CHAPTER – VII

RESULTS AND DISCUSSION

7. Results and Discussion

Part A: Tenofovir Disoproxil Fumarate

7.1 Preformulating Studies

7.1.1 Organoleptic Properties of TDF

The organoleptic properties of TDF like color, odor, and taste were observed and recorded as shown below.

Table 7.1 : Organoleptic Properties of TDF

Sr. No.	Parameter	Standard	Observed
1.	Color	White to off-white	Off-white
2.	Odor	Characteristic	Characteristic
3.	Taste	Bitter	Bitter

Inference: The organoleptic properties of TDF match with the standard reference TDF.

7.1.2 Density and Flow Properties

Density and flow properties of Tenofovir disoproxil fumarate API was evaluated and the results are given in the following table,

Table 7.2 : Density and Flow Properties of Tenofovir Disoproxil Fumarate API

Bulk Density (g/ml)	Tapped Density (g/ml)	Carr's Index (%)
0.35	**0.51**	**30.85**

Inference: Tenofovir disoproxil fumarate (TDF) API showed poor to very poor flow characteristics.

7.1.3 Solubility

The solubility of TDF in different solvents mentioned below,

Table 7.3 : Solubility Data of TDF

Solvent	Solubility (mg/ml)
0.1 N HCl	77.86 ± 2.06
Distilled water	13.27 ± 0.45
pH 6.8 phosphate buffer	68.33 ± 1.96

Solvent	Solubility (mg/ml)
Ethanol	97.42 ± 3.57
Methanol	80.09 ± 2.81
Dimethyl Sulfoxide	101.55 ± 3.32

Inference: The highest solubility among aqueous media is in 0.1 N acid and the lowest is in distilled water. Among the organic solvents, the highest solubility is found in DMSO.

7.1.4 Melting Point

The melting point of TDF was determined using capillary technique using mineral oil and differential scanning calorimetry.

Table 7.4 : Melting Point of TDF

Method	Melting Point (°C)
Capillary technique	115-118°C
Differential scanning calorimetry	115.67°C (peak)

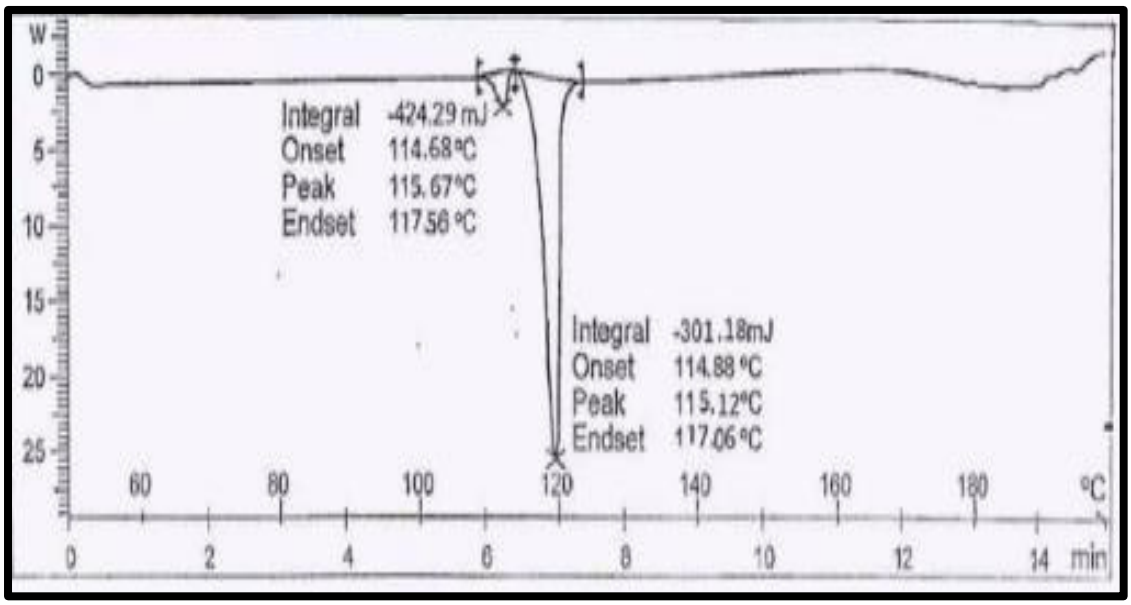

Fig. 7.1 : DSC Thermogram of TDF

Inference: The melting point is found to be complying with the acceptance criteria as per reference

7.1.5 Loss on Drying

The percent loss on drying was estimated using hot air oven till constant weight was obtained.

Inference: The % loss on drying for TDF sample was found to be 1.23 ± 0.004%.

The % LOD is found to be complying with the acceptance criteria as per Indian Pharmacopoeia i.e. NMT 3.5%.

6.2 UV-spectrophotometric Method for Measurement of TDF

Estimation of the Absorption Maxima (λmax)

The 5µg/ml solution of TDF was scanned for estimating the absorption maxima (λmax) between 200 to 400 nm. The absorption maxima were observed at 260 nm as shown below

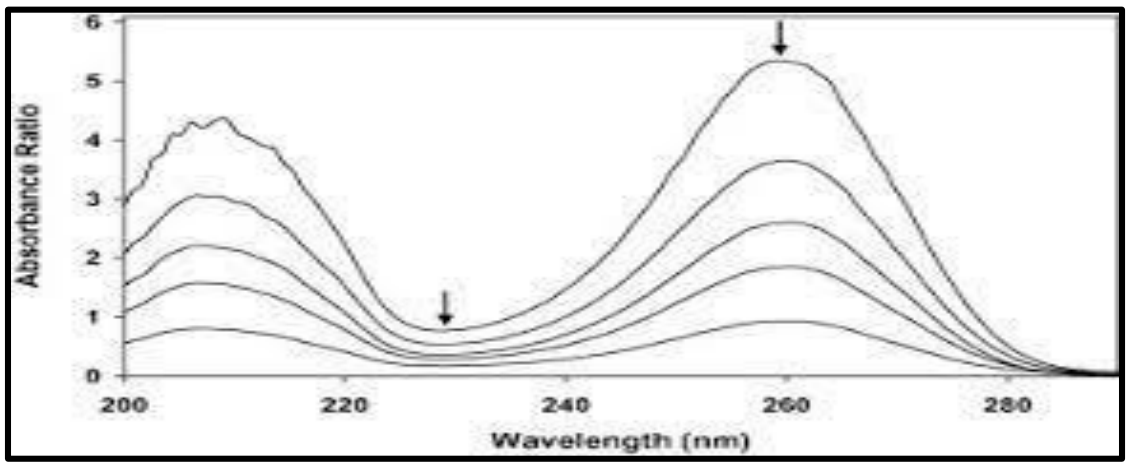

Fig. 7.2 : UV Spectrum of TDF Showing Absorption Maxima

Standard Graphs of TDF in 0.1 N Hydrochloric Acid

The working solutions of 5, 10, 15, 20, 25, 30, and 35 µg/ml concentration of TDF were prepared in 0.1N hydrochloric acid. They were scanned at 260 nm using UV-Visible spectrophotometer using 0.1N hydrochloric acid as blank. The absorbances of working solutions were recorded and tabulated against the concentration (µg/ml). A standard graph was plotted between concentration of TDF in µg/ml (x-axis) against absorbance (y-axis).

Table 7.5 : Standard Graphs of TDF in 0.1 N Hydrochloric Acid

Sr. No.	Concentration of TDF (µg/ml)	Absorbance
1.	5	0.111
2.	10	0.225
3.	15	0.343
4.	20	0.456
5.	25	0.561
6.	30	0.678
7.	35	0.796

The calibration curve of TDF in 0.1 N HCl is shown below. The straight line was obtained after plotting the curve between the concentration of TDF in µg/ml (x-axis) against absorbance (y-axis). The equation of the straight line and regression coefficient (r^2) was found to be y = 0.0227x - 0.0013 and 0.9998 respectively. The value of the regression coefficient was closer to 1 indicating the best-fitted line.

