A REVIEW OF ANALYTICAL TECHNIQUES FOR DETERMINATION OF ANTI-HIV DRUGS

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ABSTRACT

Pharmaceutical analysis plays a very prominent role in quality assurance as well as quality control of bulk drugs and pharmaceutical formulations. Rapid increase in pharmaceutical industries and production of drug in various parts of the world has brought a rise in demand for new analytical techniques in the pharmaceutical industries. As a consequence, analytical method development has become the basic activity of analysis. From the times of yore, people were trying to find safe and sound ways to treat viral infections. In the current scenario, due to the emerging of new viruses, the development of drugs for their treatment is also gaining equal importance. Before launching to the market, these drugs should undergo a validation process. High-performance liquid chromatography (HPLC) coupled with ultraviolet (UV), Photodiode array detectors (PDA), Mass spectrophotometer (MS) detectors etc. is one of the fastest, safe and precise technologies used for determination and separation of pharmaceutical drugs, impurities and biological samples. HPLC is versatile and it takes less time for quantification of drugs as compared to old liquid chromatography techniques. Tenofovir disoproxil fumarate (TDF), Emtricitabine (FTC) and Efavirenz (EFV) is an antiretroviral medicine used treat AIDS as well as chronic Hepatitis-B. It is used alone or with other HIV medications to help control HIV infection. The present review article assesses the published analytical methods and a variety of approach for investigation of TDF, FTC and EFV in bulk drug as well as pharmaceutical formulations including combinations. The present studies revealed that HPLC technique along with the spectroscopic have been most widely explored for the analysis. The investigatory review may provide the comprehensive details to the researchers who are working in the area of analytical research of TDF, FTC and EFV.

Keywords: Pharmaceutical analysis, High-performance liquid chromatography, Tenofovir disoproxil fumarate, Emtricitabine, Efavirenz

INTRODUCTION

The main goal of the pharmaceutical industry is to provide drug products with sufficient quality, efficacy and safety. The development of a new drug product and its production consist of many pharmaceutical processes, including analytical testing. The analytical data generated support further decisions on how development should be pursued or provide information on whether a drug product should be released. It is important that each such development or production process provide credible results with constant quality and therefore, it needs to be controlled and, if necessary, continually improved. By improvement of quality of a pharmaceutical process, the quality of a drug product is also improved. Analytical methods are among the most critical processes in drug product development and production. They play a key role in supporting other development and production processes throughout all stages of a drug product’s life cycle. It is essential that an analytical method be precise, accurate and reliable, making it suitable for its intended purpose [1, 2]. In most cases, the main working principle of an analytical method is separation of the analytes present in the sample. Liquid chromatography (LC) techniques are most commonly employed, such as HPLC or ultraperformance liquid chromatography (UPLC), often in reversed-phase mode with UV absorbance detection. The purposes of analysis differ depending on the number, importance and relation of analytes that are required to be determined. Analytical methods for the assay of an active pharmaceutical ingredient (API) or determination of its related substances and degradation products are most commonly applied [2]. Development of a specific and robust stability-indicating LC method for the determination of related substances and degradation products is a complex process. It requires a deliberate forced degradation of a drug substance and/or a drug product under various stress conditions, such as hydrolytic, oxidative, photolytic, or thermal conditions, to provide stressed samples containing the analyte and its degradation products. The stress conditions are more severe than the accelerated and long-term stability conditions prescribed in the ICH guidelines for stability testing. An analytical method for determination of degradation products should be capable of detecting their increase during the product’s shelf life and the method for the assay should be capable of detecting any decrease in the drug substance’s content during its shelf life. Such methods are stability indicating [3-6].

