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A Precise Review on Tenofovir Disoproxil Fumarate: An Analytical Profile

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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 anti-retroviral 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 TNF. The active substance TNF inhibits the Nucleotide Reverse Transcriptase. TDF is quickly hydrolysed into Tenofovir monophosphate in the body and gets converted into the active drug. The chemical reaction for this conversion *in vivo* is as represented in Fig. 2(Avihingsanon *et al.,* 2015). TDF is available in the market as tablets; alone and in combination with other drugs. TDF is mostly expelled with Glomerular filtration and in that being transported into renal proximal tubule cells through organic anion transporter-1 (OAT-1). TDF usually considered as a safe drug, but renal toxicities are reported with its use. The reports are available which provides cause of proximal tubulopathy of kidney, Fanconi syndrome, kidney related other toxicities including insipidus calcium and phosphorus dysregulation with bone disease and reduction in Glomerular function (Patel *et al.,* 2010). Besides the aforementioned effects, the antiretroviral therapy (ART) has transformed HIV infection into a manageable, lifelong disease. The first line regimens are critical to successful ART for its long-term treatment (Bygrave *et al.,* 2011).

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

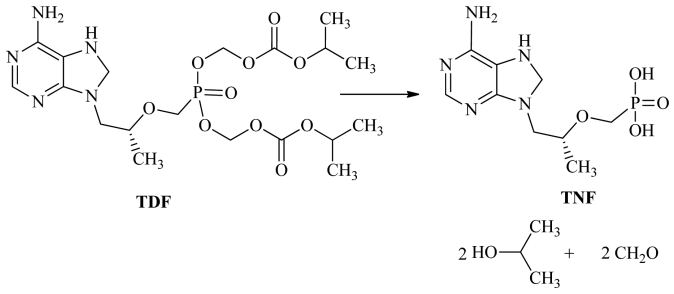

Figure 1: Chemical structure of TDF and its metabolism into TNF.

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 $C_{19}H_{32}N_5O_{10}P$ 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**].

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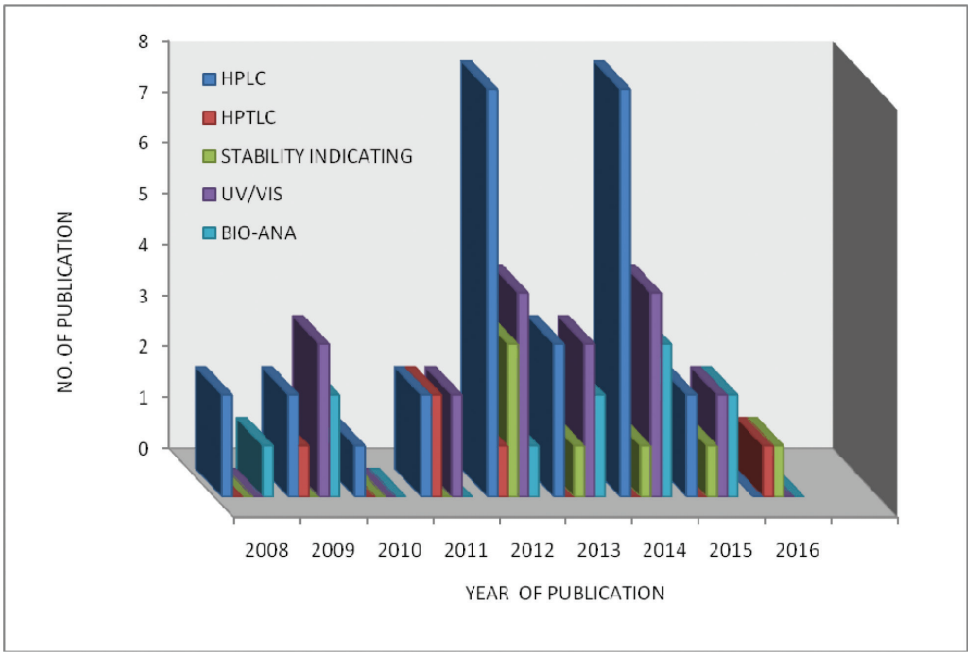

Figure 2: Method Prevalence for Pharmaceutical Determination of TDF.