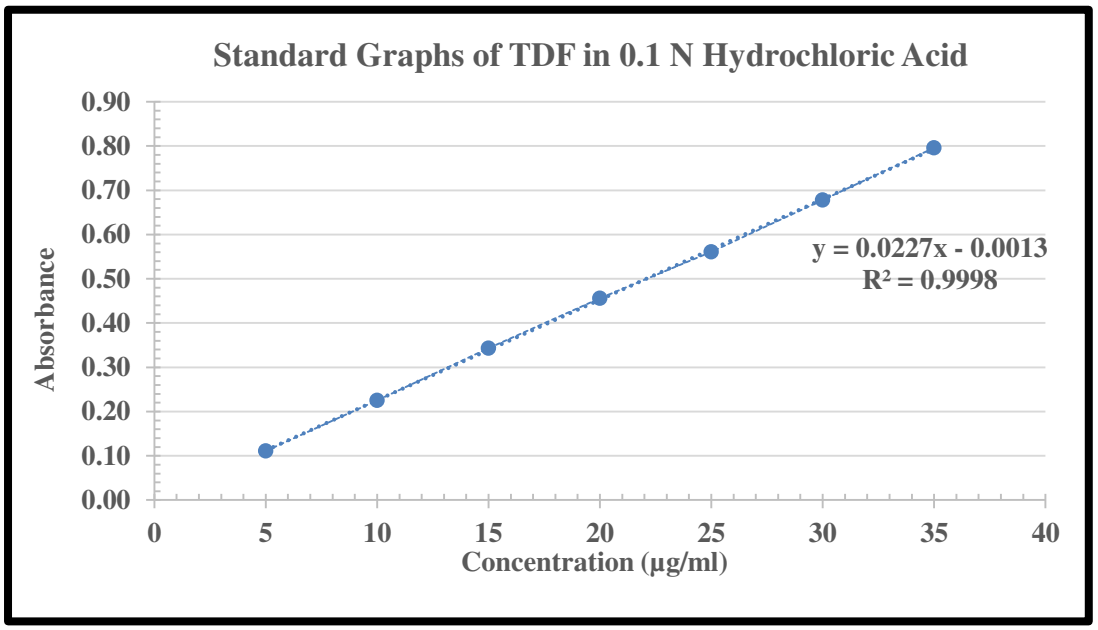

Fig. 7.3 : Standard Graph of TDF in 0.1N HCl

Standard Graphs of TDF in Distilled Water

The working solutions of 5, 10, 15, 20, 25, 30, and 35 µg/ml of TDF were prepared in distilled water. They were scanned at 260 nm using UV-Visible spectrophotometer

using distilled water as blank. The absorbances of working solutions were recorded and tabulated against the concentration (µg/ml). A standard graph was plotted between concentration of TDF in µg/ml (x-axis) against absorbance (y-axis).

Table 7.6 : Standard Graphs of TDF in Distilled Water

Sr. No.	Concentration of TDF (µg/ml)	Absorbance
1.	5	0.121
2.	10	0.231
3.	15	0.349
4.	20	0.463
5.	25	0.576
6.	30	0.687
7.	35	0.807

The calibration curve of TDF in distilled water is shown below. The straight line was obtained after plotting the curve between the concentration of TDF in µg/ml (x-axis) against absorbance (y-axis). The equation of straight line and regression coefficient (r^2) were found to be y = 0.0228x + 0.0053 and 0.9999 respectively. The value of regression coefficient (r^2) was closer to 1 indicating the best-fitted line.

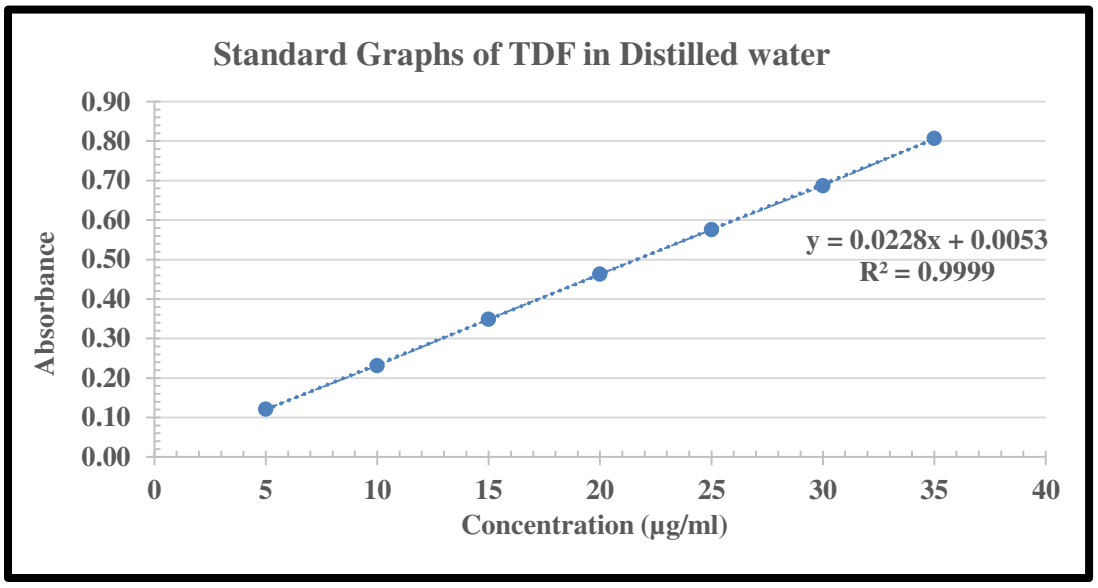

Fig. 7.4 : Standard Graph of TDF in Distilled Water

Standard Graphs of TDF in Phosphate Buffer pH 6.8

The working solutions of 5, 10, 15, 20, 25, 30, and 35 µg/ml of TDF were prepared in phosphate buffer pH 6.8 were scanned at 260 nm using UV-Visible spectrophotometer using distilled water as blank. The absorbances of working solutions were tabulated against the concentration (µg/ml). A standard graph was plotted between concentration of TDF in µg/ml (x-axis) against absorbance (y-axis).

Table 7.7: Standard Graphs of TDF in Phosphate Buffer pH 6.8

Sr. No.	Concentration of TDF (µg/ml)	Absorbance
1.	5	0.119
2.	10	0.236
3.	15	0.354
4.	20	0.472
5.	25	0.589
6.	30	0.698
7.	35	0.814

The calibration curve of TDF in phosphate buffer pH 6.8 is shown below. The straight line was obtained after plotting the curve between the concentration of TDF in µg/ml (x-axis) against absorbance (y-axis). The equation of straight line and regression coefficient (r^2) were found to be y = 0.0232x + 0.0054 and 0.9999 respectively. The value of regression coefficient (r^2) was closer to 1 indicating the best fitted line.

