International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 1, 2022

Original Article

FORMULATION AND DEVELOPMENT OF FIXED-DOSE COMBINATION OF BI-LAYER TABLETS OF EFAVIRENZ, LAMIVUDINE AND TENOFOVIR DISOPROXIL FUMARATE TABLETS 600 MG/300 MG/300 MG

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Received: 28 Jul 2021, Revised and Accepted: 06 Dec 2021

ABSTRACT

Objective: This study is to formulate bi-layer tablet as a multidrug regimen against each reference listed drugs of Brand SUSTIVA® (efavirenz tablets 600 mg), EPIVER®(lamivudine tablets 300 mg), and VIREAD®(tenofovir disoproxil tablets 300 mg) to treat human immunodeficiency virus (HIV) infections. Which provides highly active antiretroviral therapy to provide effective treatment.

Methods: Bilayer formulation was developed with each blend of layer-I (efavirenz) and layer-II (lamivudine and tenofovir disoproxil fumarate) through wet granulation process and roller compaction process, respectively. Further, both layers were compressed by using bi-layer compression followed by film coating. Layer-I and II formulations were developed by using various concentrations of diluents, surfactants, and disintegrants to improve the solubility of efavirenz and improve the flowability and uniformity of layer-II. Finally, the optimum formulation was developed to compare the *in vitro* dissolution with each branded formulation.

Results: Drug-excipients interaction results revealed that the mixtures of three drug substances in 50 °C/75 % relative humidity (RH) resulted in an increase in tenofovir IMP-E and the highest unknown impurity was significantly increased and additionally decreased tenofovir assay in the presence of efavirenz. Sodium lauryl sulfate is very critical and it acts as a wetting agent and increases the solubility of efavirenz, and directly influences the dissolution of a drug product. Microcrystalline and croscarmellose sodium have a chance to affect the dissolution and friability of tenofovir. Powdered cellulose was acting as a diluent and flow property of the lamivudine part and it also affects the uniformity and dissolution. So, these ranges were optimized. X-ray diffraction (XRD) indicates there are no polymorphic changes for the optimized formulation and there is no interaction between the three active substances, and finally, *in vitro* dissolution results for the optimized formulation against the reference drugs.

Conclusion: Optimum formulation yielded consistent drug release against each branded drug to treat human immunodeficiency virus (HIV1) infections. This formulation is robust and easily scale up for the next stage.

Keywords: Fixed-dose formulation, Human immunodeficiency virus (HIV1) infection, Bilayer compression, Efavirenz, Lamivudine, and tenofovir

DOI: https://dx.doi.org/10.22159/ijap.2022v14i1.42764. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

A combination drug or a fixed-dose combination (FDC) is a medicine that includes two or more active ingredients combined in a single dosage form [1]. Terms like "combination drug" or "combination drug product" can be common shorthand for a fixed-dose combination (FDC) product. Fixed-dose formulations can be administered as a multi-drug regimen to treat various diseases by an effect on different modes of action. From a patient perspective, they offer convenience, reduced dosing unit burden, and cost savings. From a clinical perspective, the aging population in developed countries will need multiple medications to treat age-related diseases and co-morbidities. However, the recommended fixed-dose combination (FDC) drugs such as efavirenz, lamivudine, and tenofovir disoproxil fumarate have novel approaches of multiple dosage regimens to treat human immunodeficiency virus (HIV-1) infection through nucleotide reverse transcriptase inhibitors, non-nucleoside reverse-transcriptase inhibitors.

Three active pharmaceutical ingredients (APIs) in the formulation have chances to be incompatible and may impact the related substances of the finished product. So, bilayer tablets were recommended for formulation development [2].

Human immunodeficiency virus (HIV) is a retrovirus that causes irreversible destruction of the immune system, leading to the occurrence of opportunistic infections and malignancies. The human immunodeficiency virus is found in two major forms, which are human immunodeficiency virus (HIV-1 and HIV-2). Human immunodeficiency virus (HIV-1) is the most pathogenic strain of the virus worldwide. The human immunodeficiency virus (HIV-2) is the most common in west Africa. The human immunodeficiency virus (HIV) attacks the body's immune system, especially the cluster of differentiation 4 (CD4cells) (T cells), which helps the immune system fight infections. If left untreated, human immunodeficiency virus (HIV) reduces the number of clusters of differentiation 4 (CD4cells) (T cells) in the body, making the person more likely to get infections or infection-related symptom cancers [3, 4].

Mono-therapy is no longer recommended because incomplete viral suppression can encourage the development of resistance. Similarly, the magnitude and durability of viral suppression were lower with dual antiretroviral combinations compared with combinations containing three or more agents. For example, generally, two nucleoside reverse transcriptase inhibitors (NRTIs) are combined with an antiretroviral from PI or non-nucleoside reverse transcriptase inhibitors (NNRTI) class. Similarly, mono-therapy with PI or non-nucleoside reverse transcriptase inhibitors (NNRTI) is also not advisable to prevent the emergence of resistance and subsequent drug failure. The current strategy for the treatment of human immunodeficiency virus (HIV) infection is called highly active antiretroviral therapy (HAART) and is based on cocktails of drugs that are currently approved by the food and drug administration. These drugs include compounds that target the viral entry step and the enzymes reverse transcriptase or protease. The introduction of highly active antiretroviral therapy (HAART) has dramatically changed the landscape of human immunodeficiency virus (HIV) disease. Death from acquired immune deficiency syndrome (AIDS)-related diseases have been reduced significantly since highly active antiretroviral therapy (HAART) came into use. Nevertheless, it is not clear how long clinical benefit will last,

considering the emergence of multiple drug-resistant viral strains. The addition of new anti-human immunodeficiency virus (HIV) drugs targeting other steps of the viral replication cycle may increase the potency of inhibition and delay resistance development. However, a multidrug regimen is commonly called highly active antiretroviral therapy to provide effective treatment [5, 6].

MATERIALS AND METHODS

Materials

Efavirenz, lamivudine, and tenofovir disoproxil fumarate were procured from laurus labs and a gift sample from desano. Microcrystalline cellulose, lactose monohydrate, hydroxypropyl cellulose, lactose monohydrate and powdered cellulose croscarmellose sodium, sodium lauryl sulfate, magnesium stearate, colloidal silicon dioxide, and opadry white were commercially procured and used for this study.

Preformulation study

Preformulation study was executed to understand the physical properties of drug substances and excipients that may impact on the formulation and process design and performance. Which provides knowledge for scientific foundation guidance and conserve resources in the drug development and evaluation process, enhance drug product quality [7, 8].

Evaluation of physical parameters for active substances

Physical parameters such as bulk density (g/ml), tapped density (g/ml), compressibility index, and hausner's ratio were verified to understand the flow property of active substances before initiation of the formulation development of fixed-dose formulation.

Evaluation of particle size distribution (PSD) data for active substances

The particle size of each tenofovir disoproxil fumarate (TDF) and lamivudine (LMV) active pharmaceutical ingredient (API) was determined by using CILAS particle size analyzer at 2000 mbar dispensing pressure and efavirenz active pharmaceutical ingredient (API) was determined by using CILAS particle size analyzer at 500 m bar dispensing pressure.

Solubility studies

Solubility studies were carried out for all the active substances through the high-performance liquid chromatography (HPLC) method for the selection of the dissolution method.

X-ray diffraction (XRD) studies

The active substances and their formulation were subjected to X-ray diffraction (XRD) studies to determine the crystalline structure of the formulation.

Dissolution method development

Both tenofovir disoproxil fumarate (TDF), lamivudine (LMV) have been classified as biopharmaceutics classification system (BCS) Class III compounds displaying high aqueous solubility. The target is an immediate release fixed-dose combination tablet and Tmax of each single entity reference listed drug (RLD) product is around 1 hour, so the dissolution of a target drug product is expected in the stomach and absorption in the upper small intestine is expected, hence suggesting the use of dissolution medium with low pH. Tenofovir disoproxil fumarate (TDF) is soluble in 0.1 N hydrochloric acid (HCl) (gastric media), sparingly soluble in pH 4.5 acetate buffer and in pH 6.8 phosphate buffer. Lamivudine is freely soluble in all three media.