Recent estimates indicate that 34 million people are currently living with HIV/AIDS worldwide, with approximately 2.5 million new infections occurring annually [7]. The virus is transmitted through the exchange of virus containing fluids, including blood, breast milk, semen and genital secretions [8-10]. Routes of viral infection include sexual contact, injection drug use, from mother to child during pregnancy, childbirth, or breast-feeding, and exposure of infected body fluids to exposed membranes or tissue [10, 11]. Antiretroviral therapy (ART) is the primary modality for the treatment and management of the disease and can substantially reduce HIV-related morbidity and mortality [12-14]. ART is strongly recommended for all HIV-infected individuals, regardless of pretreatment CD4 T cell count. Furthermore, ART has shown efficacy not only in disease management but also in viral prevention as pre-exposure prophylaxis in high-risk populations [15-18]. There are currently more than 25 antiretroviral (ARV) agents approved for HIV treatment by the U. S. Food and Drug Administration (FDA) in both single-and multi-drug formulations [19]. Combinatorial ART regimens are typically required for the sustained suppression of viral replication and clinical benefit [20]. Currently, more than 100 regimens exist for the treatment of HIV [21]. ARVs elicit their therapeutic effects through the targeted inhibition of various stages of the viral infection cycle. Thus, drug classes are stratified as CCR5 antagonists, viral fusion inhibitors, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrate strand transfer inhibitors (INSTIs), and protease inhibitors (PIs). Many combinatorial ART regimens incorporate drugs from more than one ARV class, and the U. S. Department of Health and Human Services (DHHS) has indicated recommended and alternative regimens for disease management [22]. In addition, new therapies are continually
beingsoughtthatspotteneviraltargets,haveactivityagainst resistantviralstrains,havealowerincidenceofadverseeffects,and offerconvenientdosing.Newagentsofexistingclassesarecurrentlyin advancedstagesofclinicaldevelopment[23].Thegrowing demandformostoftheseagentsstimulatesa searchfornew evenmore effective drugs, in a higher level of quality control of thesetherapeutic substances and preparations, so that they are in the highest possible degree free from any impurities that may come from the production process, as well as from decompositions products of active or auxiliary substances. Therefore, it seems appropriate todervenewanalyticalmethodsregardingtheir qualitativeandquantitative analysis. For this aim, different analytical methods are used for determining anti-HIV drugs. Anti- HIV drugs are the recent developments of drugs and there is a great need to review the analytical work reported so far in the literatures. Efforts have been made to collect the literature from 2000 up to the present. Analytical methods allowing the determination of TDF, FTC and EFV drugs in various media, such as pharmaceutical formulations, biological matrices and environmental samples, is discussed. At present, there are five major classes of ARV drugs viz. nucleoside reverse transcriptase inhibitor [NRTI], non-nucleoside reverse transcriptase inhibitor [NNRTI], Protease inhibitors [PI], fusion inhibitor and integrase inhibitor [IIs].

The first single-tablet fixed-dose combination (FDC) antiretroviral (ARV) has been commercially available since 2006 and is marketed as Atripla® [24]. A generic product has been commercially available in South Africa since April 2013 [25, 26] and consists of efavirenz (EFV), emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) in a ratio of 600 mg/200 mg/300 mg. TDF in this quantity is equivalent to 245 mg tenofovir disoproxil (TNF) and 136 mg of tenofovir [27]. The tablet is taken once daily for the treatment of HIV-1 infection [28, 29]. Once-daily FDC tablets are the simplest antiretroviral therapy available [30]. FDC ARV therapy is convenient for patients as it reduces the "pill burden" which in turn improves adherence to therapy [28-30]. FTC were initially indicated for treating HIV-positive antiretroviral naïve patients and HIV-positive pregnant women and those who are breastfeeding. It is now available to all patients on the recommendation of a physician [26]. Treatment with EFV, FTC and TNF is the preferred first-line therapy for antiretroviral naïve HIV-1-infected persons [30]. Bioequivalence between the dosage form containing a single molecule and the FDC in addition to favourable pharmacokinetics facilitates once daily dosing of EFV, FTC and TNF [24, 30].

Nucleoside reverses transcriptase inhibitors (NRTIs)
The first generation of ARV drugs is NRTIs permitted to treat HIV [31]. Reverse transcriptase is an HIV enzyme that converts viral RNA into DNA in host CD4 cells and the process is known as reverse transcription. NRTIs inhibit the enzyme reverse transcriptase and prevent the synthesis of DNA. Without reverse transcriptase, HIV cannot replicate and infection cannot spread. Nucleoside analogues possess structural similarity with the natural building blocks of DNA and have to undergo phosphorylation to become active in the body. NRTIs are falsely chosen by reverse transcriptase to build the faulty DNA that denies further addition of natural nucleotides. Thus, the new DNA built incorrectly led to halt HIV replication [32]. Following are some NRTIs used for HIV therapy: Zidovudine, Didanosine, Stavudine, Lamivudine, Abacavir, Adefovir, Emtricitabine (FTC), Tenofovir disoproxil fumarate (TDF).

