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Original Article

Formulation Development and Characterization of Anti-Retroviral Agents

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ABSTRACT

Emtricitabine and Tenofovir disproxil succinate 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 characterize an immediate release tablet of Emtricitabine and Tenofovir disproxil succinate using different Techniques. 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 formulated tablets were evaluated for various precompression 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 was found to be the better disintegrant when compared to sodium starch glycolate in the formulation of immediate release tablets of Emtricitabine and Tenofovir disproxil succinate. Compared to the direct compression, wet granulation with Isopropyl alcohol was found to be the best method of choice for formulation of these tablets. The absorbance of Emtricitabine and Tenofovir disproxil succinate were screened in the UV region and the maximum absorbance was found to be 282 nm and 258nm respectively. The results of the present study indicates that, the prepared tablets of Emtricitabine and Tenofovir disproxil succinate could perform therapeutically, with improved efficacy and better patient compliance like that of the marketed product.

Keywords- Emtricitabine, Tenofovir disproxil succinate, Antiretroviral, immediate release tablets.

1. 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

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safety to the patients. [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].

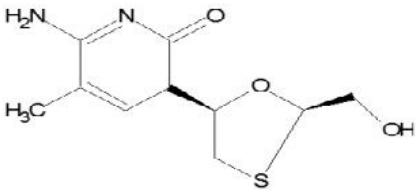

Fig 1: Structure of Emtricitabine

Tenofovir disproxil succinate (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 succinate [8,9].

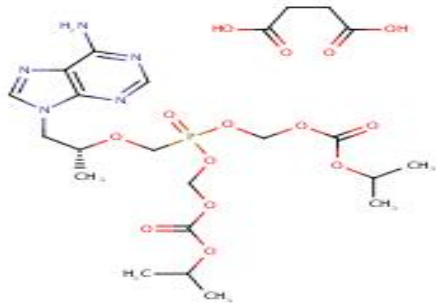

Fig 2: Structure of Tenofovir disproxil succinate

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].

2. MATERIALS AND METHODS

Emtricitabine (Shanghai Desano Chemical Pharmaceutical Co., Ltd), Tenofovir disproxil succinate (Zhejiang Jiuzhou Pharmaceutical Co., Ltd).Microcrystalline cellulose (FMC Polymer), Lactose monohydrate (DFE Pharma India Ltd), Croscarmellose sodium (Blanver), Partially Pregelatinized starch (Colorcon Asia) Glycerol triacetate (Merck), Magnesium Stearate(FACI SpA).

Formulation of Tablets

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

S. No.	Ingredients	F001	F002	F003	F004	F005	F006
A	**Intra granular (mg)**						
1	Drug A	200.00	200.00	200.00	200.00	200.00	200.00
2	Drug B	245.00	245.00	245.00	245.00	245.00	245.00
3	Microcelac 100	374.30	374.30	**	**	200.00	**
4	Lactose Anhydrous	**	**	**	96.00	**	**
5	Microcrystalline Cellulose 112	**	**	**	278.30	**	**
6	Croscarmellose sodium	60.00	60.00	30.00	60.00	60.00	30.00
7	Partially Pregelatinized	50.00	50.00	50.00	50.00	50.00	50.00
8	Lactose Monohydrate	**	**	96.00	**	**	96.00
9	Microcrystalline Cellulose 101	**	**	133.30	**	**	133.30
10	Magnesium Stearate	10.00	10.00	**	10.00	10.00	**
	Binder preparation						
11	Isopropyl Alcohol	***	**	**	**	**	qs
12	P. Water	***	**	Qs	**	QS	**
	Extra granular (mg)						
13	Microcrystalline Cellulose 112	***	**	150	**	**	240.00
14	Microcelac 100	**	**	**	**	156.80	**
15	Croscarmellose sodium	**	**	30	**	10.00	48.00
16	Magnesium stearate	5.00	5.00	10.00	5.00	12.50	10.00
Average weight (mg)		**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**	**1000.00**

F001: Formulation of Emtricitabine and Tenofovir tablets were prepared by Slugging mhod.

F002: Formulation of Emtricitabine and Tenofovir tablets were prepared by Slugging method with microniczed API.

F003: Formulation of Emtricitabine and Tenofovir tablets were prepared by wet granulation approach with purified water.

F004: Formulation of Emtricitabine and Tenofovir tablets were prepared by Roller Compactor approach using anhydrous lactose and MCC 112.