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; Kandagal *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 Acetontrile/ 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 1: HPLC analysis of TDF alone and in its combination with other drugs.

Sr. No.	Drug(s)	Mobile Phase (v/v)	Detection (λ nm)	Linearity µg/mL	tR (min)	Correlation Coefficient (r^2)	Reference
1	TDF	H_2O: MeOH (45:55)	230	0-120	5.69	0.996	[15]
2	TDF	HCCOH:ACN(50:50)	305	5-30	6.718	0.9993	[16]
3	TDF	MeOH: ACN: OPA (85:10:05)	260	5-35	2.3	0.999	[17]
4	TDF	NaH_2PO_4 buffer (pH2.3): MeOH (49:51)	260	50-300	9.437	0.9995	[18]
5	TDF	Acetate Buffer pH 2.8 : ACN : MeOH(40: 40:20)	260	20 - 60	2.47	0.9992	[19]
6	TDF	ACN: H_2O (75:25)	259	0.2– 10	1.92	0.9966	[20]
7	TDF	NaH_2PO_4 buffer (pH 2.3): MeOH (49:51)	260	50-300	9.437	0.9995.	[21]
8	TDF+ EMT	ACN:50 mM Na_2HPO_4 (pH 6.0): TEA (50:50:0.1)	TDF 260 EMT 280	TDF - 0.3–100 EMT 0.8–80	TDF 4.98 EMT 5.99	0.99996 0.99995	[22]

9	TDF+ EMT	MeOH: Phosphate buffer(65:35)	260	TDF 10-50 EMB 10-50	TDF 6.231 EMB 2.461	0.999 0.999	[23]
10	TDF+ EMT	ACN: MeOH: H_2O (30:50:20)	258	TDF 1-6 EMT 2-12	TDF 2.77 EMT 3.49	0.9998 0.9999	[24]
11	TDF+ EMT	ACN: Phosphate buffer(pH 3.5)(60:40)	270	TDF 3-15 EMT- 2-10	TDF 2.85 EMT 3.55	0.999 0.999	[25]
12	TDF+ EMT	Phosphate Buffer pH 3.0: ACN (60:40)	260	TDF 75-450 EMT- 50-300	TDF 4.5 EMT 3.3	0.999 0.999	[26]
13	TDF+ EMT	ACN: Phosphate (pH 3.5) buffers (60:40)	270	TDF- 3-15 EMT- 2 -10	TDF-2.84 EMT-3.55	TDF – 0.999 EMT- 0.999	[27]
14	TDF+ EMT	ACN: 10 mM Phosphate buffer (pH 6.8) (60:40)	260	TDF- 60-360 EMT- 40-240	TDF-7.42 EMT-2.81	TDF- 0.999 EMT- 0.993	[28]
15	TDF+ EMT	ACN: KH_2PO_4buffer (pH 3.0):TEA (70:30:0.5)	260	TDF- 5-50 EMT- 5-50	TDF- 2.27 EMT-1.78	TDF- 0.9986 EMT- 0.9995	[29]
16	TDF+ EMT	ACN: Phosphate buffer (pH 6.8)(60:40)	260	TDF- 6-36 EMT- 4-24	TDF-3.89 EMT-2.883	TDF- 0.999 EMT- 0.999	[30]
17	TDF+ EMT	MeOH: ACN : TEA (46:50:04)	259	TDF- 40-100 EMT- 40-100	TDF- 1.5 EMT- 3.5	TDF- 0.999 EMT- 0.999	[31]
18	TDF+ LMV	TEA buffer(pH5.0):ACN: MeOH(30:40:30)	260	TDF- 50-300 LMV- 50-300	9.437	0.999 0.999	[32]
19	TDF+ LMV	KH_2PO_4buffer (pH6.0):MeOH:H_2O(33:65:2)	260	TDF- 50-150 LMV- 50-150	TDF- 3.4 LMV- 2.3	TDF- 0.9989 LMV- 0.9983	[33]
20	TDF+ LMV	ACN: H_2O(80:20)	260	5-25	TDF- 2.933 LMV-6.966	TDF- 0.9987 LMV- 0.9981	[34]
21	TDF+ EMT+ RPV	MeOH : H_2O (85:15) ACN : Buffer (pH 3.5) (70:30)	265	TDF- 120-1440 EMT- 80-960 RPV- 10-120	TDF- 8.386 EMT-6.250 RPV- 10.296	0.999 0.999 1.000	[35]