Emtricitabine (FTC)
FTC is a synthetic fluoro derivative of thiaacydine with potent antiviral activity approved in 2003. Chemically it is a 4-amino-5-fluoro-1-[2R, 5S]-[2-hydroxymethyl]-1,3- oxathiolan-5-yl] pyrimidin-2-one, the solubility of which in water is 112 mg/ml with logb value of 1.4. FTC is a white to off white, crystalline powder [33, 35]. FTC has an empirical formula of C14H11FN2O2S and a relative molecular mass of 274.2 g/mol [34, 36]. FTC contains no less than 99.0 percent and not more than 101.0 percent of emtricitabine (C14H11FN2O2S), calculated with reference to the anhydrous reference material [34]. FTC, when combined with TDF, has shown together greater HIV RNA suppression compared to the combination of Zidovudine and Lamivudine [37, 38]. Co administration of FTC/TDF with antiviral drugs that eliminate through kidney by means of active tubular discharge may enhance plasma TDF or FTC concentrations and/or those of simultaneously given drugs [39]. FTC undergoes phosphorylation to form active FTC triphosphates metabolite using cellular kinase enzymes. FTC and phosphorylated metabolite give a peak eluted at a retention time of approximately 12.6 minutes mimicking normal nucleos(t)ides that are incorporated into DNA at the 3′ terminus. However FTC and TNF lack the 3′-OH and their incorporation at the 3′ terminus of the DNA therefore terminates chain elongation by preventing incorporation of additional nucleotides [28, 41, 42]. TDF diphosphate and FTC 5′-triphosphate are weak inhibitors of g. G cell culture, and the ability to inhibit both FTC and TDF is lower than that of FTC and TDF [28]. The combination of TNF and FTC has been the preferred NRTI regimen since 2010 since approval by FDA [30]. A single-tablet combination has been approved by the FDA and more recently by the Medicines Control Council (MCC) of South Africa for pre-exposure prophylaxis (PrEP) although it is not widely used clinically [43, 44]. The HIV-1 reverse transcriptase (RT) mutation K65R is a common drug resistance mutation that confers resistance to NRTI including TNF and FTC [11, 42, 45, 46] and this mutation may be responsible for cross-resistance between different NRTI [42, 46]. Cases of acute renal failure and Fanconi syndrome (FS) have been reported in patients treated with TNF [47] although clinically important renal toxicity is rare [48]. FTC and TNF undergo limited systemic metabolism [49].

Tenofovir disoproxil fumarate (TDF)
TDF is the acyclic nucleotide analogue of adenosine monophosphate approved for HIV treatment in 2004 [50]. Chemically, it is an [2R]-1-[6-aminopyrimidin-9-yl][propan-2-yl]oxymethyl phosphonic acid, the solubility of which in water is 13.4 mg/ml at 25 °C with a logb value of 1.6. TDF has an empirical formula of C19H30N5O10P, C4H4O4 and a relative molar mass of 635.5 g/mol. It occurs as a white to almost white crystalline powder [51-53]. TDF contains no less than 98.5 percent and not more than 101.0 percent of TDF (C19H30N5O10P, C4H4O4), calculated with reference to an anhydrous reference material [52]. TDF refers to the solid/raw material whereas TNF refers to TDF in solution and tenofovir peaks in chromatograms. TDF is phosphorylated twice to the active diphosphate form. High fat meal increases the bioavailability of TDF and remains unaffected by normal meal [54]. As TDF is eliminated through the kidney and is not a substrate for CYP45, its dosage regimen needs to be modified in renal complications [55, 56]. Gervasoni et al. showed that HIV-infected females with diminished body weight are in danger to be exposed to elevated TDF plasma trough concentrations, eventually bringing about a huge threat to produce long-term TDF complications [55].

