>
<td>0.9996</td>
<td>[72]</td>
</tr>
</tbody>
</table>
10 TDF+EMT I First Derivative TDF-306.88 TDF-3-21 TDF-0.9999 [73]  
   II EMT-224.38 EMT-2-14  
   III EMT-293.38 EMT-4-20 0.9996  
   ACM TDF-270.29 TDF-6-30 0.9997  
11 TDF+EMT I SEM TDF-260 TDF-5-25 TDF-0.9999  
   EMT-290 EMT-7-35 0.999  
12 TDF+EMT I SEM TDF-210, 281 TDF-4-24 0.9997  
   EMT-210, 281 0.99977  
13 TDF+LMV I SEM TNF-271 TDF-5-50 0.999  
   LAM-261 0.999  
14 TDF+LMV I SEM TDF-260 TDF-5-45 0.9999  
   LMV-280 LMV-2-16 0.998  
15 TDF+EMT+RPV I SEM TNF-259 TDF-3-21 0.9993  
   EMT-280 EM1-10 1.000  
   RPV-291 RPV-0.5-3 0.9996  

3.1.5.1 Spectrophotometry analysis as a single drug  
Three different methods have been reported for determination of TDF in tablet dosage form as a single component. All these methods reported for analysis implemented zero order, first order and Area under curve (AUC) techniques. Water was used as a solvent and analysis were carried out at appropriate wavelength (Buridi, 2013; Gnanarajan et al., 2013; Balaji et al., 2012; Mondal and Singh, 2014; Srujani et al., 2015; Himaja et al., 2014; Rani et al., 2012).

3.1.5.2 Simultaneous spectrophotometry determinations in various dosage forms  
TDF is available with many antiviral drugs in combined tablet dosage form. The range of UV-spectrophotometry method for simultaneous determination of TNF in the pharmaceutical formulation has been established. Five different methods established for determination of TDF in tablets as multiple components have been reported. All these methods studied for analysis of TNF employed Zero order, First order, Derivative method, ACM and SEM methods using water and methanol as a solvent. The combination of TDF, EMT, LMV and RPV has been estimated using ICH guidelines. (Viswanath et al., 2013; Sasikala et al., 2013; Choudhari et al., 2011; Srinivasan et al., 2014; Ananda et al., 2011; Dubbaka et al., 2015; Soumya et al., 2012; Madhuri et al., 2014)

3.2 A Typical Methods of Pharmaceutical Importance  
A simple and reliable method of capillary electrophoresis (CE) combined with field-amplified sample stacking, using hydroxypropyl methyl cellulose as electro-osmotic flow suppressant has been reported for separation and identification of TNF and Adefovir, using phosphate buffer solution containing 0.3% of hydroxypropyl methylcellulose and measured at 18 kV or 214 nm. The method has been successfully established for determination of TNF and Adefovir in bovine serum and which is suitable for pharmacokinetic study. The prominent characteristic as claimed by author is to be highly sensitive, cost-effective, relatively simple, and time-saving than other methods such as LC/MS/MS, HPLC-UV, Fluorescent derivation HPLC, and SPME-HPLC (Liu and Duan 2015). The electrochemical reduction of (TNF) was studied in Britton-Robinson (BR) buffer at the dropping mercury drop electrode (HMDE). A method based on square-wave cathodic adsorptive stripping voltammetry (SWCAdSV) was studied and validated for assay of TNF in human plasma and a tablet formulation. Sample preparation of plasma involved protein precipitation with acetonitrile. The method reported to be linear in the concentration range 0.5–5.0µg/ml. The method was reported to quite inexpensive compare to other methods (Jain and Sharma 2013). Quantification of TDF in pharmaceutical formulations via chemical derivatization using p-chloranilic acid and sodium nitroprusside as a reagent have been studied as extractions free spectrophotometric methods determination of TDF in bulk drugs and pharmaceutical formulations (tablets). The first method reported to be based upon the charge transfer complexation reaction between drug as an electron donor and p-chloranilic acid (p-CA) as a p-acceptor to form a violet chromogen measured at 531 nm. The colored product depicts linearity in the concentration range of 2–10 µg/mL. The second method was reported to be based on the formation of light green molecular complex with sodium nitroprusside in presence of hydroxylamine under alkaline conditions.
conditions and exhibiting λ-max at 401 nm and showed linearity in the concentration range of 2-10 µg/ml (Disha and Gurupadayya 2013). A solid-phase extraction (SPE) method has been studied and validated on a liquid chromatography coupled with a mass spectrometer for the determination of plasma concentrations of TDF and EMT in HIV patients. Separation was achieved with a gradient (Acetonitrile and water with formic acid 0.05%) on an Atlantis 4.6 mm×150 mm, reversed-phase analytical column. Detection of TNF, EMT, and internal standard (IS) was achieved by electrospray ionization mass spectrometry (ESI-MS) in the positive ion mode. Plasma was analyzed, and the limit of quantitation was 15.6 ng/mL for TNF and 11.7 ng/mL for EMT; limit of detection was 2 ng/mL for TNF and 1.5 ng/mL for EMT. Mean recovery of TNF, EMT, and IS were 46.5 % [relative standard deviation (RSD): 8.8 %] and 88.8 % (RSD: 1.0 %), and 81.7 % (RSD: 3.1 %), respectively. The method was applied for the estimation of drug plasma concentration of HIV patients treated with EMT and TNF, in combination with others (D’Avolio et al., 2008).

3. Conclusion

Review of literature reported during the period of 2008-2016 described analytical methods for estimation of TDF. Different analytical methods implemented for analysis of TDF in bulk and in its combined dosage forms and in plasma include LC-MS/MS, HPLC, HPTLC and UV-Spectroscopy methods. Fig. 2 depicts method prevalence for pharmaceutical analysis of TDF and Fig. 3 give comparative account on various methods published using different analytical techniques for TDF.

The detection of HPLC analysis of most of methods was monitored at 260 nm with PDA/UV detector. The mobile phase composition for the separation of TDF includes methanol, acetonitrile and buffer or their combination in appropriate proportion. The present review article can provide wisdom to the reader about the various methods employed for analysis of TDF and update them with several options for analysis of TDF. The date compiled may be useful for the further analysis of TDF.

Figure 3: Total Analytical Methods for determination of TDF

Acknowledgement

Authors are grateful to R. C. Patel Institute of Pharmaceutical Education and Research Shirpur, (MS) for providing necessary library facilities.

Abbreviations Used

- ACM- Absorbance correction method
- ADV- Adefovir
- API- Active Pharmaceutical ingredient
- AUC- Area under curve
- COBI- Cobicistat
- EFV- Efavirenz
- ELV- Elvitegravir
- EMT- Emtricitabine
- HBV- Hepatitis B virus
- HIV- Human immune- deficiency virus
- HMDE- Hanging mercury drop electrode
- HPLC- High performance liquid chromatography
- HPTLC- High performance thin layer chromatography
- LC- Liquid chromatography
- LMV- Lamivudine
- M.P- Melting point
- Na,HPO₄- Disodium phosphate
- NVP- Nevirapine
- OPA- o- phosphoric acid
References


72. Sasikala M., Deepthi M. T., Kumar K. V., Raja N. M. (2013). Development And Validation of UV Spectrophotometric Method For The Simultaneous Estimation of Tenofovir Disopropxil Fumarate And


https://doi.org/10.4103/2229-4708.81096


https://doi.org/10.14233/ajchem.2015.15412


https://doi.org/10.1149/2.105308jes


https://doi.org/10.1093/chromsci/46.6.524