22	TDF+ MT+ NVP	NH_4Ac buffer (pH -4.6): ACN	254	73.10-219.31 59.76-179.29 120.10-360.30	7.89 4.31 6.76	1.000 1.000 1.000	[36]
23	TDF+ LMV+ EFV	MeOH:Phosphate buffer (70:30)	254	TDF-1-6 LMV-1-6 EFV-2-12	TDF-3.96 LMV-2.76 EFV-10.5	0.9991 0.9992 0.9995	[37]
24	TDF+ EMT+ EFV	ACN: H_2O(60:40)	260	TDF-6-72 EMT-4-48 EFV-12-144	TDF-3.860 EMT-3.105 EFV-10.549	TDF-1.0 EMT-0.999 EFV-1.0	[38]
25	TDF+ EMT+ EFV	MeOH: buffer (pH 4.5)	260	TDF-80-160 EMT-40-120 EFV-200 –280	TDF EMT EFV	TDF-0.999 EMT-0.999 EFV-0.999	[39]
26	TDF+ LMV+ EFV	phosphate buffer (pH 4): Water: ACN (30:70)	260	TDF-75-450 LMV-75-450 EFV-150-900	TDF-2.76 LMV-3.96 EFV-10.5	TDF-0.9991 LMV-0.9992 EFV-0.9995	[40]
27	TDF+ LMV+ EFV	phosphate buffer (pH 4) : ACN (42:58)	254	TDF-10-50 LMV-10-50 EFV – 20-100	TDF-3.276 LMV-2.220 EFV-10.814	TDF-0.999 LMV-0.999 EFV-0.999	[41]

Table 2: HPLC chromatographic columns and optimized analytical parameters.

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

13	TDF+EMT	Phenomenax Luna C18	250 × 4.6, 5	UV/Visible	1.0	Isocratic	[27]
14	TDF+EMT	Phenomenax Luna C18	250 × 4.6, 5	UV	1.0	Isocratic	[28]
15	TDF+EMT	Luna C18	250 × 4.6, 5	UV	1.5	Isocratic	[29]
16	TDF+EMT	Luna C18	250 × 4.6, 5	UV	1.0	Isocratic	[30]
17	TDF+EMT	Chromosil C-18	250 × 4.6, 5	UV	0.5 – 1.5	Isocratic	[31]
18	TDF+LMV	Symmetry C8	150 × 4.6, 5	UV/Visible	1.0	Isocratic	[32]
19	TDF+LMV	Thermosil C18	150 × 4.6,3.5	UV	0.8	Isocratic	[33]
20	TDF+LMV	RP- C18	250 × 4.6, 5	UV	0.7	Gradient	[34]
21	TDF+ EMT + RPV	Inertsil ODS 3V	250 × 4.6, 5	PDA	1.5	Gradient	[35]
22	TDF+EMT +NVP	Inertsil ODS-2	150 × 4.6 , 5	DAD	1.0	Isocratic	[36]
23	TDF+LMV +EFV	Kromasil C18	150 × 4.6, 5	UV	1.0	Isocratic	[37]
24	TDF+EMT +EFV	Hypersil BDS C18	250 × 4.6, 5	PDA	0.8	Isocratic	[38]
25	TDF+EMT-EFV	Zorbax SB CN	250 × 4.6, 5	UV	1.5	Isocratic	[39]
26	TDF+LMV +EFV	Hypersil C18	150 × 4.6, 5	PDA	1.0	Gradient	[40]
27	TDF+LMV +EFV	Symmetry C18	100 × 4.6, 3.5	UV	0.5	Gradient	[41]

Table 3: Pharmaceutical Analysis of TDF/TNF in biological fluids.