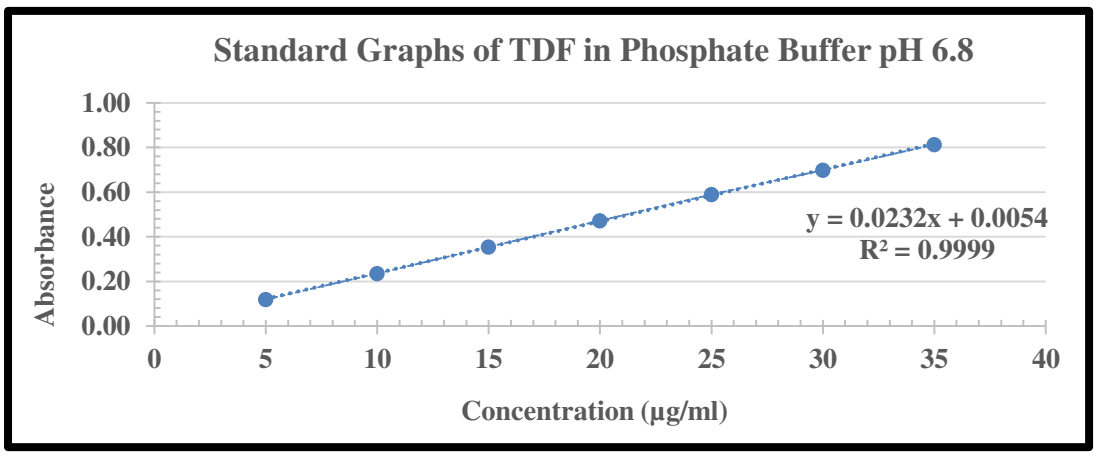

Fig. 7.5 : Standard Graph of TDF in Phosphate Buffer in pH 6.8

The above standard graphs of TDF were used for the estimation of drug content, % drug release, solubility, etc. in the further part of the research.

7.3 Excipient Compatibility Studies

The samples were evaluated for physical characteristics (physical appearance) and results of the compatibility study are reported in the following tables.

Table 7.8 : Details of Excipient Compatibility Studies

Binary mixture	Observation at Initial (0 days)	Observation after 7 days
Tenofovir Disoproxil Fumarate (API)	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Microcrystalline cellulose (Avicel PH101)	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Spray dried Lactose (DCL-11)	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Mannitol	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Polyvinyl Pyrrolidone (K-30)	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Hydroxypropyl cellulose (HPC-L)	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Magnesium stearate	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Talc	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Gelucire	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate + Precirol	Off white powder	No discoloration.
Tenofovir Disoproxil Fumarate +	Off-white wet mass	No discoloration.

Binary mixture	Observation at Initial (0 days)	Observation after 7 days
Cross Povidone		
Tenofovir Disoproxil Fumarate + Silicified Microcrystalline Cellulose (PROSOLV)	Off-white wet mass	No discoloration.
Tenofovir Disoproxil Fumarate + Alpha-tocopherol	Off white powder	No discoloration.
Mixture of Tenofovir Disoproxil Fumarate +All above excipients	Off white powder	No discoloration.

Inference: There was no change in the physical observations of the binary mixture when exposed at 50°C±2°C for 7 days. Further finalized formulation will be evaluated for accelerated stability study using all these excipients. Hence, it can be concluded that the above excipients are compatible with the drug substance.

7.4 Preparation of Drug Pellets

7.4.1 Selection of Filler

Three different fillers Microcrystalline Cellulose, Spray Dried Lactose, and Mannitol were evaluated.

Table 7.9 : Selection of Filler

Study Outcome	Formulation code		
	F1	F5	F9
Good Pellets (Fraction #16/20) quantity (%)	92	95	83
Endpoint observation remark	Good	Good	Fair

Inference:

- The manufacturing process was feasible for use of all three fillers.
- The good pellet fraction was observed more than 90% for pellets formulated using Microcrystalline Cellulose and Spray Dried Lactose. Hence, these two fillers were selected for further optimization.

7.4.2 Selection of Binder

Two binding agents, Polyvinyl Pyrrolidone (K-30) and Hydroxypropyl Cellulose (HPC-L) were evaluated for suitability of pellets formulation.

Table 7.10 : Selection of Binder

Study Outcome	Formulation code			
	F1A	F1B	F5A	F5B
Good Pellets (Fraction #16/20) quantity (%)	93	75	94	62
Endpoint observation remark	Good	Poor	Good	Poor

Inference:

- The manufacturing process was feasible for use of Polyvinyl Pyrrolidone (K-30) as binder. However, consistent extrudes were not able to produced for trials formulated using Hydroxypropyl Cellulose (HPC-L) as binder. Additionally, more fragile pellets were produced. Hence, Polyvinyl Pyrrolidone (K-30) selected as binder.
- The good pellet fraction was observed more than 90% for pellets formulated using Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11). Hence, these two fillers were selected for further optimization.

7.4.3 Optimization of Fluid Uptake

Two different fluid uptake levels were evaluated using a Polyvinyl Pyrrolidone (K-30) (2% w/v). Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 7.11 : Optimization of Fluid Uptake

Study Outcome	Formulation code			
	F1C	F1D	F5C	F5D
Good Pellets (Fraction #16/20) quantity (%)	93	88	94	86
Endpoint observation remark	Good	Fair	Good	Fair

Inference:

- The feasibility of forming extrudes and further spheronization was better with 8% fluid uptake for trials manufactured with fillers, Microcrystalline Cellulose and Spray Dried Lactose. A slightly sticky nature was observed for extrudes formulated with 12% fluid uptake.
- Hence, 8% fluid uptake was finalized for further optimization.

7.4.4 Optimization of Screen Size for Preparation of Extrudes.

Two different sieve sizes were selected for optimization. The type of binder and Fluid uptake levels were kept constant. Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 7.12 : Optimization of Screen Size for Preparation of Extrudes

Study Outcome	Formulation Code			
	F1E	F1F	F5E	F5F
Good Pellets (Fraction #16/20) quantity (%)	95	85	92	81
Endpoint observation remark	Good	Fair	Good	Fair

Inference:

- The good pellets fraction was observed higher for screen size #16 at extrusion stage, Hence, the use of #16 sieve for extrusion was selected.

7.4.5 Optimization of Spheronization Process

Three different spheronizer cross-hatch plates of 1.0, 1.2, and 1.5 mm sizes were selected for optimization. The type of binder, Fluid uptake levels and extrusion screen were kept constant. Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11)

Table 7.13 : Optimization of Spheronization Process

Study Outcome	Formulation Code					
	F1G	**F1H**	**F1I**	**F5E**	**F5F**	**F5I**
Good Pellets (Fraction #16/20) quantity (%)	84	92	71	82	93	77
Endpoint observation remark	Fair	Good	Fair	Fair	Good	Fair

Inference:

- There was observation of higher fines (#20 pass) using Spheronizer cross-hatch plates 1.0 mm. On the other hand, coarser pellets were produced using 1.5 mm plate, this lead to higher oversize on #16 mesh.
- Hence, for optimum size of pellets in the fraction of #16 passed and #20 retained, Spheronizer cross-hatch plates 1.2 mm selected.

7.4.6 Optimization of Drying Process for Pellets

Two different drying time were selected for optimization. The type of binder, Fluid uptake levels, extrusion screen and cross-hatch plate size were kept constant. Optimization was done using formulations of Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11).

Table 7.14: Optimization of Drying Process for Pellets

Study Outcome	Formulation Code			
	F1J	F1K	F5J	F5K
Good Pellets (Fraction #16/20) quantity (%)	93	91	94	90
Endpoint observation remark	Good	Good	Good	Good

Inference:

- The fraction of good pellets was comparable (>90%) irrespective of drying time (1 h and 3 h).
- The LOD of pellets was checked. The LOD of the pellets at drying times of 1 hour and 3 hour were checked.

- The LOD of pellets dried for 1 hour was 2.10%-2.57% and for 3 hour was 1.82%-1.94%. This indicates a uniform drying of pellets is achieved in 3 hours.
- On the basis of observed data, 3 hour drying time was considered.

7.4.7 Hot Melt Coating of Pellets:

7.4.7.1 Optimization Hot Melt Coating Agent and Level of coating

The TDF pellets based on Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL-11) were utilized for coating optimization. Two hot melt coating agents, Gelucire® 43/01 and Precirol® ATO 5 were used for coating evaluation, along with α- Tocopherol as an antioxidant for the coating agent. The coating levels of 3, 4 and 5% were evaluated.