Development began with the dissolution methods recommended in the dissolution methods database by FDA for the single entity reference listed drug (RLD) products: 900 ml of 0.1 N hydrochloric acid using United States pharmacopoeia (USP) apparatus 2 at 50 rpm and temperature 37.0±0.5 ℃. The results revealed that the drug release of tenofovir disoproxil fumarate (TDF), lamivudine (LMV) was not sensitive to pH (similar to 0.1 N hydrochloric acid, pH 4.5 acetate buffer, and pH 6.8 phosphate buffers). Since the target is an immediate release product and by considering Tmax, dissolution in the stomach and absorption in the upper small intestine is expected, hence suggesting using 0.1 N hydrochloric acid medium [9, 10]. Efavirenz (EFV) is known to be a biopharmaceutics classification system (BCS) Class II/IV compound displaying low aqueous solubility.

Efavirenz (EFV) is practically insoluble in 0.1 N hydrochloric acid, pH 4.5 acetate buffer, pH 6.8 phosphate buffer, and purified water. Hence, efavirenz (EFV) exhibits pH-independent solubility at 37 °C. And it is very slightly soluble in purified water with 0.5 % sodium lauryl sulfate (SLS), slightly soluble in purified water with 1 % sodium lauryl sulfate (SLS), and purified water with 2 % sodium lauryl sulfate (SLS) [11].

Drug-excipient compatibility study

Based on the literature and prior knowledge, the excipients were used for the drug-excipient compatibility study. The excipient compatibility studies were performed in two steps. The purpose of the 1st excipient compatibility study was to confirm the reaction among the drug substances and the reaction between the drug substances and sodium lauryl sulfate. The 2nd excipient compatibility study was performed to confirm the reaction between each drug substance and the excipient.

First excipient compatibility study

The samples were prepared by mixing the drug substance and the excipients in the respective ratio. And then, the resulting samples were stored in climatic chambers at 50±2 °C/75±5 % relative humidity (RH) conditions in both open and closed conditions in a labeled glass vial. The compatibility test period was performed for 2 w, because the temperature condition was severe than that of the normal compatibility test condition.

Second excipient compatibility study

The samples were prepared by mixing the drug substance and the excipients in the respective ratios. And then, the resulting samples were stored in climatic chambers at 40±2 °C/75±5 % relative humidity (RH) in open condition and 50±2 °C/65±5 % relative humidity (RH) in closed conditions in a labeled glass vial.

The physical observation data generated at 1st, 2nd, and 4th-week samples at closed condition (50 °C, 65 % relative humidity (RH)) and open condition (40 °C, 75 % relative humidity (RH)), found no significant change is observed, except tenofovir disoproxil fumarate (TDF)+lamivudine (LMV)+efavirenz (EFV) API blend at open condition (50 °C, 65 % relative humidity (RH)). The chemical stability data generated at 1st, 2nd, and 4th-week samples at open conditions (40 °C/75 % relative humidity (RH)), no significant increase in impurities were observed except microcrystalline cellulose and colloidal silicon dioxide blend samples with tenofovir disoproxil fumarate (TDF), respectively. The chemical stability data generated at 1st, 2nd, and 4th-week samples at closed conditions (50 °C/65 % relative humidity (RH)), no significant increase in impurities were observed except magnesium stearate blended samples with tenofovir disoproxil fumarate (TDF). As there was significant degradation observed with tenofovir disoproxil fumarate (TDF)+magnesium stearate sample under heat condition, the contact with tenofovir disoproxil fumarate (TDF) is limited by only using a small quantity in intragranular and the degradation tendency will be monitored and assured during stability study. Chromatographic results found, no significant change was observed. The physical observation data generated of 1st, 2nd-week samples at 50 °C/75 % relative humidity (RH) (Open and Closed conditions), a significant change was not observed except to tenofovir disoproxil fumarate (TDF)+lamivudine (LMV)+efavirenz (EFV) sample. The tenofovir disoproxil fumarate (TDF)+lamivudine (LMV)+efavirenz (EFV) sample of open condition turned brown to off-brown lumps at 1week and 2weeks. Therefore, it is considered a bilayer tablet manufacturing process. When compared tenofovir disoproxil fumarate (TDF)+lamivudine (LMV)+efavirenz (EFV) with tenofovir disoproxil fumarate (TDF)+lamivudine (LMV), tenofovir IMP-E and highest unknown impurity are significantly increased at both 1week and 2 weeks open condition and also observed a significant decrease

in the assay. Therefore, the observed tenofovir disoproxil fumarate (TDF) degradation was due to lamivudine (LMV), in the presence of efavirenz (EFV). Therefore, the test drug product manufacturing process was determined to be bilayer tablet to achieve sufficient stability of the drug product. The use of sodium lauryl sulfate (SLS) in the manufacturing process of efavirenz (EFV) granules is unavoidable, and based on the above results, tenofovir disoproxil fumarate (TDF) is incompatible in presence of efavirenz (EFV)+sodium lauryl sulfate (SLS). Hence, in this bilayer manufacturing process, tenofovir disoproxil fumarate (TDF)+lamivudine (LMV) will be in one layer, and efavirenz (EFV) will be in the other layer [12].

Experimental design

A Quality Target Product Profile (QTPP) for the new fixed-dose combination (FDC) bilayer drug product was set to achieve the desired quality, considering, safety and efficacy of the drug product. The quality targets were set considering the characterization of the single entity reference listed drug (RLD) product and general compendia standards. The objective of this development of a fixed-dose combination of efavirenz (EFV), lamivudine (LMV), and tenofovir disoproxil fumarate (TDF) Tablets 600 mg/300 mg/300 mg was to have a stable fixed-dose combination bilayer tablet, which is equivalent to the loose combination of comparator products that are VIREAD® tablets 300 mg, EPIVIR® tablets 300 mg and SUSTIVA® tablets 600 mg taken concomitantly. The product has been developed as a bilayer immediate release solid dosage form for oral administration with bilayer compression strategy considering efavirenz is layer–I and lamivudine and tenofovir disoproxil fumarate blend in layer–II.

Due to tenofovir disoproxil fumarate (TDF)+lamivudine (LMV)+efavirenz (EFV) are not compatible when mixed, a bilayer tablet, composed of one layer containing efavirenz (EFV) and the other layer containing tenofovir disoproxil fumarate (TDF)+lamivudine (LMV) was developed. The excipients were selected based on prior knowledge, excipients present in the single entity of reference listed drug product (RLD), and considering drug-excipient compatibility study.

The efavirenz (EFV) layer is prepared by using a wet granulation process to improve the flowability of the granule [13]. The tenofovir disoproxil fumarate/lamivudine (TFL) layer is prepared using a dry granulation process, to protect the tenofovir disoproxil fumarate (TDF) from degradation by absorbing moisture, as it is slightly hygroscopic [14]. During the formulation study, the manufacturing steps required to reach the quality target product profile were identified and proposed for process development. The initial proposal was to use similar qualitative compositions as those of individual reference standards [15-17], but the final composition and manufacturing method was selected based on the physicochemical characteristics of the product development. Based on literature search and previous experience, the proposed manufacturing procedure for the development was designed as below fig. 1.

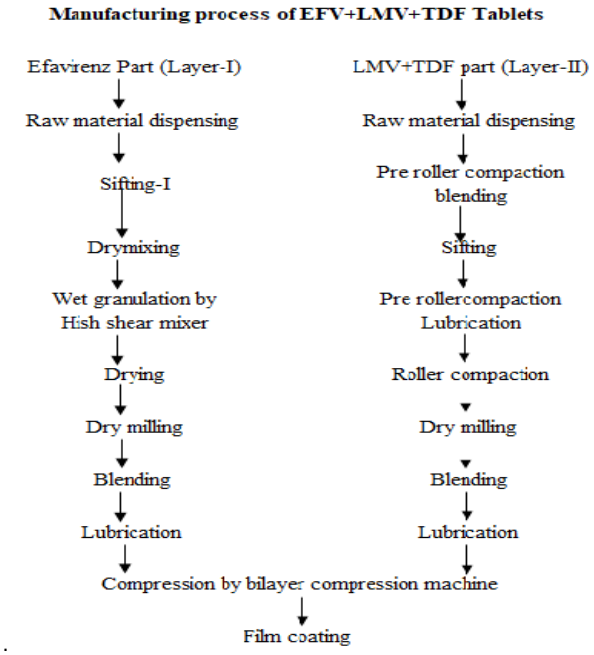

Fig. 1: Manufacturing process of FDC product

Tooling details

Based on the weight and size of individual reference drug product, bilayer tablets of the test formulation were targeted to 1550.00 mg at the compression stage by using punch tooling of 21.20X11.5 mm during the formulation and process development study.