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

9	TNF+ EMT	Human plasma	LC-MS/MS	Chromolith Speed Rod RP18 50 × 4.6, 5	MS	Lamivudine	[50]
10	TNF+ NVP+ LMV	Human plasma	LC–MS/MS	Phenomenex kinetex C18, 100 × 4.6 , 2.6	MS	Emtricitabine	[51]

3.1.2 *Bioanalytical methods of analysis*

Total ten bioanalytical methods are reported so far for determination of TNF 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; Paliwal *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 TNF 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 TNF and other drugs from biological matrix include acetonitrile, methanol, water, trichloroacetic acid, and trifluoroacetic acid etc. The internal standards viz Adefovir, fluconazole, Tenofovir, Tenofovir D6, Abacavir, Clonazepam, dideoxyuridine 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 Polar-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.

Sr. No	Name of Drug	Mobile phase composition (v/v)	Detection (λ nm)	Linearity (ng/spot)	R_f	r^2	References
1	TDF	$CHCl_3$: MeOH (8.5: 1.5)	270	200 -1200	0.54	0.9994	[52]
2	TDF	EtOAc: MeOH: HCOOH (7:2.5:0.5)	266	125-750	0.78.	0.991	[53]
3	TDF+EMT	$CHCl_3$: MeOH (9:1)	265	200 – 1000	0.47 0.18	0.9996 0.9995	[54]
4	TDF+EMT	Toluene: MeOH : EtOAc: AcOH (4:2:5:0.1)	270	120– 600 80-560	0.52 0.40	0.999 0.9992	[55]
5	TDF+ EMT+ RPV	$CHCl_3$:EtOAc: MeOH: Glacial Acetic acid (5:2:1:0.1)	272	TDF-600-3600 EMT- 600-2400 RPV- 50-300	TDF- 0.52 EMT- 0.28 RPV- 0.70	TDF-0.9993 EMT- 0.9992 RPV- 0.9995	[56]

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 (Havele 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, λmax, linearity, range, retention time and correlation coefficient is shown in Table 5.

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, λmax, linearity range, and correlation coefficient.

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

Sr. No	Name of drug(s)	Mobile phase composition(v/v)	Detection (λ nm)	Linearity µg/ml	t_R (min)	r^2	References
1	TDF	MeOH: H_2O (60:40)	260	4–20	12.09	0.9999	[57]
2	TDF	ACN: 0.025M H_2PO_4buffer (pH 3.0) (35:65)	260	0.1-50	7.5	0.999	[58]
3	TDF+EMT	NH_4Ac buffer (pH 5.5): MeOH (85:15)	260	TDF-10-60 EMT-10-60	TDF-31.83 EMT-11.6	TDF- 0.9991 EMT-0.999	[59]
4	TDF+ LMV+ NVP	A.$(NH_4)_3PO_4$: dil. TFA B. MeOH: ACN. (10:90)	260	0.05-0.50	TDF-38 LMV-11 NVP-27	TDF-0.997 LMV-0.998 NVP-0.999	[60]
5	TDF+ LMV+ EFV	TEA buffer: MeOH (35: 65)	260	TDF-75-225 LMV-75 –225 EFV-150-450	TDF-3.36 LMV-2.43 EFV-10.46	TDF-0.999 LMV-0.999 EFV-0.999	[61]
6	TDF+ EMT+ EFV	MeOH: TEA (70:30) (pH 7)	260	TDF-15-75 EMT-10-50 EFV-10-50	TDF-4.632 EMT-3.706 EFV-8.121	TDF- 0.999 EMT- 0.999 EFV- 0.999	[62]
7	TDF+ EMT+ COBI+ ELV	0.1%TFA: ACN	242	TDF-150-450. EMT-100-300 COBI-75-225 ELV-75-225	TDF- 4.75 EMT- 3.43 COBI- 5.27 ELV- 7.56	TDF- 0.999 EMT-0.999 COBI-0.999 ELV- 0.999	[63]