Table 7.15: Optimization Hot Melt Coating Agent and Level of Coating

Test	Formulation Code											
	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21
Appearance	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good
Mean particle size	850	855	867	838	857	874	843	862	873	847	859	891
Bulk Density (g/mL)	0.714 ± 0.002	0.723 ± 0.001	0.753 ± 0.001	0.749 ± 0.003	0.731 ± 0.003	0.761 ± 0.003	0.757 ± 0.002	0.764 ± 0.001	0.710 ± 0.001	0.733 ± 0.001	0.746 ± 0.001	0.769 ± 0.001
Tapped Density (g/mL)	0.787 ± 0.002	0.789 ± 0.001	0.807 ± 0.003	0.798 ± 0.002	0.813 ± 0.004	0.816 ± 0.003	0.788 ± 0.002	0.790 ± 0.002	0.801 ± 0.002	0.783 ± 0.002	0.799 ± 0.002	0.805 ± 0.002
Carr's Index	9.27	8.36	4.80	5.54	8.05	4.51	5.25	3.29	11.58	8.94	6.63	4.94
Hardness	2.85 ±0.05	2.97 ± 0.10	3.35 ± 0.05	2.98 ± 0.10	3.02 ± 0.15	3.41 ± 0.15	3.05 ± 0.10	3.11 ± 0.10	3.43 ± 0.10	3.00 ± 0.12	3.08 ± 0.10	3.51 ± 0.05
Friability	0.248 ± 0.01	0.232 ± 0.002	0.168 ± 0.001	0.215 ± 0.003	0.228 ± 0.004	0.235 ± 0.003	0.358 ± 0.005	0.228 ± 0.004	0.175 ± 0.011	0.215 ± 0.003	0.228 ± 0.004	0.235 ± 0.003
Drug content	99.86 ± 1.26	100.26 ± 2.06	99.54 ± 0.84	101.35 ± 1.98	99.02 ± 3.13	98.91 ± 0.57	100.65 ± 2.53	98.68 ± 0.21	99.34 ± 3.18	98.71 ± 0.77	101.65 ± 2.76	99.98 ± 2.29
Dissolution (%) (N=6)												
5 mins	72±7.67	66± 6.25	50± 0.12	70± 4.16	69± 6.93	59± 2.65	69 ± 5.21	64± 7.64	54± 4.15	68±7.91	68 ± 8.54	62± 4.15
10 mins	89±4.21	85 ± 3.67	79± 5.15	90± 3.12	86 ± 4.98	77± 6.84	93 ± 2.82	89 ± 5.18	85± 8.73	89±5.12	86 ±7.40	80± 8.13
15mins	96 ±2.76	93 ± 2.76	87 ± 4.72	97 ± 2.97	95 ± 3.33	84 ± 4.26	98 ± 2.01	98 ± 4.11	91 ± 5.54	99 ± 3.56	93 ±5.91	91± 7.91
30 mins	101±0.26	99 ± 0.96	98 ± 1.04	102±0.86	100± 1.16	92±3.04	102±1.20	102± 0.88	97± 4.37	101±2.05	100±4.88	98± 4.59

Inference:

- Gelucire® 43/01 and Precirol® ATO 5 with different concentration by using hot melt coating process, all the trials found feasible.
- The evaluation outcomes such as mean particle size, bulk density, tapped density, hardness, friability and drug content for all the trials were found comparable. However, the dissolution profile exhibit differences due to coating weight build-up.
- The drug release is found directly proportional to weight build.
- Dissolution of pellets having weight gain 3 and 4% gives better dissolution results than 5% weight gain. Hence 3 and 4% weight gain was selected for further evaluation.

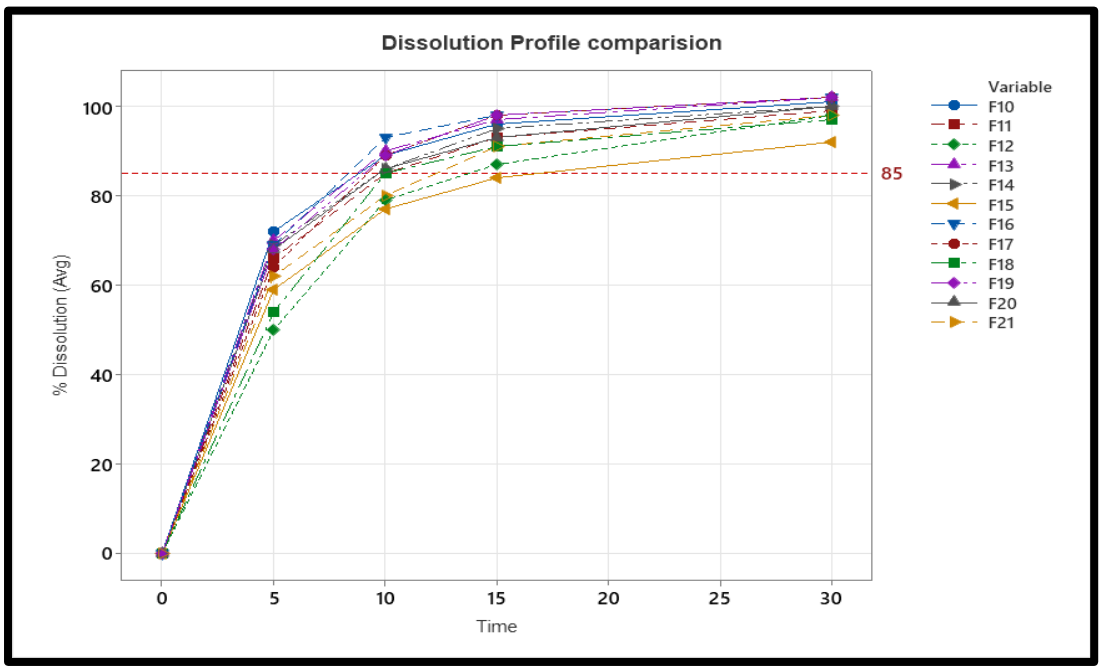

Fig. 7.6 : Dissolution Profile of Hot Melt Coated Pellets

7.4.7.2 Taste Evaluation

The taste evaluation was performed by implementing two methods,

- In-vitro Taste method
- Taste Panel Method.

The taste method evaluation was conducted on pellets coated with 3 and 4%. The uncoated pellets were used as positive control. The outcomes were tabulated as below-

The In-vitro Taste evaluation UV spectrometer was used to indicate the time after which the pellets exhibit UV absorbance at 250 nm. The UV absorbance indicates bitterness. A delay in absorbance above 2 minutes is considered effective taste masking as the time taken to swallow is generally less than 2 minutes.

Table 7.16: Taste Evaluation of Pellets

Test	Formulation Code									
	F1	**F5**	**F10**	**F11**	**F13**	**F14**	**F16**	**F17**	**F19**	**F20**
UV absorbance time (mins)	0'30''	0'30''	1'00''	2'30''	1'30''	3'00''	1'00''	2'30''	1'00''	2'30''
Taste Panel Method										
V1	+++++	+++++	+	-	-	-	++	-	-	-
V2	+++++	++++	++	-	-	-	+	-	+	-
V3	+++++	+++++	+	-	+	-	-	-	++	-
V4	++++	+++++	-	-	-	-	+	-	+	-
V5	+++++	+++++	+	-	-	-	-	-	-	-
V6	+++++	++++	+	-	-	-	+	-	-	-
V7	+++++	+++++	-	-	-	-	-	-	-	-
V8	+++++	+++++	+	-	+	-	-	-	+	-
V9	++++	+++++	+	-	-	-	-	-	-	-
V10	+++++	+++++	+	-	+	-	+	-	+	-
V11	+++++	++++	-	-	-	-	-	-	-	-
V12	+++++	+++++	++	-	-	-	+	-	+	-

Where, +++++ = very-very bitter, ++++ = very bitter, +++ = moderately bitter, ++ = bitter, + = slightly bitter, – = tasteless and V= Volunteer

Inference:

- The Taste panel of 12 volunteers indicated that the pellets coated with 3% coating lowered the bitterness potential of the pellets, however, complete masking was achieved with 4% coating level.
- Hence, pellets coated with 4% weight gain was taken for tableting.