Formulation of efavirenz part (layer-I)

The factors affecting the drug product critical quality attributes (CQAs) were investigated further based on the risk assessment (RA). It was designed to select the levels of sodium lauryl sulfate, hydroxypropyl cellulose, and croscarmellose sodium and magnesium stearate for efavirenz (EFV) layer optimization. In the case of the wet granulation process, the level of binder solution varies depending on the composition. If the composition of intra-granules was changed, the result may be distorted by the level of binder solution. For this reason, the major composition of the intra granule was fixed from ELT04 to ELT08. The levels of microcrystalline cellulose and croscarmellose sodium used in the intra granules were selected as 80 mg and 30 mg, respectively. Sodium lauryl sulfate was used as a wetting agent and the level was investigated from 5.00 mg to 12.00 mg.

Hydroxypropyl cellulose was used as a binder and the levels investigated ranged from 10.00 mg to 25.00 mg. Extra granular croscarmellose sodium was used as a disintegrant and the levels investigated ranged from 15.00 mg to 70.00 mg. Magnesium stearate was used as a lubricant and the levels investigated ranged from 8.00 mg to 12.00 mg. These levels are considered based on knowledge and inactive ingredient database (IID) levels and are within the

recommended range in the handbook of pharmaceutical excipients [18, 19]. Lactose monohydrate was used as a diluent and hydrophilic agent and it was used to adjust the weight of the tablet. Therefore, the level of lactose monohydrate would be changed according to the formulation variables. Formulation trials were carried out as bilayer tablets with a target weight of 1550.00 mg. while evaluation of efavirenz formulation, lamivudine, and tenofovir composition was kept constant to achieve the total weight. Further, the formulation variable's impact on dissolution was studied.

Formulation of lamivudine (LMV)+tenofovir disoproxil fumarate (TDF) part (Layer-II)

For formulation development, the dry granulation method was selected as the granulation process instead of wet granulation due to mitigating risk for hydrolysis of tenofovir disoproxil fumarate. The direct compression process was not selected due to flowability problems.

The selection of appropriate excipients was determined and confirmed by excipient compatibility studies and formulation studies. The selected excipients for the tenofovir disoproxil fumarate and lamivudine tablets formulation were similar to those used in single entity products. All selected excipients were commonly used in pharmaceutical oral dosage forms and were compliant with United States pharmacopoeia (USP) compendial requirements.

Tenofovir disoproxil fumarate and lamivudine have been classified as biopharmaceutics classification system (BCS) class III compounds displaying high aqueous solubility. The target is an immediate release fixed-dose combination tablet and the time to maximum plasma concentration (Tmax) of each reference listed drug (RLD) product is around 1 hour, so the target drug is expected to be dissolved in the stomach and absorbed in the upper small intestine when the suggested dissolution medium was used with low pH. Therefore, equivalence with individual reference listed drug (RLD) was evaluated by dissolution study in 0.1N hydrochloric acid (HCl) medium. Tenofovir Disoproxil Fumarate and lamivudine have poor flowability as shown in the compressibility index (tenofovir disoproxil fumarate (TDF): value = 36, poor, lamivudine (LMV): value = 34, very poor) and hausner ratio (tenofovir disoproxil fumarate (TDF): value = 1.56, poor, lamivudine (LMV): value = 1.52, very poor) listed in table 4. Poor flowability of drug substances may cause problems such as high weight and variable content uniformity of the drug product. Uneven blend distribution and bulk density may cause uneven filling of die cavities on the tablet press. Because of the poor flowability of tenofovir disoproxil fumarate and lamivudine, it is not suitable to use a direct compression process for tenofovir disoproxil fumarate and lamivudine tablets, especially when the ratio of active pharmaceutical ingredients (APIs) is high in the formulation.

The wet granulation process is commonly used to improve powder flowability and compatibility. However, tenofovir disoproxil fumarate has hydrolysis characteristics. Thus, the wet granulation process was also excluded.

Finally, the dry granulation process by roller compacting was chosen to improve the flowability and compatibility of tenofovir disoproxil fumarate. In the dry granulation process, by roller compacting, the particles of drug substances and excipients are aggregated by roller compaction to form a ribbon and then milled to produce granules by the oscillator. Enlargement of particles through the dry granulation process helps to have adequate flowability, density and compactibility that are particularly important characteristics for the high-speed production of tablets.

Table 1: Formulation trials of efavirenz part (layer-I)

S. No.	Ingredients	ELT01	ELT02	ELT03	ELT04	ELT05	ELT06	ELT07	ELT08
		Mg							
Intra granular materials									
1	Efavirenz	600.00	600.00	600.00	600.00	600.00	600.00	600.00	600.00
2	Microcrystalline cellulose	N/A	N/A	130.00	130.00	130.00	130.00	130.00	130.00
3	Lactose monohydrate	155.00	110.00	N/A	37.00	N/A	N/A	20.00	N/A
4	Sodium lauryl sulfate	N/A	N/A	5.00	8.00	12.00	12.00	10.00	10.00
5	Colloidal silicon dioxide	N/A	N/A	10.00	N/A	4.00	4.00	4.00	6.00
6	Croscarmellose sodium	22.00	20.00	N/A	30.00	30.00	30.00	30.00	30.00
7	Hydroxy propyl cellulose	25.00	20.00	25.00	10.00	15.00	15.00	20.00	10.00
8	Purified water	368.00	368.00	368.00	410.00	410.00	410.00	410.00	410.00
Extra granular materials									
9	Croscarmellose sodium	40.00	48.00	70.00	25.00	10.00	15.00	24.00	30.00
10	Microcrystalline cellulose	N/A	43.00	N/A	N/A	N/A	N/A	N/A	N/A
11	Lactose monohydrate	N/A	N/A	N/A	N/A	39.00	34.00	N/A	24.00
12	Magnesium Stearate	8.00	9.00	10.00	10.00	10.00	10.00	12.00	10.00
Layer-I weight (mg)		850.00	850.00	850.00	850.00	850.00	850.00	850.00	850.00
Layer-II (Lamivudine and tenofovir disoproxil fumarate)									
13	Tenofovir Disoproxil Fumarate	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
14	Microcrystalline cellulose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
15	Lactose monohydrate	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
16	Croscarmellose sodium	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
17	Magnesium Stearate	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Extra granular materials									
18	Lamivudine	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
19	Lactose monohydrate and powdered cellulose	104.00	104.00	104.00	104.00	104.00	104.00	104.00	104.00
20	Croscarmellose sodium	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
21	Colloidal silicon dioxide	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
22	Magnesium Stearate	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Layer-II weight (mg)		800.00	800.00	800.00	800.00	800.00	800.00	800.00	800.00
Total weight of core tablets		1650.00	1650.00	1650.00	1650.00	1650.00	1650.00	1650.00	1650.00
Film coating									
23	Opadry white	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00
24	Purified water	215.00	215.00	215.00	215.00	215.00	215.00	215.00	215.00
25	Total weight of coated tablets	1688.00	1688.00	1688.00	1688.00	1688.00	1688.00	1688.00	1688.00

As per the compatibility study and reference listed drug (RLD) composition, all the excipients were considered for the formulation development. Based on the literature, patents data, and previous experience, the total target weight was fixed. While developing the lamivudine and tenofovir formulations, the optimum formulation of efavirenz was used and it was kept constant to achieve the target weight of core tablet such as 1550.00 mg. Generally consideration of bilayer compression, layer-I weight should be higher than layer-II. Hence, layer-I and layer-II was fixed. Film coating was performed for each formulation with target weight buildup of 2.3% w/w with 15% of solid concentration. Each formulation, in-process and finished product characteristics were verified to compare with the reference product.