F005: Formulation of Emtricitabine and Tenofovir disproxil succinate tablets were prepared by Roller Compactor approach using Microcelac 100.

F006: Formulation of Emtricitabine and Tenofovir disproxil succinate tablets were prepared by Wet granulation approach with IPA.

Different excipients have been used as per suitable method mentioned in the Table 1. The tablets were compressed using 19.0 × 9.0 mm capsule shaped punches, plain on both sides.

EVALUATION OF TABLETS.

The tablets of all the batches were evaluated for weight variation, drug content, hardness, thickness, friability, disintegration time and in-vitro dissolution study.

- **Weight Variation:** 10 tablets were selected randomly from each batch and weighed individually to check for weight variation. The following percentage deviation in weight variation is allowed

Table 2: Weight variation tolerances

Average weight of a tablet	Percentage deviation
130 mg or less	10
> 130 mg and < 324 mg	7.5
324 mg or more	5

- **Thickness and Diameter:**

The thickness and diameter of 4 tablets from each formulation were recorded during the process of compression using Vernier caliper.

- **Hardness:**

Pharmatorn hardness tester was used for the determination of hardness of tablets. Tablet was placed in between the plungers and the force of the fracture was recorded.

- **Friability:**

6.5 gm. of tablets were accurately weighed and placed in the friabilator (Electrolab, EF-2 Friabilator) and operated for 100 revolutions. The tablets were de-dusted and reweighed. Percentage friability was calculated using the following formula

$$F = (1- W0 / W) \times 100$$

Where,

W0 is the weight of the tablets before the test and W is the weight of the tablet after the test. The tablets that loose less than 1% weight were considered to be satisfactory.

- **Disintegration Time:**

Six tablets were taken and introduced in each tube of disintegration apparatus, and the tablet rack of the disintegration apparatus was positioned into a one liter beaker containing 900 ml of distilled water and the time of disintegration was recorded. To discriminate between the formulations disintegration was done at room temperature and disk was not used for the study.

- ***In-vitro* Dispersion Time:**

In- Vitro dissolution studies were carried out using USP apparatus typeII at 50 rpm. Dissolution medium consist of 0.01N HCL maintained at 37°C . Drug release at different time intervals was measured by UV- Visible Spectrophotometer at 281 nm for Emtricitabine and 259 for Tenofovir disproxil succinate. In- vitro drug release drug release profile of all batches was compared with conventional formulation for drug release.

3. RESULTS AND DISCUSSION

Identification tests for Emtricitabine and tenofovir Disproxil succinate

a) **Melting point:**

The melting point of the Emtricitabine was found to be 278-281 °C and The melting point of the Tenofovir Disoproxil succinate was found to be 137-140 °C which complies with melting point reported one.

b) Loss on Drying (15 minutes at 105˚C±2 ˚C):-

Table no:3 LOD of API was shown in following

S. no	Sample	Initial	Final	LOD after 15 min
1	Emtricitabine	1.0726	1.060	0.8%
2	Tenofovire	1.106	1.056	0.9%

c) XRD study:

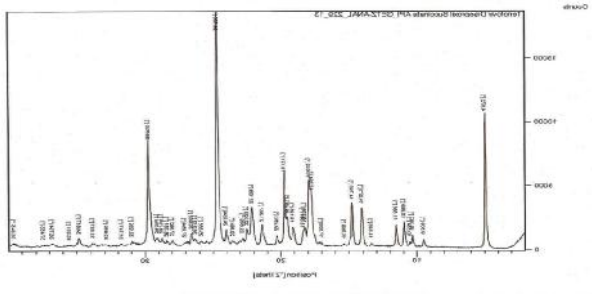

Fig 3: XRD data of Tenofovir Disoproxil succinate

Inference: Tenofovir disoproxil exhibits different polymorphism and from above XRD data it was confirm that Tenofovir Disoproxil Succinate was found to be crystalline in nature.

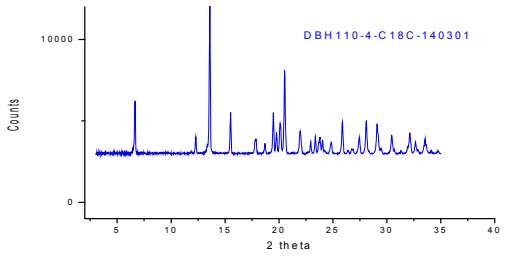

Fig 4: XRD data of Emitricitabine

Inference: from above XRD data it was confirmed that Emtricitabine was found to be crystalline in nature.

