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Formulation development and evaluation of Emtricitabine and Tenofovir Disproxil Fumarate Tablets

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Abstract

Emtricitabine and Tenofovir disproxil fumarate belongs to class of anti-retroviral drugs known as nucleotide analogue reverse transcriptase inhibitors. The main objective of the present study is to formulate and evaluate an immediate release tablet of Emtricitabine and Tenofovir disproxil fumarate using different disintegrants. Preformulation studies were performed prior to compression. The tablets were compressed using microcrystalline cellulose, lactose, pregelatinized starch, croscarmellose sodium, talc, sodium starch glycolate, magnesium stearate and opadry II blue was used for coating the tablets. The fabricated tablets were evaluated for various micromeritic properties like bulk density, tapped density, compressibility index, Hausner's ratio, angle of repose and post compression characteristics like thickness, hardness, friability, disintegration time and drug release. Croscarmellose sodium is found to be the better disintegrant when compared to sodium starch glycolate in the formulation of immediate release tablets of Emtricitabine and Tenofovir disproxil fumarate. Compared to the direct compression, wet granulation with pregelatinized starch as binder was found to be the best method of choice for formulation of these tablets. The absorbance of Emtricitabine and Tenofovir disproxil fumarate were screened in the UV region and the maximum absorbance was found to be 282 nm and 258nm respectively and this was used for HPLC analysis. The results of the present study indicates that, the prepared tablets of Emtricitabine and Tenofovir disproxil fumarate could perform therapeutically, with improved efficacy and better patient compliance like that of the marketed product.

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Key words:
Emtricitabine, Tenofovir disproxil fumarate, Anti-retroviral, immediate release tablets.

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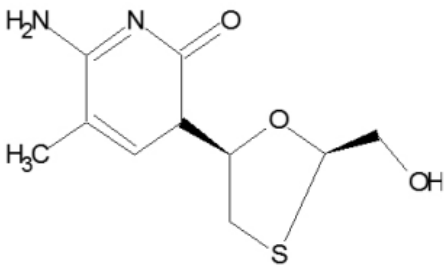
INTRODUCTION

Oral drug delivery is the most desirable and preferred method of administering therapeutic agents for their systemic effects[1,2]. For many substances, conventional immediate release

formulation provide clinically effective therapy maintaining the required balance of pharmacokinetic and pharmacodynamic profiles with an acceptable level of safety to the patients. The Indian National AIDS Control Organization (NACO) projects that there will be 90 lakh HIV cases by 2010 [3,4,5].

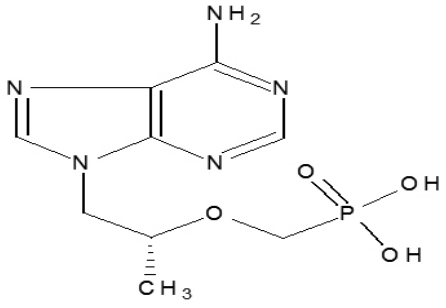
Emtricitabine (EM) is a nucleotide reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus (Type I) (HIV-1)[6]. Chemically, it is 4-amino-5fluoro-1-[2-(hydroxymethyl]-1, 3-oxathiolan-5-yl]-pyrimidin-2-one[7].

Figure 1: Structure of Emtricitabine



Tenofovir disproxil fumarate (TDF) belongs to class of anti-retroviral drugs knows as nucleotide analogue reverse transcriptase inhibitors (NtRTIs) which blocks reverse transcriptase an enzyme crucial to viral production in HIV-infected people[6]. Chemically, TDF is 9[(R)-2-[[bis [[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] propyl] adenine fumarate [8,9].

Figure 2: Structure of Tenofovir disproxil fumarate



Tenofovir is a nucleotide analog of deoxyadenosine monophosphate, while Emtricitabine, the fluorinated derivative of lamivudine, is an analog of deoxycitidine are active against HIV-1, -2 and hepatitis B virus. Their long half-lives in plasma and in peripheral blood mononuclear cells allow once-daily dosing in a single tablet, thus providing the nucleotide backbone for once-daily dosing, as a component of highly active antiretroviral therapy (HAART)[10,11].

The introduction of potent antiretroviral agents and the combined use of these drugs have markedly reduced the replication of HIV in many patients, and improved survival rates. HAART is now the standard of care in the treatment of HIV infection. It is successful in reducing viral load, extending the asymptomatic phase of infection and improving the quality of life for many infected individuals[12].