Table 2: Formulation trials of lamivudine and tenofovir disoproxil fumarate part (layer-II)

S. No.	Ingredients	ELT09	ELT10	ELT11	ELT12	ELT13	ELT14	ELT15	ELT16
		Mg							
Intra granular materials									
1	Efavirenz	600.00	600.00	600.00	600.00	600.00	600.00	600.00	600.00
2	Microcrystalline cellulose	130.00	130.00	130.00	130.00	130.00	130.00	130.00	130.00
3	Sodium lauryl sulfate	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
4	Colloidal silicon dioxide	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
5	Croscarmellose sodium	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
6	Hydroxy propyl cellulose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
7	Purified water	410.00	410.00	410.00	410.00	410.00	410.00	410.00	410.00
Extra granular materials									
8	Croscarmellose sodium	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
9	Lactose monohydrate	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
10	Magnesium Stearate	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Layer-I weight (mg)		850.00	850.00	850.00	850.00	850.00	850.00	850.00	850.00
Layer-II (Lamivudine and tenofovir disoproxil fumarate)									
11	Tenofovir Disoproxil Fumarate	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
12	Microcrystalline cellulose	N/A	23.00	25.00	48.00	25.00	N/A	25.00	10.00
13	Lactose monohydrate	23.00	N/A	23.00	N/A	23.00	23.00	N/A	15.00
14	Croscarmellose sodium	25.00	25.00	N/A	N/A	N/A	25.00	23.00	23.00
15	Colloidal silicon dioxide	N/A	N/A	N/A	N/A	N/A	2.00	2.00	N/A
16	Magnesium Stearate	2.00	2.00	2.00	2.00	2.00	N/A	N/A	2.00
Extra granular materials									
17	Lamivudine	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
18	Lactose monohydrate and powdered cellulose	N/A	N/A	N/A	100.00	100.00	60.00	120.00	104.00
19	Lactose monohydrate	100.00	N/A	50.00	N/A	N/A	N/A	N/A	N/A
20	Microcrystalline cellulose	N/A	96.00	46.00	N/A	N/A	40.00	N/A	N/A
21	Croscarmellose sodium	36.00	40.00	40.00	36.00	28.00	38.00	20.00	30.00
22	Colloidal silicon dioxide	6.00	6.00	6.00	4.00	10.00	N/A	N/A	6.00
23	Magnesium Stearate	8.00	8.00	8.00	10.00	12.00	12.00	10.00	10.00
Layer-II weight (mg)		800.00	800.00	800.00	800.00	800.00	800.00	800.00	800.00
Total weight of core tablets		1650.00	1650.00	1650.00	1650.00	1650.00	1650.00	1650.00	1650.00
Film coating									
24	Opadry white	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00
25	Purified water	215.00	215.00	215.00	215.00	215.00	215.00	215.00	215.00
26	Total weight of coated Tablets	1688.00	1688.00	1688.00	1688.00	1688.00	1688.00	1688.00	1688.00

Table 3: Process parameters used for the manufacturing process

Layer-1 (efavirenz)				**Layer-II (lamivudine and tenofovir disoproxil fumarate)**		
Wet granulation process				**Direct blending process/roller compaction**		
Process step		**Parameters**		**Process step**	**Parameters**	
Dry mixing		Dry mixing time	10 min	Pre roller compaction blending	Blending time	10 min
		Impeller speed	95RPM			
		Chopper speed	1500RPM			
Granulation	Binder addition	Binder addition time	10 min	Pre roller compaction lubrication	Lubrication time	5 min
		Impeller speed	95RPM	Roller compaction	Roller speed	5-10RPM
		Chopper speed	1500RPM		Roller gap	2.0-4.0 mm
	Kneading	Kneading time	5 min		Feed augur speed	0-15RPM
		Impeller speed	115RPM		Roller force	2-7bar
		Chopper speed	2500RPM			
Wet milling		Wet milling speed	1500RPM	N/A		
		Screen size	5.0 mm			
Drying		Inlet temp	60±10 °C			
		LOD	NMT2.5%			
Dry milling		Mill speed	500RPM	Dry milling	Mill speed	1000RPM
		Screen size	2.0 mm		Screen size	1.0 mm
Blending		Blending time	10 min	Blending	Blending time	10 min
Lubrication		Lubrication time	5 min	Lubrication	Lubrication time	5 min

By considering two active substances in the formulation, formulation tenofovir disoproxil fumarate (TDF) was used for the roller compaction and lamivudine, with other extra materials recommended to add at the blending stage. The roller compaction process followed by direct blending of layer-II yielded consistency content uniformity and assay results after compression of bilayer tablets. Further, all the in-process and finished product characteristics were verified after all the formulation trials.

The level of microcrystalline cellulose may affect the assay, content uniformity, and dissolution, and compressibility index (CI) values. Microcrystalline cellulose was used as a diluent and the levels investigated ranged from 0.00 mg to 119.00 mg. The level of croscarmellose sodium may affect the dissolution and friability. Croscarmellose sodium was used as a disintegrant and the levels investigated ranged from 28.00 mg to 63.00 mg. Lactose monohydrate investigated ranged from 0.00 mg to 123.00 mg and powdered cellulose was used as a diluent to adjust the weight of the tablet. The investigated are ranged from 0.00 mg to 130.00 mg. Colloidal silicon dioxide and magnesium stearate were changed to improve the flow properties of the final blend and avoid the sticking of core tablets during bi-layer compression. Because it was expected to have the least impact on the critical quality attributes (CQAs) among the selected excipients. Therefore, the amount of these excipients would be changed accordingly when the level of the factors was changed These levels are considered based on the knowledge and inactive ingredient database (IID) level and are within the recommended range in the handbook of Pharmaceutical Excipients [18, 19]. The detailed process parameters used for the execution of formulation trials were mentioned in table 3.

RESULTS

From the physical evaluation of active substances data, it is evident that the tenofovir disoproxil fumarate (TDF) and lamivudine (LMV) active pharmaceutical ingredients (APIs) have very poor flow properties. In the case of efavirenz (EFV), though the bulk density (BD) result is very low, the flowability character is passable. The angle of repose test for each active pharmaceutical ingredient (API) was unable to measure because the flow of each active pharmaceutical ingredient (API) was impossible to measure in the flowability tester.

Table 4: Physical property results of active pharmaceutical ingredients (APIs)*

Batch No.	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility index (CI)* (%)	Hausner ration (HR)**	Flow character
Tenofovir Disoproxil Fumarate	0.3261±0.02	0.5095±0.01	36±0.03	1.56±0.01	Very poor
Lamivudine	0.5318±0.01	0.8057±0.01	34±0.01	1.52±0.02	Very poor
Efavirenz	0.2266±0.02	0.2981±0.02	24±0.06	1.32±0.01	Passable

*n=3, mean±SD

Compressibility index (CI)

The simplest way of measuring the free flow property of a powder is compressibility, an indication of the ease with which a material can be induced to flow given by % compressibility index (% CI). It can be calculated from the unsettled apparent volume, P_{bulk}, and the final tapped volume, $p_{tapped,}$ of the powder after tapping the material until no further volume changes occur.

$$\text{Compressibility Index (CI)} = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} \times 100$$

Hausner ratio may be calculated using measured values for bulk and tapped density. (tapped density and bulk density (g**/ml**)

$$\text{Hausner Ratio (HR)} = \frac{\rho_{tap}}{\rho_{bulk}}$$

Table 5: Flow properties according to carr's index and hausner ratio

Consolidation index (carr's %)	Flow	Hausner's ratio
≥10	Excellent	1.00-1.11
11-15	Good	1.12-1.18-
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Very, very poor	>1.6

Angle of repose

The Angle of repose is defined as the maximum angle possible between the surfaces of a pile of powder and the horizontal plane. This was determined by passing required quantities of drug granules through a funnel from a particular height (2 cm) onto a flat surface until it formed a heap, which touched the tip of the funnel. Experiments for the angle of repose were not measured because of very, very poor flow of drug substances, and heap were not formed to the tip of the funnel.

Particle size distribution (PSD data) for active substances

The particle size of each tenofovir disoproxil fumarate (TDF) and lamivudine (LMV) active pharmaceutical ingredients (API's) was determined by using CILAS particle size analyzer at 2000 mbar dispensing pressure and efavirenz (EFV) active pharmaceutical ingredient (API) was determined by using CILAS particle size analyzer at 500 m bar dispensing pressure and results are mentioned below.