Non-nucleoside reverse transcriptase inhibitor [NNRTI]
NNRTIs restrain the process of viral DNA synthesis by directly binding to the hydrophobic pocket of reverse transcriptase enzyme [31]. Unlike NRTIs, which must be phosphorylated to prevent HIV from infecting the cell, NNRTIs are active in the form administered. NNRTIs are classified as 1st generation and 2nd generation NNRTIs. 1st generation NNRTIs include Nevirapine and Efavirenz [47] and 2nd generation NNRTIs are Etravirine and Rilpivirine. HIV-2 is naturally resistant to NNRTIs.

Efavirenz (EFV)
EFV is a benzoazin analogue approved by FDA in 1998 for the treatment of patients infected with HIV [57]. Chemically, it is an [4S]-6-chloro-4-[2-cyclopropylethynyl]-4-[trifluoromethyl]-1H,3- benzoazin-2-one and occurs as a white to slightly pink crystalline powder [58-60]. The empirical formula for EFV is C14H9ClF3NO2 and the relative molar mass is 315.7 g/mol [58, 60, 61]. EFV contains no less than 97.0 percent and not more than 103.0 percent of C14H9ClF3NO2, calculated with reference to the anhydrous reference material [58]. The solubility of which in water is 0.93 mg/ml at 25 °C with logb value of 4.6. The dosing of EFV is once-daily due to its long half-life. EFV is usually preferred to treat HIV patients co-infected with tuberculosis [78]. Both the diseases are life-threatening and treatment becomes very difficult due to drug-drug interactions.
between EFV and rifampicin [62, 63]. Side effects of EFV are found to be associated with the EFV plasma concentration. Various side effects are associated with high and low plasma levels of EFV particularly in HIV-TB co-infected patients for which TDM studies become necessary. EFV levels are directly correlated with optimum therapeutic output and central nervous system side effects. Therefore, TDM of EFV in clinical practice is essential for optimum therapeutic output, especially in HIV-TB co-infected patients who are under treatment with the combination of EFV and rifampicin. On the other hand, EFV possesses high protein binding property [5-99%] and thus gets penetrated into male genital tract through blood. High penetration in male genital tract makes it an important candidate to study its concentration for prophylaxis use. HIV replication took place inside the cell, so ARV drugs have to enter the cells at an adequate concentration to restrain viral replication. Subsequently, studying intracellular drug concentration is a valuable tool to ascertain effective levels of ARVs in target cells mainly in virological failure regardless of efficient plasma level concentrations.

**Dissociation constant (pKa)**

EFV is a weak acid with a pKa of 10.2. It is therefore ionised at high pH, at which the carbonate moiety undergoes deprotonation to form a negatively charged species. The trifluoromethyl and ethylene moieties are most likely responsible for the lowering of the pKa [64, 65]. The pKa of FTC and TNF are 2.65 and 3.75 respectively [35, 36, 66]. EFV is a weak acid whereas FTC and TNF are weak bases.

**Solubility**

EFV is practically insoluble in water (90 μg/ml) but is freely soluble in methanol [1-4, 31, 38, 39]. FTC is freely soluble in methanol and water (112 mg/ml) and is practically insoluble in dichloromethane R [34-36, 58]. TNF has a solubility of 13.4 mg/ml in distilled water at 25 °C [51].

**Biopharmaceutical classification system (BCS)**

The BCS provides a framework to classify molecules into categories based on their aqueous solubility and membrane permeability. Class 1 drugs have high solubility and high permeability, class 2 drugs low solubility and high permeability, class 3 drugs high solubility and low permeability and class 4 drugs low solubility and low permeability. EFV has low aqueous solubility and high intestinal permeability and is classified as a Class 2 molecule. FTC has high aqueous solubility and high intestinal permeability and is classified as a Class 1 molecule. TNF, the form of tenofovir that is absorbed, has high aqueous solubility and low intestinal permeability and is classified as a Class 3 molecule [67, 68].

**Meling range**

EFV melts within the range of 139-141 °C [69]. FTC melts within the range of 136-140 °C [70]. TNF melts within the range of 276-280 °C [71].

**Spectrophotometric methods**

Many analytical methods involving spectroscopic analysis of the drug individually as well as multicomponent samples have been reported. These methods include a simultaneous equation method, derivative spectrophotometric method, absorption ratio and a method based on Q analysis.