MATERIALS AND METHODS

Emtricitabine (Cipla Pvt. Ltd, India), Tenofovir disproxil fumarate (Matrix laboratories, India) were received as Gift sample. Microcrystalline cellulose 102 (Vijilak Pharma, India), Lactose monohydrate (Micro pellet, India), Pregelatinized Starch (Signet Chemical Corporation Pvt. Ltd), Croscarmellose sodium (Colorcon Asis Pvt. Ltd.), Talc (Ganesh Scientifics Ltd.), Sodium starch glycolate (Sigma chemicals India.), Magnesium Stearate (Amistri Drugs Ltd,) and Opadry II blue (Y-30-1070) (Colorcon Asia Pvt, Ltd.) were commercially procured and used for this study.

Formulation of Tablets

Formulation of Emtricitabine and Tenofovir disproxil fumarate tablets were prepared by direct compression and wet granulation method employing various excipients as mentioned in the Table 1. Emtricitabine, Tenofovir disproxil fumarate, lactose monohydrate, 50% microcrystalline cellulose and

sodium starch glycolate or croscarmellose sodium were passed through #40 mesh and mixed well. Binding solution was prepared separately by dissolving weighed quantity of pregelatinized starch in the water. The blended mixture was granulated with the above prepared binding solution and granules were dried in tray drier at 60°C. The dried granules were passed through #20 mesh and magnesium stearate was individually passed through #60 mesh. The dried granules were lubricated with remaining 50% microcrystalline cellulose and magnesium stearate. The tablets were compressed using a 27 station tablet compression machine using 19.2 × 9 mm capsule shaped punches. (Rimek, Ahmedabad).

Table 1: The formulation composition of Emtricitabine and Tenofovir disproxil fumarate tablets

Sl. No	Ingredients	Quantity per Tablet (mg)					
		T - I	T - II	T - III	T - IV	T - V	T - VI
01	Emtricitabine	200	200	200	200	200	200
02	Tenofovir disproxil fumarate	300	300	300	300	300	300
03	Pregletanized starch	---	50	50	50	50	50
04	Lactose monohydrate	80	80	80	80	80	80
05	Mircrocrystalline cellulose pH 102	355	305	295	305	295	285
06	Sodium starch glycolate	50	50	60	---	---	---
07	Croscarmellose sodium	---	---	---	50	60	70
08	Magnesium stearate	15	15	15	15	15	15
09	Purified water	---	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
10	Opadry II Blue	30	30	30	30	30	30

Characterization of Blend [2,13,14,15, 16]

Micromeritic Properties

Prior to compression, the blend was evaluated for their micromeritic properties such as bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose.

Bulk Density[2,13,16]

Weighed quantity of granules (W) was transferred into a graduated measuring cylinder without tapping and the volume occupied by granules was measured. Bulk density was measured by using the following formula

Bulk Density (BD) = Weight of granules / untapped volume of granules.

Tapped Density[2,13,16]

Weighed quantity of granules was taken into graduated cylinder; volume occupied by granules was measured. The graduated cylinder was fixed in the "Tapped Densitometer" (Electrolab, Mumbai, India) and tapped until the difference in the volume after consecutive tappings was less than 2%. The percentage volume variation was calculated by the following formula

Tapped Density (TD) = Weight of granules / tapped volume of granules

Compressibility Index[2,13,16]

Compressibility is the ability of powder to decrease in volume under pressure using bulk density and tapped density the percentage compressibility of granules were determined, which is given as carr's compressibility index. It is indirectly related to the relative flow rate. Table 2 shows the percentage compressibility index and its flow characteristics.

$$\text{Compressibility Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100$$

Hausner's ratio [2,13,16]

Hausner's ratio indicates the flow properties of powder and measured by the ratio of tapped density to bulk density. Table 2 shows the flow characteristics and corresponding Hausner's ratio

Hausner's ratio = Tapped Density / Bulk Density.

Angle of Repose [2,13,16]

Angle of repose (θ) is the maximum angle between the surface of a pile of powder and horizontal plane. It is usually determined by fixed funnel method and is the measure of flow ability of powder/ granules. Table 2 shows the flow properties and corresponding angle of repose.