Table 6: Flow properties of active pharmaceutical ingredients (APIs)

Batch No.	Height (mm)	Angle of repose (θ)	Flow character
Tenofovir Disoproxil Fumarate	Impossible to measure	Impossible to measure	N/A
Lamivudine	Impossible to measure	Impossible to measure	N/A
Efavirenz	Impossible to measure	Impossible to measure	N/A

Table 7: Particle size distribution results of active pharmaceutical ingredients (APIs)

d 10 (µm)	d 50 (µm)	d 90 (µm)	Mean particle size (µm)
Tenofovir Disoproxil Fumarate			
1.42±2.24	6.36±3.28	27.29±2.22	11.69
Lamivudine			
22.42±1.28	58.96±4.28	96.93±3.14	59.44
Efavirenz			
0.62±0.18	1.95±2.28	3.42±4.15	2.00

*n=3 mean±SD

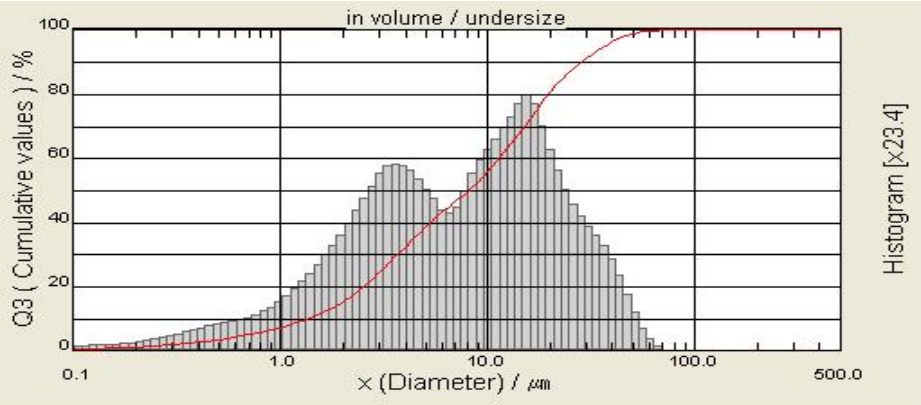

Fig. 2: Particle size (PSD data) of tenofovir disoproxil fumarate. The data is expressed as mean±SD, n=3

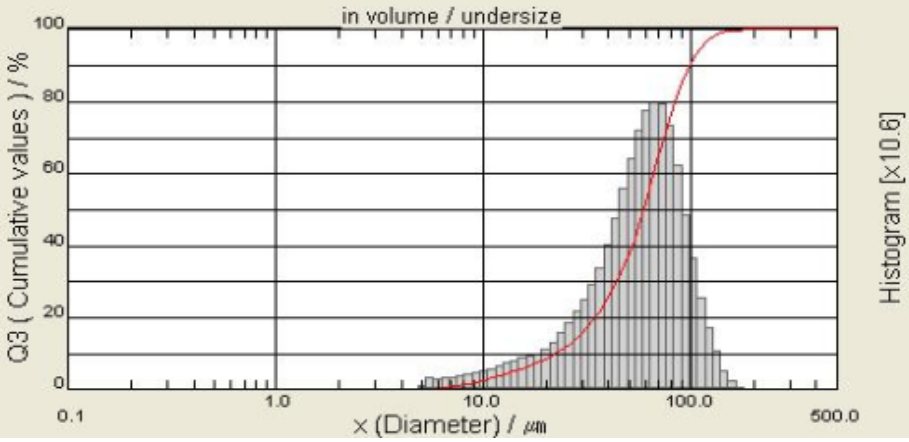

Fig. 3: Particle size data (PSD data) of lamivudine. The data is expressed as mean±SD, n=3

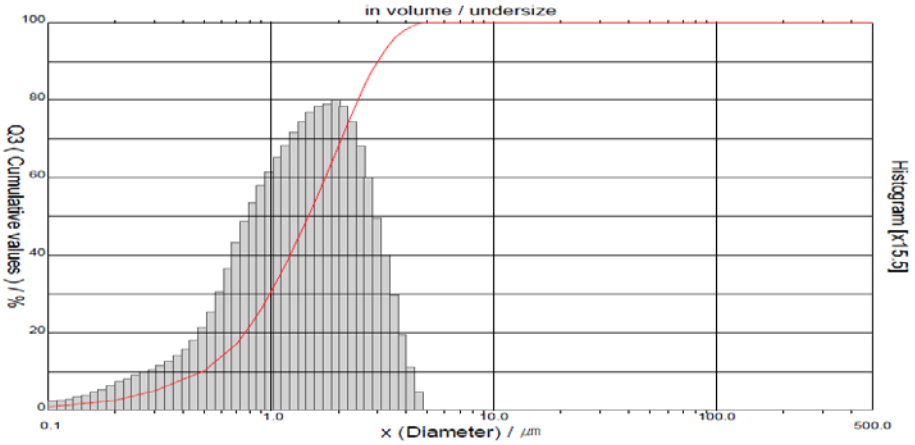

Fig. 4: Particle size data (PSD data) of efavirenz. The data is expressed as mean±SD, n=3

Solubility studies

Tenofovir disoproxil fumarate (TDF) is categorized under the biopharmaceutics classification system (BCS) class III compound (high soluble and low permeable). In this test, tenofovir disoproxil fumarate (TDF) exhibits pH-dependent solubility at 37 °C. Tenofovir disoproxil fumarate (TDF) is soluble in 0.1 N hydrochloric acid (HCl) and sparingly soluble in pH 4.5 acetate buffer and pH 6.8 phosphate buffer based on the United states pharmacopeia (USP) solubility scale. Lamivudine (LMV) is categorized under the biopharmaceutics classification system (BCS) class III compound (high, soluble and low-permeable). In this test, lamivudine (LMV) exhibits pH-independent solubility at 37 °C. Lamivudine (LMV) is freely soluble in 0.1 N hydrochloric acid (HCl), pH 4.5 acetate buffer, and pH 6.8 phosphate buffer. The solubility study results were interpreted based on the United States pharmacopeia (USP) solubility scale. Efavirenz (EFV) is categorized under biopharmaceutics classification system (BCS) class II or IV compound (Low soluble). In this test, efavirenz (EFV) exhibits pH-independent solubility at 37 °C. Efavirenz (EFV) is practically insoluble in 0.1 N hydrochloric acid (HCl), pH 4.5 acetate buffer, pH 6.8 phosphate buffer and in purified water. Results of the solubility study were interpreted based on the United States pharmacopeia (USP) solubility scale [20]. Based on the solubility studies of three active substances, dissolution methods such as purified water with 1.0% sodium lauryl sulfate (SLS) for efavirenz and 0.1N hydrochloric acid (HCL) for lamivudine and tenofovir disoproxil fumarate were selected.

Table 8: Solubility in various media at 37 °C

Medium/Buffer (100 ml at 37 °C)	Solubility (mg/ml)	Dose solubility volume (ml)	Part of solvent required for 1 Part of Solute	Descriptive term united states pharmacopeia (USP)
Efavirenz				
0.1 N HCl	0.014±0.03	42,857	71,429	Practically insoluble
pH 4.5 acetate buffer	0.022±0.19	27,273	45,455	Practically insoluble
pH 6.8 phosphate buffer	0.013±0.23	46,154	76,923	Practically insoluble
Purified Water	0.015±0.16	40,000	66,667	Practically insoluble
Purified Water with 0.5 % SLS	0.918±0.08	654	1090	Very slightly soluble
Purified Water with 1 % SLS	2.229±0.15	269	449	Slightly soluble
Purified Water with 2 % SLS	4.650±0.07	129	215	Slightly soluble
Tenofovir Disoproxil Fumarate				
0.1N HCl	43.530±0.11	7	23	soluble
pH 4.5 acetate buffer	22.894±0.15	13	44	sparingly soluble
pH 6.8 phosphate buffer	15.735±0.04	19	64	sparingly soluble
Lamivudine				
0.1N HCl	185.778±0.03	2	5	freely soluble
pH 4.5 acetate buffer	168.693±0.09	2	6	freely soluble
pH 6.8 phosphate buffer	153.677±0.06	2	7	freely soluble

*n=3, mean**±**SD

Table 9: United States pharmacopeia (USP) solubility scale

Descriptive term	Parts of solvent required for 1 part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble, or Insoluble	10,000 and over

Table 10: Final blend physical characteristics-efavirenz part (layer-I)*

Batch No.	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility index (%)	Hausner's ratio	LOD (%)
ELT01	0.62±0.02	0.76±0.01	18±0.08	0.82±0.02	1.52
ELT02	0.57±0.03	0.71±0.05	19±0.011	1.24±0.03	1.92
ELT03	0.64±0.01	0.80±006	20±0.12	1.25±0.02	1.78
ELT04	0.55±0.08	0.69±0.04	20±0.12	1.25±0.01	2.02
ELT05	0.59±0.04	0.74±0.06	20±0.01	1.25±0.06	2.40
ELT06	0.61±0.01	0.78±006	22±0.12	1.28±0.02	1.58
ELT07	0.58±0.04	0.76±0.02	24±0.13	1.31±0.06	1.46
ELT08	0.56±0.01	0.67±006	16±0.12	1.20±0.02	1.58