7.4.7.3 Optimization of Coating Process Parameters: Pan Speed

The TDF pellets based on Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL-11) were utilized for coating optimization. Two Hot Melt Coating agents, Gelucire® 43/01 and Precirol® ATO 5 were used for coating evaluation, along with α- Tocopherol. The experiments were conducted with two different pan speeds keeping coating weight build-up constant.

Table 7.17: Optimization of Coating Process Parameters: Pan Speed

Study Outcome	Formulation Code							
	F22	F23	F24	F25	F26	F27	F28	F29
Mean particle size	849	810	855	826	858	801	867	819
Hardness	2.92 ±0.05	2.55 ± 0.10	3.18 ± 0.05	2.69 ± 0.10	3.16± 0.06	2.49 ± 0.11	3.09 ± 0.05	2.72 ± 0.13
Appearance	Good	Fines	Good	Fines	Good	Fines	Good	Fines

Inference:

- The hot melt coating process was found to be feasible for pan 15 rpm, as for 20 rpm fines were visually observed in coating pan.
- The coating was observed uniform on visual monitoring for 15 rpm, same was confirmed with surface morphology.
- The mean particle size and hardness data for 20 rpm was comparatively lower indicates the generation fines due to attrition in coating pan.

Surface morphology of pellets:

- The hot melt coated pellets on 15 rpm were spherical in shape and uniform in size.

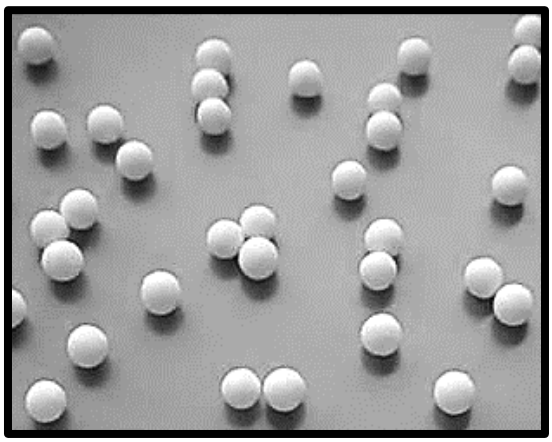

Fig. 7.7 : Surface Morphology of Hot Melt Coated Pellets

7.4.7.4 Optimization of Coating Process Parameters: Temperature

The TDF pellets based on Microcrystalline Cellulose (Avicel PH 101) and Spray-dried Lactose (DCL-11) were utilized for coating optimization. Two hot melt coating agents, Gelucire® 43/01 and Precirol® ATO 5 were used for coating evaluation, along with α- Tocopherol. The experiments were conducted with two different bed temperatures keeping pan rpm and coating weight build up constant.

Table 7.18: Optimization of Coating Process Parameters: Temperature

Study Outcome	Formulation Code							
	F30	F31	F32	F33	F34	F35	F36	F37
Appearance	Agglomeration	Good	Agglomeration	Good	Agglomeration	Good	Agglomeration	Good

Inference:

- The hot melt coating process was found to be feasible for 60º C bed temperature, as 40º C agglomeration of pellets was observed in the coating pan. The agglomeration is due to rapid solidification of the coating agent on lower temperature.

7.4.7.5 Stability Evaluation

- Stability charged experimental Batches: F31, F33, F35 and F37

- Pack: Equivalent to unit dose were filled in the self-sealing Aluminium pouch.
- Stability Condition: At temperature 25±2°C & 60±5% RH and 40 ± 2°C & 75 ± 5% RH for 6 months in the stability chamber (Remi Laboratory Instrument, CHM-6).
- Frequency:
    - For 40 ± 2°C & 75 ± 5% RH: Initial, 1month, 2 Month, 3 month and 6 months
    - For 25±2°C & 60±5% RH : Initial, 1month, 2 Month, 3 month , 6 months.
- Testing: Physical appearance, drug content and in-vitro drug release.

Table 7.19: Accelerated Stability Data (Pellets)

40 ± 2°C & 75 ± 5% RH				
Formulation code	**Station**	**Appearance (White to off-white pellets)**	**Drug Content (%) (90.0 -110.0)**	**Drug Release (%) (NLT 80%(Q) in 30 mins)**
F31	Initial	Complies	100.3	98
	1 M	Complies	99.0	95
	2M	Complies	98.3	97
	3M	Complies	101.0	95
	6M	Complies	100.0	93
F33	Initial	Complies	98.0	94
	1 M	Complies	102.0	96
	2M	Complies	99.8	97
	3M	Complies	97.9	91
	6M	Complies	99.5	98
F35	Initial	Complies	101.1	100
	1 M	Complies	99.0	98
	2M	Complies	98.7	95
	3M	Complies	100.0	101
	6M	Complies	96.9	94
F37	Initial	Complies	97.8	99

40 ± 2°C & 75 ± 5% RH				
Formulation code	**Station**	**Appearance (White to off-white pellets)**	**Drug Content (%) (90.0 -110.0)**	**Drug Release (%) (NLT 80%(Q) in 30 mins)**
	1 M	Complies	102.4	102
	2M	Complies	101.0	101
	3M	Complies	100.2	99
	6M	Complies	99.8	99

Table 7.20: Long Term Stability Data (Pellets)

25±2°C & 60±5% RH				
Formulation Code	**Station**	**Appearance (White to off-white pellets)**	**Drug Content (%) (90.0 -110.0)**	**Drug Release (%) (NLT 80%(Q) in 30 mins)**
F31	Initial	Complies	99.8	99
	1 M	Complies	98.6	100
	2M	Complies	100.2	98
	3M	Complies	98.7	97
	6M	Complies	99.2	99
F33	Initial	Complies	100.6	98
	1 M	Complies	98.8	98
	2M	Complies	97.9	97
	3M	Complies	98.2	100
	6M	Complies	98.0	101
F35	Initial	Complies	102.1	99
	1 M	Complies	99.5	98
	2M	Complies	100.4	97
	3M	Complies	99.7	100
	6M	Complies	98.3	99
F37	Initial	Complies	100.5	101

25±2°C & 60±5% RH				
Formulation Code	**Station**	**Appearance (White to off-white pellets)**	**Drug Content (%) (90.0 -110.0)**	**Drug Release (%) (NLT 80%(Q) in 30 mins)**
	1 M	Complies	99.4	99
	2M	Complies	98.7	98
	3M	Complies	96.9	102
	6M	Complies	99.5	98

Inference:

- The accelerated stability data up to six months indicates that Hot Melt Coated pellets in designated packs were stable, without any borderline compliance. This gives assurance of product quality.
- The long-term stability data was also found consistent.
- Based on overall stability data, it can be assured to provide the quality product with new technology of taste masking i.e. hot melt coating.