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

Sr. No	Name of drug	Method/ order		Detection (λ, nm)	Linearity (µg/ml)	(r^2) Value	References
1	TDF	I	Zero	451	8-40	0.998	[64]
2	TDF	I	Zero	261	5- 90	0.9983	[65]
3	TDF	I II	Zero AUC	260 250-270	5-30	0.999 0.999	[66]
4	TDF	I	Zero	259	5-45	0.9995	[67]
5	TDF	I II	Zero AUC	260 250-270	5-25	0.992 .9917	[68]
6	TDF	I II	Zero First	260 273	4-24	0.999 0.998	[69]
7	TDF	I	Zero	260	10-50	1.00004	[70]
8	TDF+EMT	I	ACM	TDF-260 EMT-290	TDF- 5-25 EMT-7-35	0.999 0.999	[71]
9	TDF+EMT	I	Zero	TDF-240 EMT-280	10-80	0.9996 0.9993	[72]

10	TDF+EMT	I II III	First Derivative ACM	TDF-306.88 EMT-224.38 EMT-293.38 TDF-270.29	TDF-3-21 EMT-2-14 EMT-4-20 TDF- 6-30	TDF- 0.9999 0.9996 0.9997	[73]
11	TDF+EMT	I	SEM	TDF-260 EMT- 290	TDF- 5-25 EMT- 7-35	0.999 0.999	[74]
12	TDF+EMT	I	SEM	TDF-210, 281 EMT-210, 281	4-24	0.9997 0.99977 0.99978 0.99991	[75]
13	TDF+LMV	I	SEM	TNF-271 LAM-261	5-50	0.999 0.999	[76]
14	TDF+LMV	I	SEM	TDF- 260 LMV-280	TDF- 5-45 LMV-2-16	0.999 0.998	[77]
15	TDF+ EMT+ RPV	I	SEM	TNF-259 EMT-280 RPV-291	TNF-3-21 EMT-1-10 RPV-0.5-3	0.9993 1.000 0.9996	[78]

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 and exhibiting λ-max at 401 nm and showed linearity in theconcentration 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.

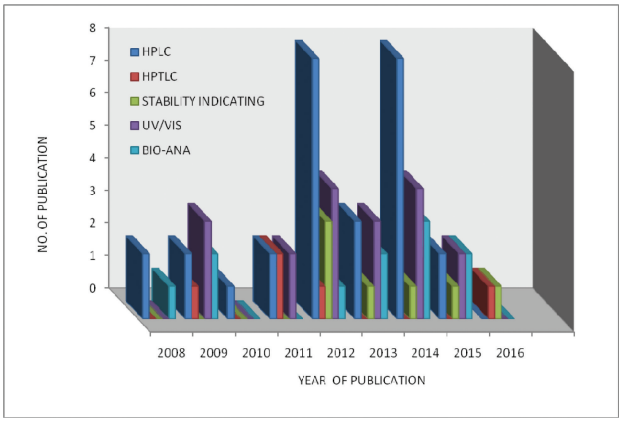

Figure 2: Method Prevalence for Pharmaceutical Determination of TDF.

One of the most commonly used solvents for sample extraction of TDF from pharmaceutical formulation and also from biological fluids is methanol and acetonitrile. 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.

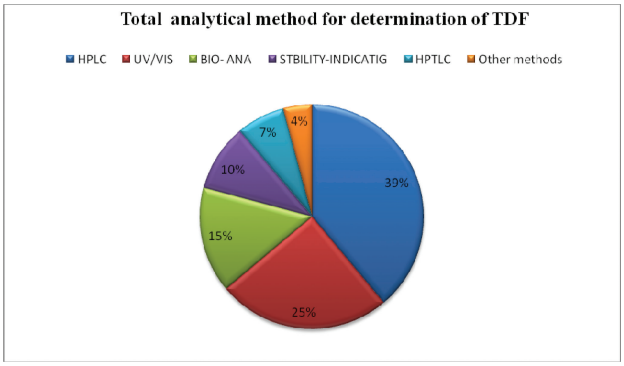

Figure 3: Total Analytical Methods for determination of TDF

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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_2HPO_4-Disodium phosphate
- NVP- Nevirapine
- OPA- o- phosphoric acid

- p-CA- p-chloranilic acid
- pH- power of hydrogen
- RF- Retention factor
- RPV- Rilpivirine
- RT- Retention time
- SEM-Simultaneous equation method
- SIM- stability indicating method
- SPME- solid phase microextraction method
- TDF- Tenofovir Disofroxil fumarate
- TEA- Triethylamine
- TNF- Tenofovir

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