*n=3, mean**±**SD

Table 11: Final blend physical characteristics (lamivudine and tenofovir disoproxil fumarate part (layer-II)*

Batch No.	Bulk density (g/ml)	Tapped density (g/ml)	Compressibility index (%)	Hausner's ratio	LOD (%)
ELT09	0.55±0.01	0.69±0.004	21±0.12	1.27±0.02	1.02
ELT10	0.57±0.02	0.73±0.001	21±0.05	1.27±0.01	1.20
ELT11	0.59±0.01	0.76±0.002	23±.0.04	1.30±0.01	1.18
ELT12	0.60±0.06	0.74±0.001	19±0.03	1.23±0.03	1.50
ELT13	0.60±0.01	0.74±0.002	19±0.14	1.23±0.04	1.45
ELT14	0.52±0.03	0.69±0.001	25±0.08	1.33±0.05	1.62
ELT15	0.58±0.04	0.75±0.004	22±0.11	1.29±0.02	1.50
ELT16	0.55±0.02	0.71±0.002	22±0.02	1.28±0.01	1.11

*n=3, mean±SD

Physical property of final blend

The flowability of the final blend for layer–I (efavirenz part) and layer-II (lamivudine and tenofovir disoproxil fumarate part in table 10 and table 11 indicate fair to passable as per the united states pharmacopoeia (USP) flow property guidance and there is no flowability issues was observed during the bilayer compression. Further, loss on drying (LOD) of final blends was maintained within the limit of not more than (NMT) 2.5% w/w.

Bilayer compression results

Bilayer compression was carried out by using parle elizabeth compression machine with 10station by using punch tooling of 21.20X11.5 mm. All the compression process parameters and physical parameters were evaluated during the formulation trials from ELT01 to ELT16 and are presented in table 12. The physical appearance of the compressed tablets was satisfactory. All the formulation study were carried out by using a hardness range of 18-28 kP during the compression stage.

Polymorphism

The polymorphic study was carried out for the three active pharmaceutical ingredients (APIs) such as efavirenz, lamivudine, and tenofovir and confirmed that these drug substances comply Form-1 (efavirenz and tenofovir) and Form-II for lamivudine by considering the 2-theta values. Further, these polymorphic forms were reconfirmed from the finished drug product as per fig. 5-10, and which indicates that the manufacturing process has no impact on the polymorphic character of the three-drug substances. It can also conclude that there is no interaction between active substances for the bi-layer formulation.

Table 12: Compression parameters and physical parameters of the core tablets

Parameters *		ELT 01	ELT 02	ELT 03	ELT 04	ELT 05	ELT06	ELT07	ELT08	ELT09	ELT10	ELT11	ELT12	ELT13	ELT14	ELT15	ELT16
Turret speed		10															
Feeder speed	S1	10 RPM (efavirenz part)															
	S2	10 RPM (lamivudine and tenofovir disoproxil fumarate part)															
Fill depth (mm)	S1	4.80 ± 0.3	5.05 ± 0.2	6.01 ± 0.3	5.98 ± 0.4	4.95 ± 0.2	6.02± 0.3	6.75± 0.25	6.02± 0.3	6.15± 0.3	6.40± 0.6	6.10± 0.5	6.18± 0.1	6.24± 0.8	6.21± 0.4	5.95± 0.2	6.01± 0.24
	S2	13.90± 0.2	12.90± 0.4	13.40± 0.01	12.60± 0.01	13.80± 0.2	11.90 ± 0.5	13.90 ± 0.3	13.60 ± 0.8	13.80 ± 0.2	12.40 ± 0.02	13.10 ± 0.3	12.90 ± 0.6	13.22 ± 0.4	13.18 ± 0.2	13.20 ± 0.2	13.80 ± 0.2
PCF position (mm)	S1	3.2	2.9	3.6	3.3	4.2	2.6	5.5	4.8	5.2	3.8	4.5	6.2	5.4	2.9	4.5	4.8
	S2	4.9	2.6	6.2	4.5	5.2	4.8	3.9	4.6	6.2	4.8	4.2	2.9	3.9	2.8	3.8	5.5
MCF position (mm)	S1	9.2	7.8	8.5	7.0	9.2	8.6	9.2	8.9	7.8	8.6	9.2	88.0	9.1	8.9	7.9	8.7
	S2	3.8	4.5	4.7	3.9	4.8	5.3	4.6	3.9	7.6	4.6	5.3	4.6	4.2	5.4	5.0	4.9
Average force of PCF(kN)	S1	3.10	3.50	4.50	4.19	2.92	3.80	4.20	2.92	4.2	5.20	4.8	3.97	2.18	4.77	5.12	6.12
	S2	4.10	3.90	2.90	3.60	2.92	2.20	3.29	3.19	2.28	2.28	3.21	2.22	3.24	2.29	3.62	2.21
Average force of MCF(kN)	S1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S2	24.05± 0.28	23.04± 0.35	22.02± 0.22	23.05± 0.29	22.04± 0.24	23.08 ± 0.28	21.09 ± 0.31	22.02 ± 0.25	21.05 ± 0.4	22.31 ± 0.22	23.24 ± 0.24	22.12 ± 0.39	21.08 ± 0.32	21.95 ± 0.48	22.15 ± 0.17	22.05 ± 0.02
Weight variance	S1a nd S2	1556 .0±1. 20	1548 .0±1. 50	1562 .0±1. 28	1550 .0±1. 35	156 0.0± 1.42	1548. 0±2.2 0	1552. 0±1.8 0	1555. 0±1.5 0	1565. 0±1.4 2	1548. 0±2.2 4	1539. 0±2.5 0	1547. 0±1.0 5	1552. 0±2.2 0	1534. 0±2.4 0	1548. 0±1.8 9	1550. 0±1.6 7
Hardness range (kP)		22± 1.95	22± 2.02	22± 1.57	22± 2.30	22± 1.97	22± 2.02	22± 2.47	22± 1.87	22± 2.87	22± 1.48	22± 2.10	22± 1.59	22± 2.56	22± 1.56	22± 2.40	22± 1.62
Thickness (mm)		7.98 ±0.1 0	8.00 ± 0.20	8.00 ± 0.20	8.10 ± 0.1	7.95 ± 0.2	8.05± 0.20	8.10± 0.10	8.05± 0.12	8.12± 0.22	8.01± 0.0.1 2	7.98± 0.01	8.05± 0.21	8.01± 0.2	8.12± 0.14	8.00± 0.2	8.12± 0.1
Disintegration time (min)		8	11	9	9	10	9	7	7	11	9	10	9	8	9	8	7
Friability		0.1± 0.01	0.1± 0.01	0.1± 0.01	0.2± 0.01	0.2± 0.01	0.2±0 .01	0.2±0 .01	0.1±0 .01	0.2±0 .01	0.3±0 .02	0.2±0 .02	0.2±0 .01	0.3±0 .01	0.2±0 .01	0.1±0 .02	0.2±0 .01

* n=3, mean±SD

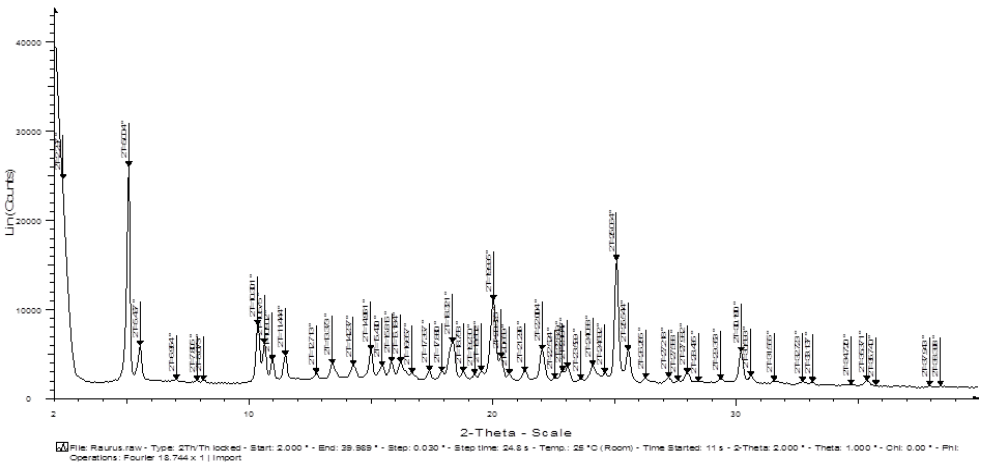

Fig. 5: The XRD profiles of tenofovir disoproxil fumarate (Form I)