Weighed quantity of granules was passed through a funnel kept at a height of 2cm from the base. The powder is passes until it forms heap and touches the tip of funnel. The radius was measured and angle of repose was calculated using the following formula

$\theta = \text{Tan}^{-1} (h/r)$

Where, θ = Angle of repose

h = Height of heap of pile

r = Radius of base of pile

Table 2: Compressibility Index, Hausner's Ratio, Angle of repose with corresponding Flow characters.

Type of Flow	Compressibility Index	Hausner's ratio	Angle of repose (θ)
Excellent	1-10	1-1.1	25 – 30
Good	11-15	1.12 – 1.18	31 – 35
Fair	16-20	1.19 – 1.25	36 – 40
Passable	21-25	1.26 – 1.34	41 – 45
Poor	26-31	1.35 – 1.45	46 – 55
Very Poor	32-37	1.46 – 1.59	56 – 65
Extremely Poor	>38	>1.6	>66

Evaluation of Tablets [2,13,16,17]

The formulated tablets were evaluated for the following physicochemical parameters.

Hardness[2,13,16,17]

Tablets require certain amount of strength to have a resistance from breakage, while transportation and handling before use. It was measured by Monsanto Hardness Tester (Tab machines, India).

Thickness[2,13,16,17]

The thickness of tablet can vary without any change in weight. This is generally due to the differences of density of granules, pressure applied for compression and the speed of compression. It was measured by vernier caliper (Mitutoyo, Japan).

Friability[2,13,16,17]

Friability was performed by using friability test apparatus (Electrolab, ET2, India). Specified number of tablets were weighed and placed in the tumbling chamber and roated for four minutes at a speed of 25 rpm. During each revolution, tablets fall from a distance of 6 inches to undergo shock. After 100 revolutions the tablets are dusted and reweighed. The loss in weight indicates friability and loss of less than 1% in weight is considered to be acceptable. It was determined by the following formula.

$F = W_1 - W_2 / W_1 \times 100$

Where, W_1 = Initial weight of tablets

W_2 = Final weight of tablets

Weight variation test [2,13,16,17]

Twenty tablets were selected randomly and weighed individually. Average weight of tablets were calculated and compared with that of the individual tablets. Weight not more than two of the individual weight deviate from the average weight by more than the percentage shown in table 3.

Table 3: Average weight with corresponding percentage deviation

Average weight of Tablets (Mg)	Maximum percentage deviation (%)
130 or less	10
130 – 324	7.5
324 or more	5

Disintegration time[18,19]

The disintegration time was performed using an USP disintegration test apparatus (TD2, Tab machines, India) with distilled water at 37±0.5°C. The disintegration time was taken to be the time when no granules of any tablets were left on the mesh of the apparatus. The time reported to obtain complete disintegration of six tablets were recorded and mean value was reported.

Dissolution studies[1]

The release rate of Emtricitabine and Tenofovir disproxil fumarate from the tablets was determined using USP dissolution testing apparatus II (Electro lab, India). The dissolution testing was performed using 900ml of 0.01N HCl at 37±0.5°C temperature and speed 50 rpm. Sample of 10ml was withdrawn at regular interval of 5th, 10th, 15th 30th and 45th minutes and replaced with fresh medium to maintain sink condition and the percentage of drug release was determined using HPLC.

Standard Stock Solution[20]

Tenofovir disproxil fumarate and Emtricitabine were weighed separately (100 mg) and dissolved in buffer and made up to 100ml in volumetric flasks to get a concentration of 1000µg/ml.

Selection of λ max[20]

The standard stock solutions of TDF and EM were further diluted separately to get a concentration of 10µg/ml. The absorbance of the solutions were screened in the UV region and found that TDF showed maximum absorbance at 258 nm and EM at 282 nm. Thus λ_{max} of TDF was found to be 258nm and then EM to be 282 nm.

Emtricitabine and Tenofovir disproxil fumarate working Standard

22.2 mg of Emtricitabine Working Standard and 33.3 mg of Tenofovir working standard were accurately weighed and transferred separately into a 100 ml clean dry volumetric flask, add about 60 ml of diluents, sonicate for 5 minutes until it dissolves. Cool the solution to room temperature and dilute to volume with dissolution medium.

Sample Preparation

Place one tablet in each of six dissolution flasks containing 900ml of dissolution medium, previously maintained at 37°C ±0.5° C. After completion of specified time interval, a portion of solution from the zone of midway between the surface of dissolution medium and top of the rotating blade not less than 1cm from vessel wall was withdrawn and filtered through 0.45 µ membrane filter.