7.5 Compression of Tablets

The TDF pellets coated at 4% weight build-up were blended with extragranular material and compressed into tablets. The evaluation was performed for pellets manufactured using Microcrystalline Cellulose (Avicel PH 101) and Spray Dried Lactose (DCL-11) as a filler. Both types of pellets were compressed at low, medium and High hardness. The study was repeated for both the coating agents. Below is the tabulated compilation of experiment outcomes,

Table 7.21: Hot Melt Coated Pellets Using Gelucire® 43/01

Study Outcome	**Formulation Code**							
	F38	**F39**	**F40**	**F41**	**F42**	**F43**	**F44***	**F45***
Hardness	L	M	H	L	M	H	M	M
Weight Variation (%) (n=10)	-0.9% to 2.1%	-1.8% to 0.5%	-2.0% to 1.6%	-1.9% to 1.3%	-1.0% to 2.3%	-1.6% to 1.9%	-2.1% to 1.7%	-1.1% to 1.4%

Limit: 413 mg ±5%								
Hardness (n=10) (kg/cm2)	6.1 ± 0.2	9.2 ± 0.1	11.8 ± 0.3	5.5 ± 0.3	8.8 ± 0.2	12.1 ± 0.4	9.0 ± 0.3	9.2 ± 0.2
Thickness (mm) (n=10)	3.41 ± 0.22	3.34 ± 0.19	3.26 ± 0.28	3.44 ± 0.30	3.37 ± 0.24	3.25 ± 0.14	3.24 ± 0.20	3.20 ± 0.16
Friability (%) (6.5 g, Triplicate)	0.29 ± 0.12	0.18 ± 0.15	0.11 ± 0.12	0.34 ± 0.27	0.26 ± 0.60	0.14 ± 0.18	0.36 ± 0.46	0.43 ± 0.36
Disintegration time (mins) (n=6)	Max. 4	Max. 6	Max. 7	Max. 5	Max. 7	Max. 8	Max. 6	Max. 5
Drug content (%)	99.2± 1.2	97.7± 1.4	101.0± 0.9	101.3 ± 0.2	100.2± 0.6	98.9± 0.6	102.1 ± 0.2	101.6± 0.7
Dissolution (NLT 80Q% in 30 mins)	96± 2	95± 1	92± 4	95± 2	94± 4	93± 2	97± 1	96± 3
Taste Evaluation – UV absorbance time (mins)	>10	>10	>10	>10	>10	>10	2	2

*Compressed tablets weight 400 mg, being uncoated pellets.

L: Low Hardness, M: Medium Hardness, H: High Hardness

Table 7.22: Hot Melt Coated Pellets Using Precirol® ATO 5

Study Outcome	Formulation Code							
	F46	**F47**	**F48**	**F49**	**F50**	**F51**	**F52***	**F53***
Hardness	L	M	H	L	M	H	M	M
Weight Variation (%) (n=10) Limit: 413	-2.1% to 2.3%	-1.9% to 1.6%	-2.0% to 1.3%	-2.5% to 2.1%	-2.2% to 1.5%	-0.8% to 2.2%	-1.4% to 0.7%	-1.6% to 1.4%

Study Outcome	Formulation Code							
	F46	**F47**	**F48**	**F49**	**F50**	**F51**	**F52***	**F53***
mg ±5%								
Hardness (n=10) (kg/cm2)	5.9 ± 0.3	8.6 ± 0.1	10.7 ± 0.3	4.9 ± 0.5	9.2 ± 0.1	11.7 ± 0.3	9.7 ± 0.2	9.6 ± 0.3
Thickness (mm) (n=10)	3.51 ± 0.31	3.43 ± 0.34	3.55 ± 0.17	3.49 ± 0.22	3.34 ± 0.23	3.21 ± 0.28	3.37 ± 0.33	3.32 ± 0.21
Friability (%) (6.5 g, Triplicate)	0.39 ± 0.17	0.14 ± 0.25	0.12 ± 0.14	0.37 ± 0.22	0.29 ± 0.51	0.20 ± 0.28	0.34 ± 0.16	0.37 ± 0.13
Disintegration time (mins) (n=6)	Max. 5	Max. 7	Max. 8	Max. 4	Max. 6	Max. 8	Max. 7	Max. 5
Drug content (%)	101.3 ± 0.9	99.2± 0.8	100.1±1.3	99.3± 0.7	99.4± 1.6	97.9± 0.5	101.3 ± 0.4	100.6± 1.1
Dissolution (NLT 80Q% in 30 mins)	97± 2	98± 1	96± 4	99± 2	96± 4	93± 2	96± 1	94± 3
Taste Evaluation – UV absorbance time (mins)	>10	>10	>10	>10	>10	>10	2	2

*Compressed tablets weight 400 mg, being uncoated pellets.

L: Low Hardness, M: Medium Hardness, H: High Hardness

Inference:

- The compression process for hot melt coated pellets found to be feasible.
- Tablets compressed at different hardness are found to have acceptable friability, disintegration time, and dissolution.
- The tablets manufactured with coated pellets of both the coating materials exhibit taste-masking ability at all three hardness levels.

7.6 In-vitro Dissolution Study

The in-vitro drug release study from tablets prepared with coated pellets and marketed tablets was performed. Drug release is found to be complete within 30 min which complies with the Pharmacopoeial standards. The drug release from marketed tablets and tablets prepared from hot melt coated pellets was found to similar, which was confirmed from similarity factor (f_2). The values of similarity factor were found to be >50 using marketed sample (Viread 300) as reference sample. It indicates similar drug release profile.

Table 7.23: Similarity Factor (f2) Data

Reference batch (Viread 300)	**Test Batch**			
Reference	Test_F39	Test_F42	Test_F47	Test_F50
(f2)	70	74	66	80

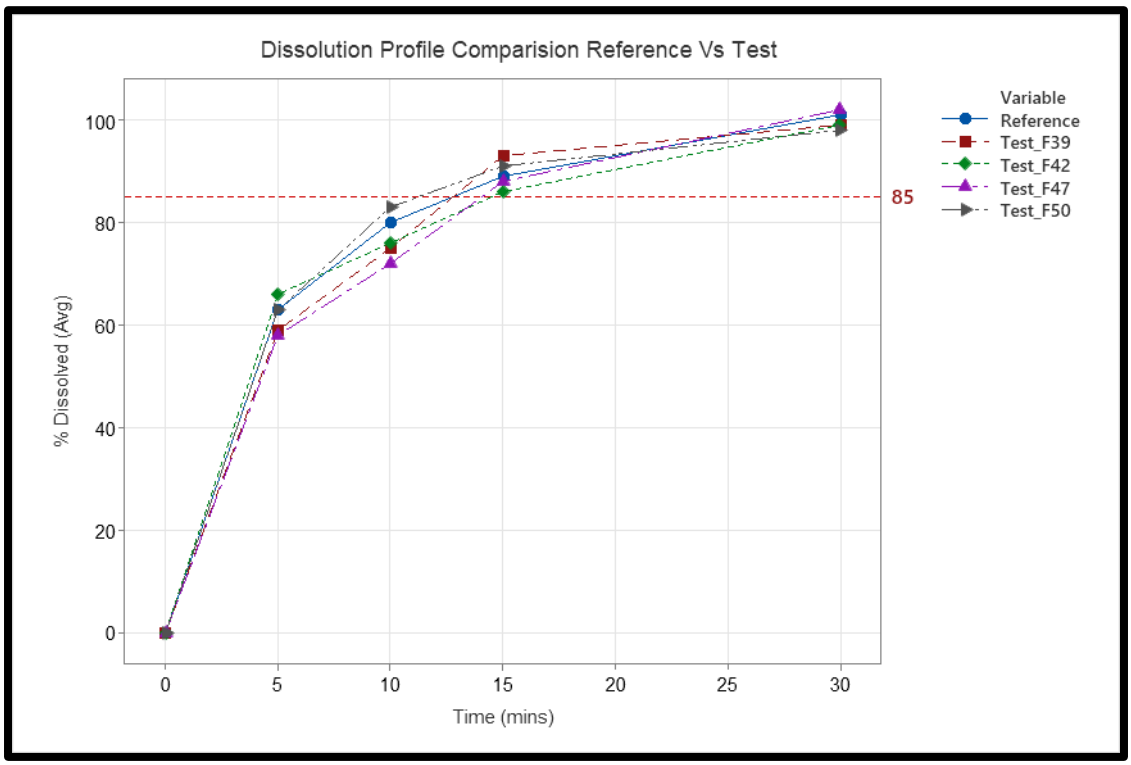

Fig. 7.8 : Comparison of Dissolution Profile of Reference (Viread) and Developed Formulations (Test)

Inference:

- Dissolution profile of reference batch against test batches found comparable.
- The f2 value > 50 shows that dissolution profile of both reference batch and test batches are similar.