**Chromatographic method**

Liquid chromatographic analysis for the determination of TDF, FTC and EFV individually and in combination has been reported covering different phases of analytical research viz; profiling of impurities, stability indicating analytical methods, bioanalytical method development in different biological fluids to determine the concentration of TDF, FTC and EFV in human serum and to determine simultaneously in synthetic mixture or combination dosage form.

**Stability indicating method**

Stability indicating method is used to check drug stability under different conditions. Here, TDF, FTC and EFV are studied by RP-HPLC and UPLC for stability studies.

**Table 1: RP-HPLC/UPLC methods for determination of TDF, FTC and EFV**

| S. No. | Name of drug/formulation/biological fluid | Column | Mobile phase composition | Detection (nm) | Ref. |
|--------|----------------------------------------|--------|--------------------------|----------------|-----|
| 1      | FTC-Tablet                             | Peerless basic C18 (50 mm x 4.6 mm, 3 μm) | Buffer (pH 3.0): methanol-90:10 (v/v) | 280 nm | 72  |
| 2      | FTC-Nanoparticles                      | Phenomenex C18 (250 mm x 4.6 mm, 5 μm) | 40 mmol phosphate buffer (pH 6.8), methanol and 2% acetonitrile (83:15:2, v/v/v) | 280 nm | 73  |
| 3      | FTC-Tablet                             | Phenomenex C18 (250 mm x 4.6 mm, 5 μm) | 10 mmol phosphate buffer (pH 6.8) methanol-2% acetic acid (73: 25: 2, v/v/v) | 280 nm | 74  |
| 4      | FTC-Capsule                            | Luna RP-18(2)250X4.6 mm, 5 μm | Buffer: acetonitrile (85:15 (v/v) | 280 nm | 75  |
| 5      | FTC-Capsule                            | Phenomenex (Torance,CA) C18 250×4.6 mm | Buffer: acetonitrile (40:20:40) (v/v/v) | 280 nm | 76  |
| 6      | FTC/TDF/Etvdragavir/Cobici stat-Tablet | Inertsil ODS 3V C18 (250 mm x 4.6 mm, 5 μm, 100 Å) | A = H2PO4 (0.02M) pH 2.5, B = acetonitrile | 240 nm | 77  |
| 7      | FTC/TDF/Tablet                         | Hypersil, 250 X 4.6 mm, 5 μm | Buffer (pH 3.7): acetonitrile 60:40 (v/v) | - | 78  |
| 8      | FTC/TDF/Bilpiravirine-Tablet           | Inertsil C18 (150 x 4.6 mm, 5 μm) | 0.1N Phosphate buffer (pH: 4.5): acetonitrile (40:60(v/v) | 275 nm | 79  |
| 9      | FTC/TDF/Tablet                         | Inspire C18 (150 x 4.6 mm) 5 μm | Buffer (pH 2.5): methanol (30:70 (v/v) | 272 nm | 80  |
| 10     | FTC/TDF/Tablet                         | Inspire C18 (4.6×250 mm) 5 μm | Mixed buffer (KH2PO4 and K2HPO4) pH 3:ACN | 273 nm | 81  |
| 11     | FTC/TDF/Bilpiravirine-Tablet           | Kromasil C18 (250 mm x 4.6 mm, 5 μm) | 0.01N Potassium dihydrogen phosphate and acetonitrile 65:35 (v/v/v) | 279 nm | 82  |
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| 12 | FTC/TDF/Evlitegravir/Cobicistat-Tablet | Atlantis C18(100×4.6 mm, 5 μm) | Gradient mixture of 0.1% trifluoroacetic acid and acetonitrile sodium dihydrogen orthophosphate buffer (pH 6.9) and methanol (96:4) | 240 nm | 83 |
| 13 | FTC/TDF-Plasma | Hypersil C18(250 mm ×4.0 mm, 5 μm) | Acetonitrile and phosphate buffer pH 3 (60:40) | 259, 265,280 nm | 84 |
| 14 | FTC/TDF/Rilpivirine | Thermo Hypersil ODS C18 (150×4.6 mm, 5 μ) | Phosphate buffer (pH 5.6) and methanol 60:40 (v/v) | 240 nm | 86 |
| 15 | FTC/TDF-Tablet | HDMIA (50 x 2.1 mm, 1.7μ) | Methanol and phosphate buffer pH 2.5 (65:35 v/v) | 261 nm | 87 |
| 16 | FTC/TDF-Tablet | Hypersil TM BDS C18 120A (250 × 4.60 mm, 5μ) | Buffer and Acetonitrile (55:45 v/v) | 272 nm | 88 |
| 17 | FTC/TDF/Bictegravir-Tablet | Zodiac C18 150x6.6 mm, 5μ | 10 mmol phosphate buffer (pH 6.8): acetonitrile; 40: 60 (v/v) | 269 nm | 89 |