Chromatographic conditions [21]

Chromatographic separations were achieved by using Pursophere star – RP18 (250 x 4.6 mm, 5µ) analytical column. The mobile phase consist of mixture of buffer and Acetonitrile in the ratio of 970: 30 v/v respectively mobile phase A and Mixture of buffer and acetonitrile in the ratio of 60:40 as mobile phase B with gradient program as follows. The flow rate was maintained at 1.0 ml/min with injection volume of 10µl and the absorbance was measured at 258nm for TDF and 282 nm for EM. The column and the HPLC system were kept in ambient temperature.

Percentage content of Emtricitabine / Tenofovir disproxil fumarate = TA/SA × SW/100 × 900/1 × P/100 × 100/LA

Where,

TA – Peak area response due to Emtricitabine / Tenofovir disproxil fumarate from sample preparation.

SA – Peak area response due to Emtricitabine / Tenofovir disproxil fumarate from standard preparation.

SW – Weight of Emtricitabine / Tenofovir disproxil fumarate working standard taken in mg.

P – Purity of Emtricitabine / Tenofovir disproxil fumarate working standard taken on as a basis

LA – Labelled amount of Emtricitabine / Tenofovir disproxil fumarate.

Similarity and Dissimilarity factors[22,23,24]

Similarity Factor (f2) stresses on the comparison of closeness of two comparative formulations. Generally similarity factor in the range of 50-100 is acceptable according to US FDA. It can be computated using the formula

$f2 = 50 \times \log \{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$

where, n is the number of dissolution sample times, R_t and T_t are the individual or mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively.

Dissimilarity factor (f1) focuses on the difference in percent dissolved between marketed product and formulation trials at various time intervals. It can be mathematically computed by using

$f1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\} \times 100$

Therefore the factors directly compare the difference between percent drug dissolved per unit time for the formulation trials and marketed product.

STABILITY STUDIES [25,26,27,28]

In order to determine the change in *in-vitro* release profile on storage, stability study of batch **T-VI** was carried out at 40°C in a humidity chamber having 75% RH. Samples were withdrawn at regular intervals during the study of 60 days. Formulation is evaluated for change in average weight, *in-vitro* drug release pattern, thickness, hardness and disintegration time.

RESULTS AND DISCUSSION

Preformulation Studies

Compatibility test between the drug and tablet components was carried out at 40°C ± 2°C / 75±5% and 25°C ± 2°C / 60 ±5% for three months. The mixture does not show any visible change, thus indicating drug and other tablet components do not have any physical incompatibility.

Evaluation of Tablets

Micrometric Properties

Bulk Density for Emtricitabine and Tenofovir disproxil fumarate granules was found to be in the range of 0.323 to 0.500. Tapped density for granules were found to be between 0.547 and 0.620. Compressibility index and Hausner's ratio were obtained in the range of 16.66 to 40.95 and 1.20 to 1.69 respectively. Angle of repose was observed in the range of 26.00' to 37.20'. The results indicate passable flow property and compressibility (Table 4).

Table 4: Flow properties of Tablet Blend.

Parameters	Formulation Trials					
	T - I	T - II	T - III	T - IV	T - V	T – VI
Bulk Density (gm/ml)	0.320	0.420	0.449	0.478	0.502	0.462
Tapped Density (gm/ml)	0.549	0.592	0.568	0.582	0.598	0.619
Carr's Index (%)	41.01	27.81	21.89	16.73	16.50	24.83
Hausner's Ratio	1.68	1.40	1.27	1.20	1.20	1.30
Angle of Repose (θ)	35.20	33.20	31.30	28.40	26.00	28.70

Post-compression parameters

Prepared granules were compressed and these compressed tablets were evaluated for average weight, thickness, friability, hardness, disintegration and dissolution. The average percentage deviation of 20 tablets of each formula was less than 3%. The thickness and hardness of the tablet ranged from 6 – 7.5 mm and 15 - 25 kg/cm² respectively. The percentage friability of all batches ranged from 0.020 to 0.099 %W/W. The disintegration time was ranged from 3 minute 20 seconds to 11 minutes 12 seconds for uncoated tablets. The thickness and hardness of

the tablet ranged from 7.5 – 8.3 mm and 15 - 25 kg/cm² respectively. The percentage friability of all batches ranged from 0.020 to 0.099 %W/W. The disintegration time was ranged from 4 minute 08 seconds to 13 minutes 34 seconds coated tablets (Table 5 & 6).