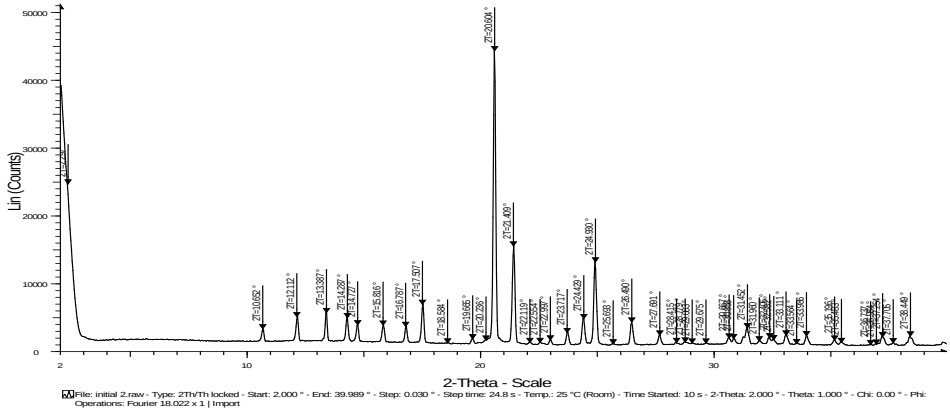

Fig. 6: The XRD profiles of lamivudine (Form I)

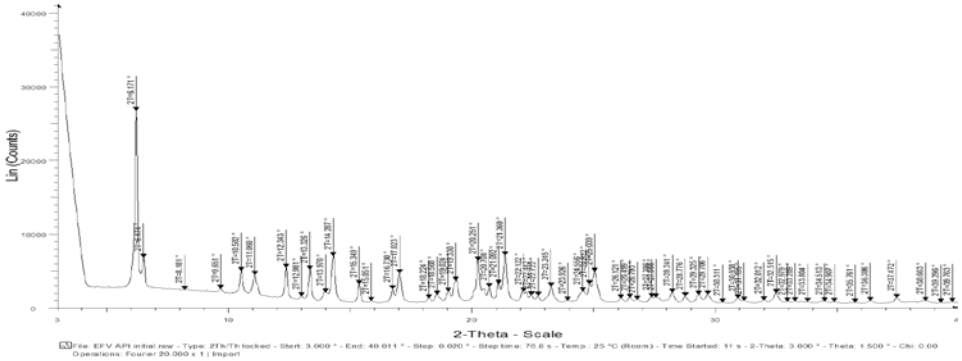

Fig. 7: The XRD profiles of efavirenz (Form I)

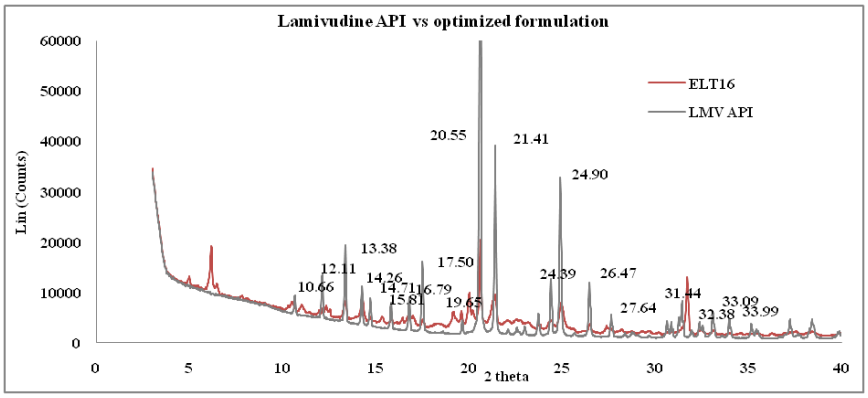

Fig. 8: XRD profile of active substance and finished product of optimized formulation (lamivudine)

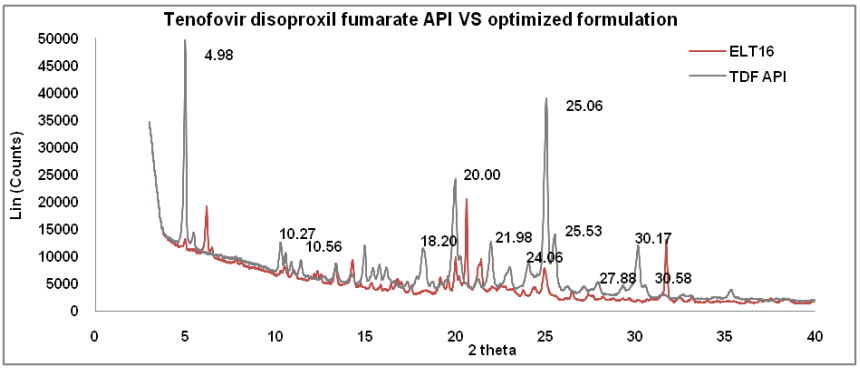

Fig. 9: XRD profile of active substance and finished product of optimized formulation (tenofovir disoproxil fumarate)

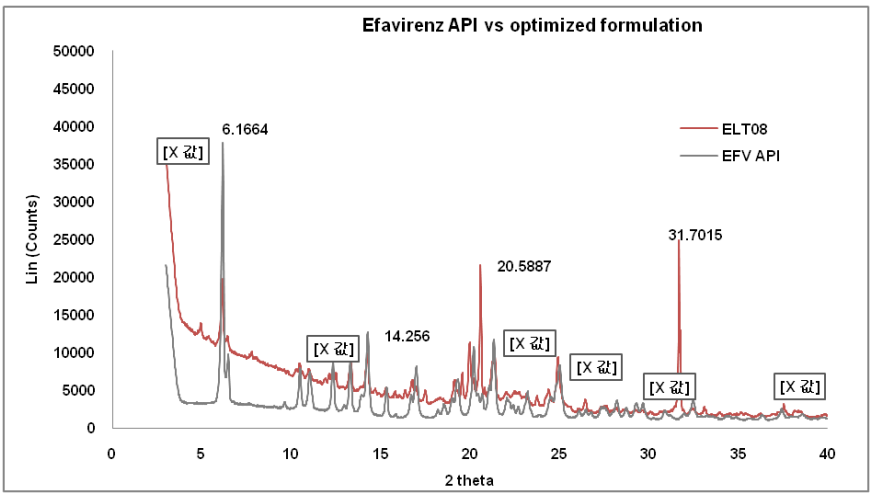

Fig. 10: XRD profile of active substance and finished product of optimized formulation (efavirenz)

In vitro drug release studies

Dissolution was carried out in purified water with 0.5% sodium lauryl sulfate (SLS), volume 900 ml, paddle speed 75 rotates per minute (RPM) for efavirenz and 0.1N hydrochloride, volume: 900 ml, paddle speed, and apparatus: Type-II was used together for the lamivudine and tenofovir disoproxil fumarate. Coated tablets were used for the study of dissolution for the formulation study from ELT0 to ELT16. Further dissolution results for the optimum formulation compared with each innovator product. Results are presented in fig. 11-14 for reference.

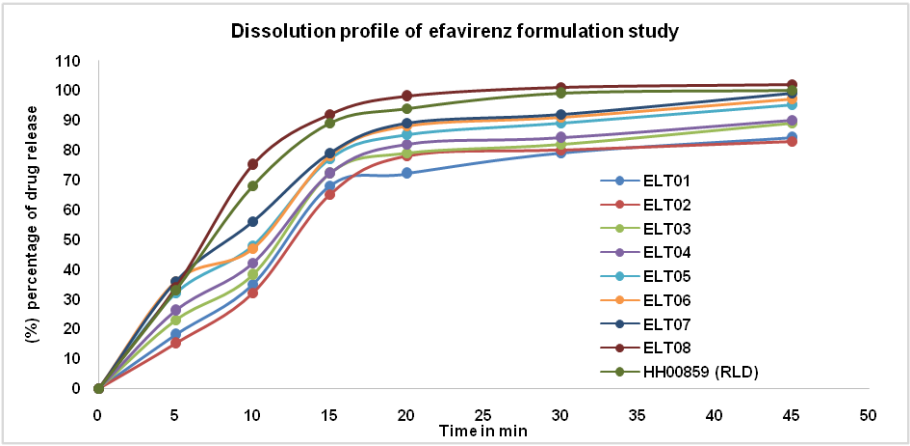

Fig. 11: Dissolution profile of efavirenz formulation study. The data is expressed as mean±SD, n=3