| 18 | FTC/TDF/Evlitegravir/Cobicistat-Tablet | Phenomenex-Luna C18 (25 cm × 4.60 mm, 5 μm) | Acetonitrile: sodium dihydrogen orthophosphate, pH 2.5 B= (acetonitrile) 55:45 v/v | 250 nm | 90 |
| 19 | FTC/TDF/Evlitegravir/Cobicistat-Tablet | ODS (250 × 4.6 mm, 5 μm) | Acetonitrile: methanol: water 30:50:20 (v/v) | 258 nm | 91 |
| 20 | FTC/TDF-Tablet | Phenomenex Luna C18 (150 mm x 4.6 mm, 5 μm) | 0.68% potassium dihydrogen orthophosphate buffer of pH = 6 and methanol 45:55 v/v | 261 nm | 92 |
| 21 | FTC/TDF-Tablet | BRH C18 (100 mm x 2.1, 1.8 μm) | Acetonitrile: potassium dihydrogen phosphate buffer (pH 3.0±0.05); triethylamine 70:30:0.5(v/v) | 260 nm | 93 |
| 22 | FTC/TDF-Tablet | Luna C18 (25 cm x 4.60 mm, 5 μm) | Methanol: Phosphate cushion 68:32 % v/v. | 259 nm | 94 |
| 23 | FTC/TDF-Tablet | Promosil C18, (250 mm, 4.6 mm, 5 μm) | 0.1% trifluoro acetic acid (TFA) buffer and methanol 39:61 (v/v) | 261 nm | 95 |
| 24 | FTC/TDF-Tablet | Inertsil ODS C18 (250 mm x 4.6 mm, 5 μm) | Orthophosphoric acid buffer: acetonitrile (55:45 v/v%) | 240 nm | 96 |
| 25 | FTC/TDF-Tablet | Kromasil C18 (250+4.6 mm, 5 μm) | Methanol: phosphate buffer pH 3 (70:30:0 v/v) | 258 nm | 97 |
| 26 | FTC/TDF-Tablet | Phenomenex Luna C18 (250 mm x 4.6 mm, 5 μm) | Ammonium acetate (10 mmol) pH 3.0, B= acetonitrile, ammonium acetate (10 mmol) pH 3.0 and methanol 70:15:15%v/v/v | 260 nm | 98 |
| 27 | FTC/TDF/Dolutegravir-Tablet | Phenomenex Kinetex Bipheryl 250×4.6 mm, 5 μm | 0.01N KH2PO4 (pH 2.5) and acetonitrile (43:57v/v) | 254 nm | 99 |
| 28 | FTC/TDF/Dolutegravir-Tablet | Kromasil C18 (250 mm x 4.6 mm x 5μ) | 0.05MPH3PO4 buffer pH 3.0 and acetonitrile 95:5 | 240 nm | 100 |
| 29 | FTC/TDF/Elvitegravir-Tablet | Hypersil BDS C18 250x4.6 mm, 5 μ, 100A | 0.1%Formic acid: acetonitrile (65:35%, v/v) | 250 nm | 101 |
| 30 | FTC/TDF/Elvitegravir-Tablet | Agilent C18 (250 × 4.6 mm, 5 μm) | Orthophosphoric acid (pH 3.0): acetonitrile: methanol (40:50:10 v/v) | 254 nm | 102 |
| 31 | FTC/TDF-Tablet | Phenomenex Luna C18 (250 mm x 4.6 mm, 5 μm) | Acetonitrile and phosphate buffer pH 3.5 | 260 nm | 103 |
| 32 | FTC/TDF-Tablet | Hypersil TM BDS C18 120A (250×4.60 mm, 5μ) | 6.5 mmol Phosphate buffer pH 2.5 and acetonitrile (50:50 v/v) | 260 nm | 104 |
| 33 | FTC/TDF-Tablet | Phenomenex Column (15 cm x 4.6 mm, 5 μm) | 0.1% Formic acid and acetonitrile | - | 105 |
| 34 | FTC/TDF-Tablet | Acuity UPLC BEH C18 (1.7 μ, 1 mm X 50 mm) | | | |
| 35 | FTC/TDF-Tablet | Waters X-terra RP18 (150 x 4.6 mm, 3.5 μm) | | | |
| 36 | FTC/TDF-Tablet | Waters X-Terra Shield, C18 50 × 4.6 mm, 3.5 μm | | | |
| 37 | FTC/TDF-Tablet | Hypersil BDS C18 250 mm x 4.6 mm; 5μ | | | |
| 38 | FTC/TDF-Tablet | Hypersil BDS C18 250 mm x 4.6 mm, 5 μ, 100A | | | |
| 39 | FTC/TDF-Tablet | Hypersil BDS C18 250 mm x 4.6 mm, 5 μ | | | |
| 40 | FTC/TDF-Tablet | Waters XBridge (4.6 x 250 mm, 5 μ) | | | |
| 41 | FTC/TDF-Tablet | Acquity UPLC BEH Shield RP18 (50 × 3 mm, 1.7 μ) | | | |
| 42 | FTC/TDF-Tablet | Waters XBridge (4.6 x 250 mm, 5 μ) | | | |
| 43 | FTC/TDF-Tablet | Waters XBridge (4.6 x 250 mm, 5 μ) | | | |
| 44 | FTC/TDF-Tablet | Acquity UPLC BEH Shield RP18 (50 × 3 mm, 1.7 μ) | | | |
| 45 | FTC/TDF-Tablet | Chromolith Performance RP-18e (100 × 4.6 mm) | | | |
| 46 | FTC/TDF-Tablet | Chromolith Performance RP-18e (100 × 4.6 mm) | | | |
| 47 | FTC/TDF-Tablet | SHISEIDO C18 (250 x 4.6 mm, 5μ) | | | |
| 48 | FTC/TDF-Tablet | Zorbax SB-Phenyl, (250 mm X 4.6 mm), 5 μ | | | |
| 49 | FTC/TDF-Tablet | Inertsil ODS JV (250 x 4.6 mm, 5μ) | | | |
| 50 | FTC/TDF-Tablet | Chromolith Performance RP-18e (100 × 4.6 mm) | | | |
Table 2: Spectrophotometric methods used for determination of TDF, FTC and EFV alone and in combined dosage form