The drug release was found to be ranged from 91.0% to 100.7% for Emtricitabine and 88.3% to 100.1% for Tenofovir disproxil fumarate (Figure 3&4)

Table 5: Physical characteristics of uncoated tablets.

Parameters	Formulation Trials				
	T - II	T - III	T - IV	T - V	T - VI
Average Weight (mg)	1020 ± 1.5	1021 ± 0.5	1018 ± 1.5	1019 ± 1.4	1016 ± 1.0
Thickness (mm)	7.06 ± 0.5	7.34 ± 0.5	6.86 ± 0.8	6.34 ± 0.5	7.02 ± 0.3
Hardness (kg/cm²)	8.4 ± 0.4	9.4 ± 0.3	11.4 ± 0.9	10.0 ± 0.5	11.0 ± 0.3
Friability (%)	0.09 ± 0.1	0.07 ± 0.02	0.03 ± 0.03	0.04 ± 0.02	0.02 ± 0.04
Disintegration Time (Min)	2 min 57 sec ± 2 sec	6 min 38 sec ± 3 sec	8 min 48 sec ± 5 sec	9 min28 sec ± 5 sec	10 min 50 sec ± 6 sec

Table 6: Physical characteristics of coated tablets.

Parameters	Formulation Trials				
	T - II	T - III	T - IV	T - V	T - VI
Average Weight (mg)	1031 ± 1.5	1038 ± 0.8	1036 ± 2.5	1037 ± 1.0	1035 ± 1.7
Thickness (mm)	7.24 ± 0.5	7.29 ± 0.9	6.96 ± 0.4	7.02 ± 0.5	6.99 ± 0.7
Hardness (kg/cm²)	9.0 ± 0.4	10.4 ± 0.4	11.4 ± 0.9	11.0 ± 0.4	11.5 ± 0.3
Friability (%)	0.07 ± 0.1	0.06 ± 0.03	0.02 ± 0.04	0.02 ± 0.05	0.01 ± 0.05
Disintegration Time (Min)	3 min 26 sec ± 2 sec	7 min 10 sec ± 3sec	9 min 48 sec ± 1 sec	10 min 40 sec ± 5 sec	11 min 40 sec ± 6 sec

Figure 3: Comparative *in vitro* release profile of Emtricitabine in the formulation with Marketed Product.

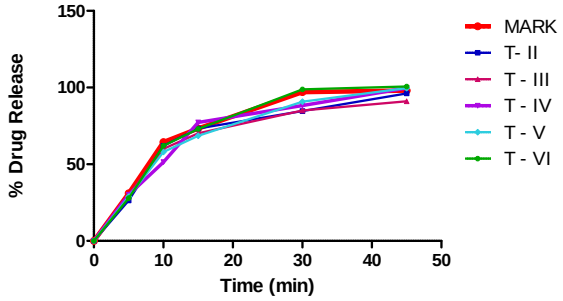
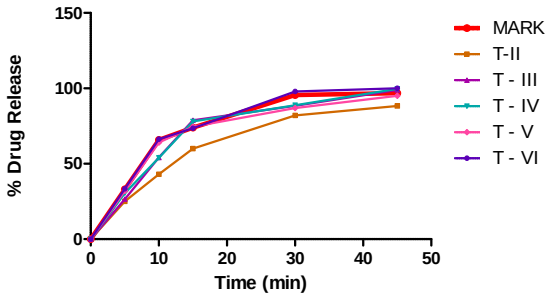


Figure 4: Comparative *in vitro* release profile of Tenofovir disproxil fumarate in the formulation with Marketed Product.



Formulation trial T - I was performed to select the method of preparation, primarily with direct compression. There was poor powder flow and capping was detected in this method.

Trial T – II was taken by changing the method of preparation as wet granulation method using Pregelatinized starch as binder, tablet came good but poor flow of powder was observed and disintegration was not satisfactory.

Formulation Trial T – III was planned to improve the disintegration time by increasing the concentration of sodium starch glycolate. The disintegration slightly increased but not satisfactory.

Formulation Trial T – IV was planned to change the disintegrating agent from sodium starch glycolate to croscarmellose sodium and all the physical parameters of tablets were found to be satisfactory, and *in vitro* dissolution profile was not satisfactory.