7.7 Taste Evaluation

The taste evaluation was performed by implementing Taste Panel Method on volunteers.

The taste method evaluation was conducted on tablets with optimized HMC coated pellets. The tablets manufactured using un-coated pellets were used as positive control. The outcomes were tabulated as below-

Table 7.24: Taste Evaluation by Taste Panel Method

Volunteers Code	Formulation Code						
	F52	F53	Viread 300	F39	F42	F47	F50
V1	++++	++++	-	-	-	-	-
V2	++++	++++	-	-	-	-	-
V3	+++++	+++	-	-	-	-	-
V4	++++	++++	-	-	-	-	-
V5	++++	++++	-	-	-	-	-
V6	+++++	++++	-	-	-	-	-
V7	+++	++++	-	-	-	-	-
V8	++++	++++	-	-	-	-	-
V9	++++	++++	-	-	-	-	-
V10	+++++	+++++	-	-	-	-	-
V11	+++++	++++	-	-	-	-	-
V12	+++++	+++++	-	-	-	-	-

Where, +++++ = very-very bitter, ++++ = very bitter, +++ = moderately bitter, ++ = bitter, + = slightly bitter, – = tasteless and V= Volunteer

Inference:

- The Taste panel of 12 volunteers indicated that the tablets prepared with coated pellets are capable of bitter taste masking similar to the marketed coated tablet product.

7.8 Stability Evaluation

- Stability charged experimental Batches: F39, F42, F47 and F50
- Pack: Polycarbonate bottles sealed with Aluminum foil.
- Stability Condition: At temperature 25±2°C & 60±5% RH and 40 ± 2°C & 75 ± 5% RH for 6 months in the stability chamber (Remi Laboratory Instrument, CHM-6).
- Frequency:

- For 40 ± 2°C & 75 ± 5% RH: Initial, 1month, 2 Month, 3 month and 6 months
- For 25±2°C & 60±5% RH : Initial, 1month, 2 Month, 3 month , 6 months.

- Testing: Physical appearance, drug content and in-vitro drug release.

Table 7.25: Accelerated Stability Data

40 ± 2°C & 75 ± 5% RH				
Formulation Code	**Station**	**Appearance (White to off white tablets)**	**Drug Content (%) (90.0 -110.0)**	**Drug Release (%) (NLT 80% (Q) in 30 minutes)**
F39	Initial	Complies	101.2	100
	1 M	Complies	91.0	100
	2M	Complies	98.3	98
	3M	Complies	100.0	94
	6M	Complies	99.0	98
F42	Initial	Complies	99.9	93
	1 M	Complies	98.0	99
	2M	Complies	98.8	98
	3M	Complies	96.9	95
	6M	Complies	100.5	98
F47	Initial	Complies	102.1	99
	1 M	Complies	98.0	99
	2M	Complies	98.8	98
	3M	Complies	100.9	100
	6M	Complies	99.4	96
F50	Initial	Complies	98.8	97
	1 M	Complies	101.9	101
	2M	Complies	99.0	99
	3M	Complies	99.2	101
	6M	Complies	101.8	100

Table 7.26: Long-term Stability Data

25±2°C & 60±5% RH				
Formulation Code	**Station**	**Appearance (White to off white tablets)**	**Drug Content (%) (90.0 -110.0)**	**Drug Release (%) (NLT 80% (Q) in 30 minutes)**
F39	Initial	Complies	99.7	100
	1 M	Complies	99.3	99
	2M	Complies	11.2	95
	3M	Complies	99.7	99
	6M	Complies	100.2	99
F42	Initial	Complies	101.6	95
	1 M	Complies	100.8	99
	2M	Complies	99.8	99
	3M	Complies	98.9	99
	6M	Complies	97.9	101
F47	Initial	Complies	100.1	100
	1 M	Complies	100.5	95
	2M	Complies	100.4	99
	3M	Complies	98.9	101
	6M	Complies	98.9	98
F50	Initial	Complies	102.5	99
	1 M	Complies	100.4	100
	2M	Complies	99.6	101
	3M	Complies	99.8	100
	6M	Complies	99.9	99

Inference:

- The final product (tablets) formulated with hot melt coated pellets (agent – Gelucire and Precirol) shown the consistent results on accelerated stability

condition up to six months. This indicates that, the formulated product is capable to withstand up to its shelf life (target 24 months) in the designated pack.

- The long-term stability data up to six moth shows no negative trends.
- Based on overall stability data, it can be assured to provide the quality product with new technology of taste masking i.e., hot melt coating.

Part B: Sitagliptin Phosphate Monohydrate

7.9 Preformulating Studies

7.9.1 Organoleptic Properties

The organoleptic properties of SPM like color, odor and taste were observed. The shape of the crystal was observed under microscope. The observations were tabulated in below table.

Table 7.27 : Organoleptic Properties of SPM

Sr. No.	Parameter	Observation
1.	Description	Crystalline, off-white in powder
2.	Bulk density	0.461 g/ml
3.	Tapped density	0.579 g/ml
4.	Carr's index	20.4 (Fair)

Inference: The organoleptic properties of SPM were compared with the Pharmacopoeial standards and were matches with the standard reference indicates that SPM sample.

7.9.2 Melting point

The melting point is traditionally used as a measure of purity. The change in the melting point value than the standard affect quality and purity of the model drug. The melting behavior was determined using a Mettler-Toledo MP70 melting point system (Greifensee, Switzerland). A capillary was used with a closed bottom, applying a heating rate of 10°C min^{-1} up to a temperature limit of 400°C. The melting point of sitagliptin phosphate monohydrate was found to be 216.48°C which closely matches with Pharmacopoeial standard.

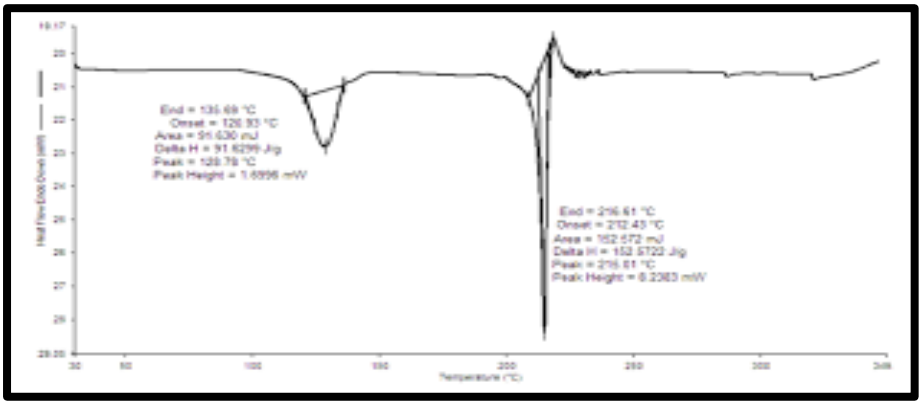

Fig. 7.9: DSC Thermogram of SPM

The sitagliptin phosphate monohydrate differential scanning calorimetry studies indicated a sharp peak at 216.61 °C with an enthalpy change of 131.5 J/ g, corresponding to the melting of pure sitagliptin phosphate monohydrate. So, it was inferred that the given sample of the drug was pure. The DSC thermogram of Sitagliptin phosphate monohydrate showed a characteristic sharp endothermic peak at 135.69 ºC due to water liberation from the drug as it is a monohydrate salt.