| S. No. | Name of drug | Sample matrix | Method | Detection (nm) | Ref. |
|--------|--------------|---------------|--------|---------------|-----|
| 1      | EFV          | Tablet        | Zero order | 247           | 148 |
| 2      | TDF          | Tablet        | Zero order | 250           | 149 |
| 3      | EFV/TDF/Lamivudine-Plasma | Tablet | Simultaneous equation | 247, 260, 272 nm | 150 |
| 4      | FTC          | Tablet        | Zero order and area under curve | 280 nm, 272-286 nm | 151 |
| 5      | TDF          | Tablet        | Zero order | 260 nm        | 152 |
| 6      | EFV/TDF/Lamivudine | Tablet | Zero order | 247, 260, 272 nm | 153 |
| 7      | FTC/TDF      | Bulk          | Simultaneous equation, Q-absorbance ratio method | (1)280 nm, 260 nm (2) 251,237 nm | 154 |
| 8      | FTC/TDF      | Tablet        | Simultaneous equation eethod | 282,261 nm | 155 |
| 9      | FTC/TDF      | Tablet        | Simultaneous Equation Method | 282, 210 nm | 156 |
| 10     | FTC/TDF/EFV  | Tablet        | Simultaneous equation method | 260, 241, 240 nm | 157 |
| 11     | FTC/TDF/Cobicistat/Elvitegravir | Tablet | Simultaneous equation method | 283, 259, 240, 258 nm | 158 |
| 12     | FTC          | Tablet        | Zero order, first order derivative | 241.1 and 232.7 nm | 159 |
| 13     | FTC/TDF      | Tablet        | Least square, first order, area under curve | 281,260.5 nm; 234.5, 281 nm; 278-283 nm, 258-262 nm | 160 |
| 14     | TDF          | Tablet        | Zero order, first order | 260 nm, 273 nm | 161 |
| 15     | EFV/TDF/Lamivudine | Tablet | Simultaneous equation method, multicomponent analysis and derivative spectroscopy method | 247, 259 and 272 nm | 162 |
| 16     | EFV/TDF/Lipirivirine | Tablet | Simultaneous equation method | 240.8, 257.6, and 305.6 nm | 163 |
| 17     | EFV/TDF      | Tablet        | Simultaneous equation and Absorbance ratio method | 250, 274, 255, 274 nm | 164 |
| 18     | EFV/TDF      | Tablet        | Rate derivative spectra, first-order, absorption corrected method | 271.07 and 302.17 nm; 224.38 and 306.88 nm | 165 |
| 19     | EFV/TDF      | Tablet        | Zero order | 298 nm | 166 |
| 20     | TDF/Lamivudine | Tablet       | Simultaneous equation method | 271.6 and 261 nm | 167 |
Table 3: HPTLC methods for determination of TDF, FTC and EFV