In formulation trial T – V, the percentage of croscarmellose sodium was increased and the *in vitro* drug release increased but was not matching to the marketed product.

Formulation Trial T –VI was planned to increase the *in vitro* drug release to match with the marketed product. All the parameters are found to be satisfactory *in vitro* drug release profile is nearly matching with the marketed product.

The dissimilarity factor (F_1) and Similarity factor (F_2) is found to be 2.15, 1.10 and 82.23, 91.50 (Table 7).

Table 7: Dissimilarity F_1 and Similarity factor F_2 of T – II to T - VI

Trials (EMT)	F_1	F_2
T – II	4.39	61.34
T – III	3.54	70.89
T – IV	2.85	73.56
T – V	2.72	75.50
T – VI	2.15	82.23
Trials (TDF)	**F_1**	**F_2**
T – II	6.00	46.67
T – III	5.75	54.89
T – IV	4.83	60.45
T – V	3.75	70.39
T – VI	1.10	91.50

Stability Studies

Stability studies performed on batch T - VI as per ICH guidelines for 60 days at 40°C±2°C / 75% RH±5%. That shows no remarkable changes in the physical properties of the tablets (Table 8) as well as no remarkable changes in the release profile as indicated in Figure 5 & 6. The studies shows tablets after stability studies are in acceptable range.

Table 8: Stability data for T - VI Film Coated Emtricitabine and Tenofovir disproxil fumarate Tablets

Sl. No	Test	Initial Analysis	Stability Results	
			1 Month	2 Month
			40°C / 75% RH	40°C / 75% RH
1	Average Weight (mg)	1031 ± 1.5	1031 ± 1.4	1032 ± 1.2
2	Thickness (mm)	7.24 ± 0.05	7.24 ± 0.04	7.24 ± 0.05
3	Hardness (Kg/cm²)	11.00 ± 0.5	11.25 ± 0.7	11.65 ± 0.4
4	Disintegration time (min)	10 mins 40 sec ± 5 sec	10 mins 50 sec ± 3 sec	09 mins 47 sec ± 7 sec

Figure 5: *In vitro* drug release profile of T - VI for Emtricitabine after stability studies.

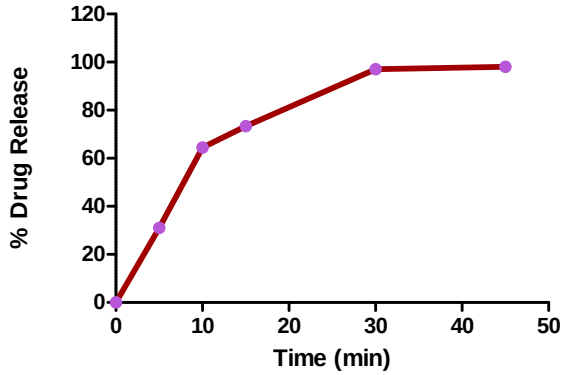
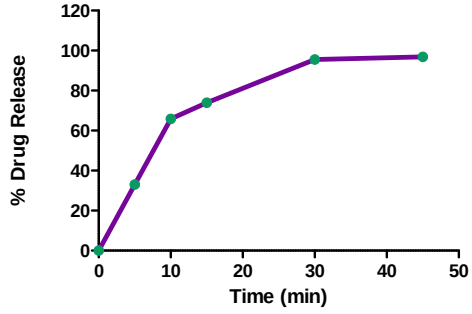


Figure 6: *In vitro* drug release profile of T - VI for Tenofovir disproxil fumarate after stability studies



CONCLUSION

The immediate release tablets of Emtricitabine and Tenofovir disproxil fumarate have been developed with wet granulation method and it was compared with that of marketed product. Compared to the direct compression, wet granulation with pregelatinized starch as binder was found to be the best method of choice for formulation of these tablets. Various trials were performed to optimize the disintegrants concentration of sodium starch glycolate and croscarmellose sodium. Dissolution studies were performed in media pH 0.01N HCl and found to be comparable with that the marketed product. Formulation trial T – VI during accelerated stability studies does not show any remarkable changes in their characteristics. Therefore, it was concluded that the T – VI trial was the satisfactory formulation that could perform therapeutically, with improved efficacy and better patient compliance like that of the marketed product.

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