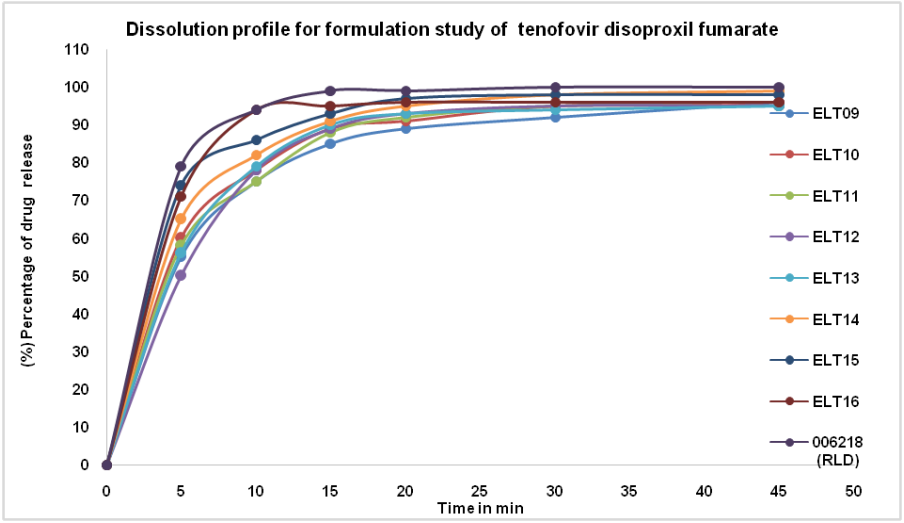

Fig. 12: Dissolution profile for formulation study of tenofovir disoproxil fumarate. The data is expressed as mean±SD, n=3

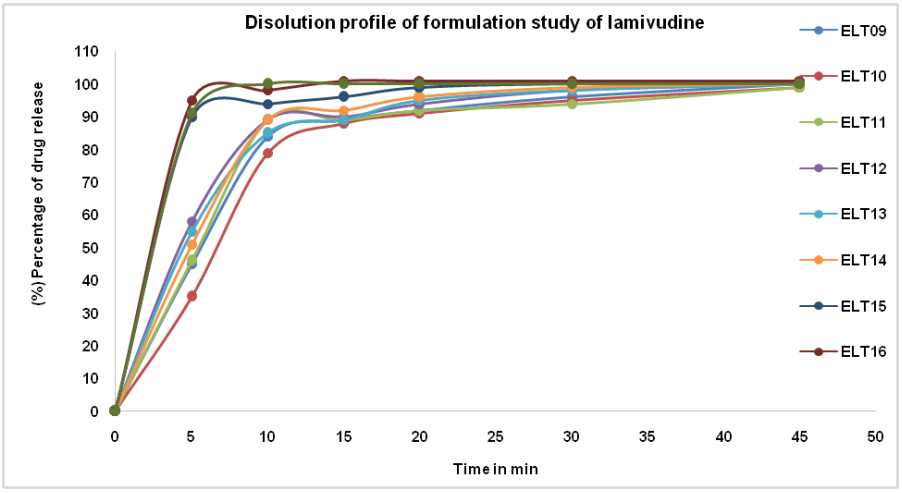

Fig. 13: Dissolution profile for formulation study of lamivudine. The data is expressed as mean±SD, n=3

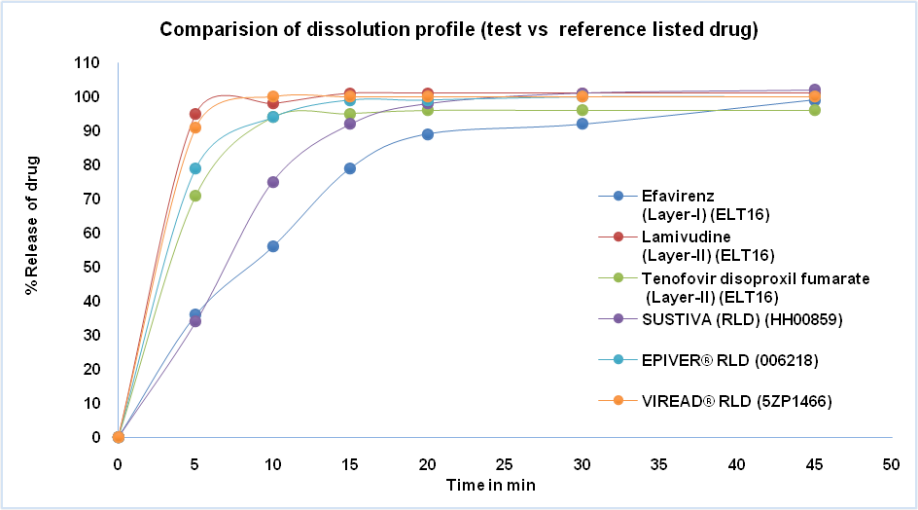

Fig. 14: Comparison of dissolution profile for test and reference drug product. The data is expressed as mean±SD, n=3

DISCUSSION

The formulation was designed by a preformulation study, which was suggested to understand the physical properties of the active substances and in-process blends to understand the flow properties. As per the Tibalinda, p etel [8], pre-formulation data and previous experience, lamivudine and tenofovir part manufacturing was recommended to use direct blending followed by roller compaction process instead of the wet granulation process. Based on pre-formulation results also conclude that tenofovir is slightly sensitive to moisture and not suitable for the wet granulation process [10]. Drug-excipients results revealed that there was no interaction of active substances if the process was designed as bi-layer tablets [12]. Because of the poor flow properties of efavirenz, wet granulation process was recommended [13]. Further, excipients selection for both the layers of blends was selected as per the reference listed drugs [15-18]. In the case of efavirenz formulation, sodium lauryl sulfate is very critical and it acts as a wetting agent and increases the solubility of efavirenz, and directly influences the dissolution of drug product and this range was optimized [13]. Further, the croscarmellose sodium and microcrystalline cellulose range were optimized to improve the dispersion and enhance the disintegration of efavirenz in the formulation. Hydroxy propyl was added to the dry mix to ensure the proper binding with other excipients and to form good granules and this range was optimized. In the case of lamivudine and tenofovir formulation, microcrystalline and croscarmellose sodium has chances to affects the dissolution and friability of tenofovir. Powdered cellulose was acting as a diluent and flow property of the lamivudine part and it also affect the uniformity and dissolution of lamivudine. So, these ranges were optimized to ensure the uniformity and dissolution of tablets. Bi-layer tablets were compressed with blends of the first layer is efavirenz followed by the second layer is Lamivudine and tenofovir through hardness range of 18-28kp. Which have good compressibility and DT is comparable to the reference formulations [9, 13]. *In vitro* dissolution media and conditions were selected based on solubility studies and other references [11, 13, 14, 20]. Finally, the optimized formulation of the dissolution profile was compared with each reference drug product and confirmed that the results were similar. Further, all the excipients quantities were used for the both layers of formulation followed to be within the inactive ingredients (IID) list as per the united states of food drug administration (USFDA) [18].

CONCLUSION

Fixed-dose formulations of efavirenz, lamivudine, and tenofovir disoproxil fumarate tablets 600 mg/300 mg/300 mg was successfully manufactured with various percentages of surfactants, diluents, disintegrants, binders, and lubricants and finalized the stable formulation. Based on the solubility studies of three active substances, dissolution methods such as purified water with 1.0% sodium lauryl sulfate (SLS) for efavirenz and 0.1N hydrochloric acid (HCL) for lamivudine and tenofovir disoproxil fumarate were

selected. Based on drug-excipient studies, results confirmed that there is no interaction between both layers by using three active substances. Sodium lauryl sulfate is acting as a wetting agent and enhances the solubility of efavirenz and hydroxypropyl cellulose was added in the dry mix for uniform mixing with purified water as solvent in the wet granulation process and this range is critical for the dissolution. The roller compaction process and process parameters were designed to reduce the interaction of tenofovir and lamivudine along with magnesium stearate and improve the consistent uniformity and dissolution. X-ray diffraction (XRD) data also revealed that there was no change in polymorphism for the optimum formulation compared to active substances. It can be concluded that fixed-dose formulations are available as a single dosage regimen and show better *in vitro* drug release compared with innovator formulations.

ACKNOWLEDGEMENT

The authors are very much thankful to the management of remedium laboratories, hyderabad. for allowing utilizing the facilities for my research work.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest regarding the publication of the paper.

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