| S. No | Name of drug               | Formulation | Stationary phase plates | Mobile phase composition                                                                 | Ref. |
|-------|----------------------------|-------------|-------------------------|------------------------------------------------------------------------------------------|------|
| 1     | EFV / Lamivudine           | Tablet      | silica gel 60 G F254    | Ethyl acetate: methanol: formic acid 7:6:2.5:0.5 (v/v/v/v)                               | 168  |
| 2     | FTC / TDF / EFV            | Tablet      | silicagel 60 F 254      | Chlo roform: methanol (90:10)                                                             | 169  |
| 3     | FTC / TDF / Rilpivirin     | Tablet      | silica gel 60 F 254     | Methanol: toluene: ethylacetate: ammonia (1.5:5:5:1:5:0.1 v/v/v/v/v/v)                    | 170  |
| 4     | TDF / Lamivudine           | Tablet      | silicagel 60 F254, (20 x 10 cm) | Chlo roform: methanol: toluene (8: 2: 2, v/v/v)                                           | 171  |
| 5     | EFV                        | Tablet      | silica gel 60 F 254     | Toluene: ethyl acetate: formic acid (10: 3: 1 v/v)                                        | 172  |
| 6     | EFV                        | Plasma      | silica gel 60 F254      | Dichloromethane: methanol (5:0.3 v/v)                                                     | 173  |
| 7     | TDF                        | Tablet      | silica gel GF aluminum  | Ethyl acetate: methanol: formic acid (7:2.5:0.5 %/v/v)                                   | 174  |
| 8     | FTC / TDF / Rilpivirin     | Tablet      | silicagel 60 F254       | Chlo roform: ethyl acetate: methanol: glacial acetic acid (5:2:1:0.1 v/v/v/v/v)          | 175  |

CONCLUSION

The present review discussed about different analytical approach employed for the assessment of TDF, FTC and EFV. Profuse examinations have been accomplished including, Bio-analytical, HPLC, UPLC, HPTLC, UV/Vis-Spectroscopy, LC-MS, LC-ESI-MS etc. for evaluation of TDF, FTC and EFV in bulk and in its combination with other drugs from pharmaceutical formulations and also biological fluids. Liquid chromatography with UV detection has been found to be most studied for estimation of TDF, FTC and EFV in bulk as well as pharmaceutical dosage forms, while hyphenated LS-MS, LSMS/MS methods reported for determination of TDF, FTC and EFV and its metabolite in plasma and other biological fluids. Few chromatography approaches like HPTLC and Stability-indicating HPLC, UPLC and HPTLC are also reported. Few simple UV-Spectrophotometric methods may be used for routine analysis of TDF, FTC and EFV alone and in combination with other drugs. These compiled data may of use for research for further studies in analysis of TDF, FTC and EFV.

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CONFLICT OF INTERESTS

Declared none

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