Table 4: Core tablet parameters as follows.

Parameters	Specification
Description	White to off white colored, capsule shaped, tablets, plain on both sides
Theoretical mass of tablet	1000.00 mg
Average mass	1000.00 mg ± 5.0 %
Uniformity of mass	NMT 2/20 individual mass deviate from ± 5% of the average mass and none by ± 10 % of the average mass.
Punch size	19.00 x 9.00 mm capsule shaped punches
Thickness	6.00 ± 0.30 mm
Hardness	100 - 200 N
Friability	NMT 1.0 % w/w

Table 5: Flow properties measurement

B. NO:	Emtricitabine	Tenofovir Disoproxil succinate
B.D.	0.370g/ml	0.476 g/ml
T.D.	0.500g/ml	0.500g/ml
C.I.	25.92%	47.62%
H.R.	1.35	1.05

Table 6: Flow properties of the blended powders of different formulations

S. No	Formulation Code	Bulk Density	Tapped Density	Hausner's Ratio	Compressibility Index	Angle of Repose $\theta = \tan^{-1} h/r$
1	F001	0.600	0.833	1.38	28.00	35 ± 0.65
2	F002	0.476	0.740	1.55	35.71	40 ± 0.72
3	F003	0.512	0.740	1.44	30.76	37 ± 0.77
4	F004	0.471	0.753	1.55	35.84	28 ± 0.29
5	F005	0.576	0.750	1.30	24.00	33 ± 0.81
6	F006	0.464	0.593	1.27	21.62	30 ± 0.72

*All values are expressed as mean ± S.D, n = 5.

Table 7: Dissolution Profile (% drug release) of different formulations at different time points.

Time (min)	% of Drug Release, Media: 0.01N HCl, Paddle, 900ml, 50 rpm													
	Reference		F001		F002		F003		F004		F005		F006	
0	Drug A	Drug B	Drug A	Drug B	Drug A	Drug B	Drug A	Drug B	Drug A	Drug B	Drug A	Drug B	Drug A	Drug B
5	46.1	49.1	30.5	26.9	40.0	43.7	53.8	54.3	53.0	57.1	40.8	43.7	44.3	47.9
10	90.8	90.4	65.3	61.9	89.8	92.0	91.2	90.5	91.0	94.0	81.8	83.6	87.4	90.2
15	99.3	97.7	88.3	86.5	97.2	99.1	94.0	93.6	94.0	96.5	94.7	94.6	97.8	96.2
30	100.1	98.5	97.1	97.8	98.3	99.9	95.1	94.3	94.8	97.0	99.3	98.2	98.8	99.1
45	100.5	98.8	99.1	99.8	98.5	100.3	95.5	94.6	95.6	97.6	100.4	99.5	100.1	100.5

*All values are expressed as mean ± S.D, n = 6

In- vitro Drug Release from Anti retroviral Tablets:-

In- Vitro dissolution studies were carried out using USP apparatus typeII at 50 rpm. Dissolution medium consist of 0.01N HCL maintained at 37°C . Drug release at different time intervals was measured by UV- Visible Spectrophotometer at 281 nm for Emtricitabine and 259 for Tenofovir disproxil succinate. In- vitro drug release drug release profile of all batches was compared with conventional formulation for drug release.

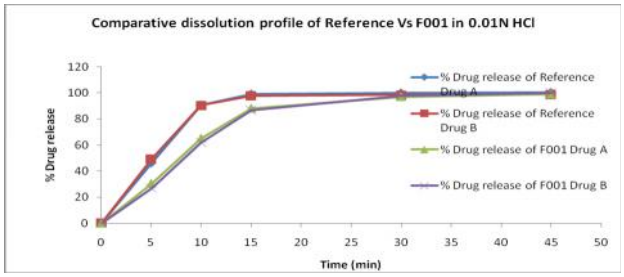

Fig 5: Comparative Dissolution profile of Reference product with F001

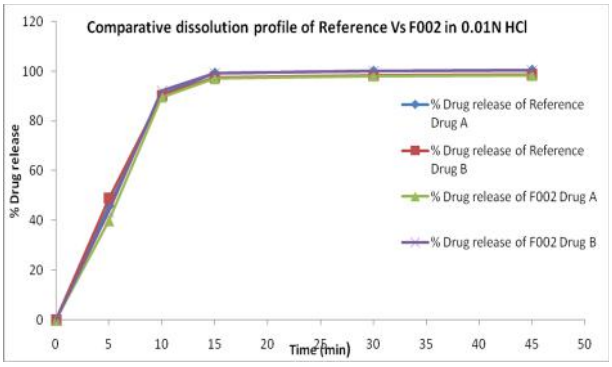

Fig 6: Comparative Dissolution profile of Reference product with F002

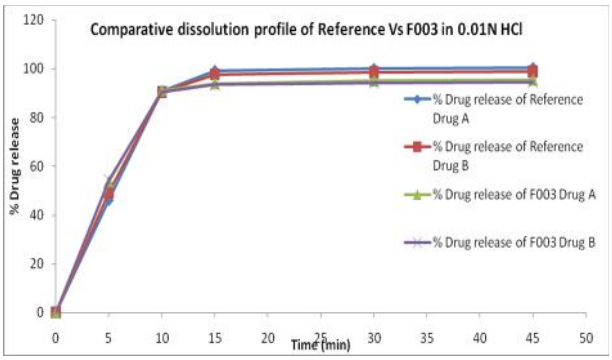

Fig 7: Comparative Dissolution profile of Reference product with F003

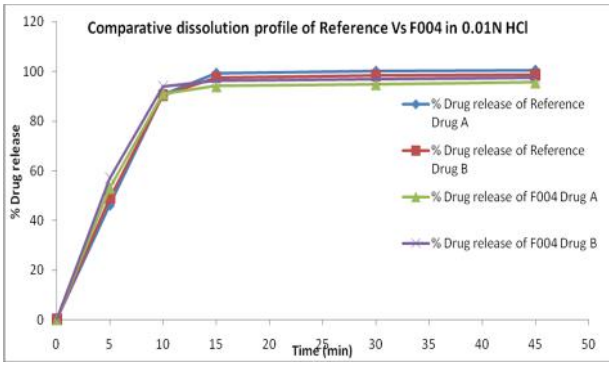

Fig 8: Comparative Dissolution profile of Reference product with F004

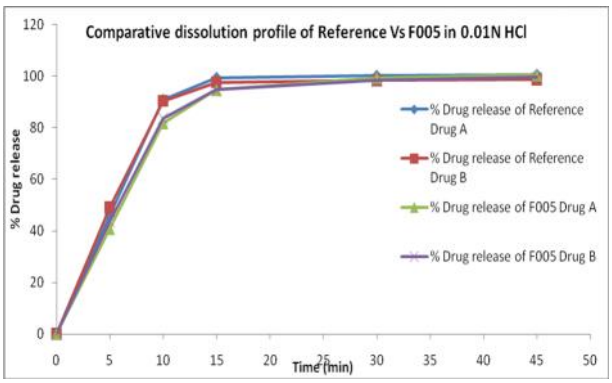

Fig 9: Comparative Dissolution profile of Reference product with F005

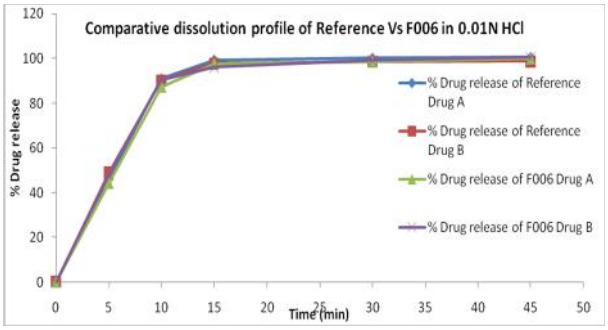

Fig 10: Comparative Dissolution profile of Reference product with optimized formulation 'F006'

4. SUMMARY AND CONCLUSION

The results obtained from F006 revealed that wet granulation approach by IPA as granulating fluid shows satisfactory *in-vitro* drug release as well other chemical parameters. With the combination of Croscarmellose sodium, partially pregelatinised maize starch, lactose, MCC, microcelac 100 and magnesium stearate shows well release pattern and other physico-chemical parameters. Dissolution graphs were shown individually for all formulation trials. There was no chemical interaction found between the drug and excipients during Fourier Transform Infrared Spectroscopy (FTIR).

5. CONCLUSION

Hence, combinely Emtricitabine and Tenofovir with efficient amount of IPA at a suitable concentration of all excipients can effectively be used to prepare immediate release tablet formulation.

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