7.9.3 Identification by Fourier Transform Infrared (FTIR) Spectroscopy

The infrared spectra were recorded on a Bruker Alpha-P FTIR spectrometer (Ettlingen, Germany) using attenuated total reflection (ATR) in the wavelength range of 4000 to 400 cm^{-1}, with a nominal resolution of 4 cm^{-1} and accumulation of 32 scans.

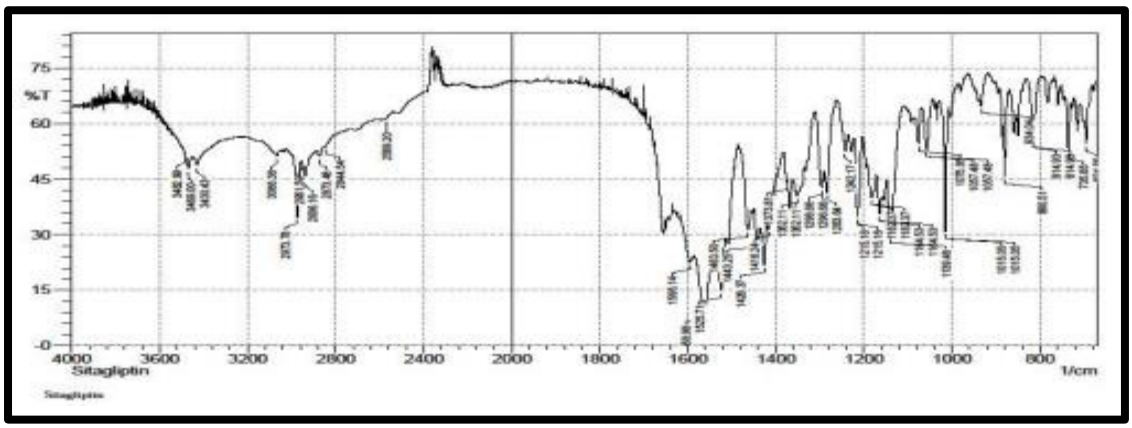

Fig. 7.10: IR spectrum of Sitagliptin Phosphate Monohydrate

Interpretation of FTIR Spectrum: The table below shows the peaks observed at different wave numbers and the functional group associated with these peaks. The major peaks are identical to the functional group of Sitagliptin phosphate monohydrate. Hence, the sample was confirmed as Sitagliptin phosphate monohydrate used for the study purpose.

Table 7.28 : Interpretation of FTIR Spectrum of Sitagliptin Phosphate Monohydrate

Sr. No.	Functional Groups	Wave number (cm^{-1})
1	O-H Stretching	3430
2	N-H stretching	3307
3	C -H stretching (Aromatic)	3049
4	C-O stretching	1631
5	C-N stretching	1517
6	N-H bending	1580
7	C-H bending	742

7.9.4 UV Spectrophotometric Method for Measurement of SPM

7.9.4.1 Estimation of the Absorption Maxima (λmax)

The 10 µg/ml SPM solution was scanned to estimate the absorption maxima (λmax) between 200 to 400 nm. The absorption maxima were observed at 267 nm.

7.9.4.2 Standard Graph of SPM in Distilled Water

The working solutions of 10, 20, 35, 40, and 50 µg/ml concentrations of SPM were prepared in distilled water. They were scanned at 267 nm using a UV-visible spectrophotometer using double distilled water as blank. The absorbances of working solutions were recorded and tabulated against the concentration (µg/ml). A standard graph was plotted between the concentration of SPM in µg/ml (x-axis) against absorbance (y-axis).

Table 7.29: Standard Graph of SPM in Distilled Water

Sr. No.	Concentration of SPM(µg/ml)	Absorbance
1.	10	0.196
2.	20	0.374
3.	35	0.559
4.	40	0.640
5.	50	0.754

The calibration curve of SPM in double distilled water is shown in the figure. The straight line was obtained after plotting the curve between the concentration of SPM in µg/ml (x-axis) against absorbance (y-axis). The equation of straight line and regression coefficient (r^2) were found to be y = 0.0138x + 0.0763 and 0.9944 respectively. The value of regression coefficient was closer to 1 indicating the best fitted line.

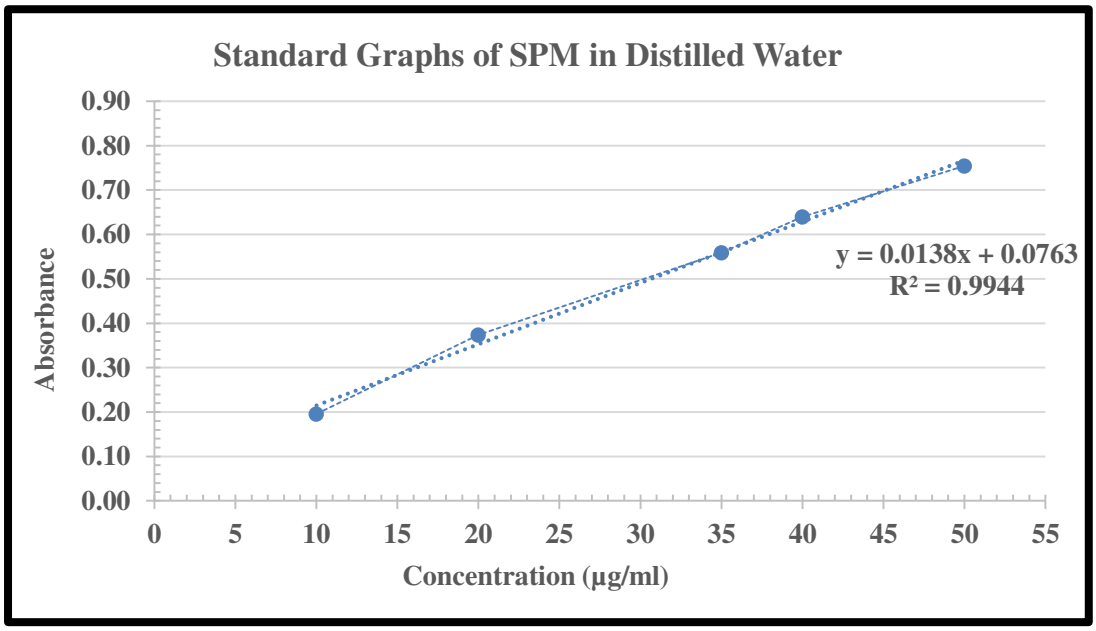

Fig. 7.11: Standard Graph of SPM in Distilled Water

7.9.4.3 Standard Graph of SPM in 0.01 M Hydrochloric Acid

The working solutions of 10, 20, 35, 40 and 50 µg/ml concentration of SPM were prepared in 0.01M hydrochloric acid. They were scanned at 267 nm using UV-Visible spectrophotometer using 0.01M hydrochloric acid as blank. The absorbances of working solutions were recorded and tabulated against the concentration (µg/ml). A

standard graph was plotted between concentration of SPM in µg/ml (x-axis) against absorbance (y-axis).

Table 7.30: Standard Graph of SPM in 0.01 M Hydrochloric Acid

Sr. No.	Concentration of SPM(µg/ml)	Absorbance
1.	10	0.192
2.	20	0.379
3.	35	0.669
4.	40	0.755
5.	50	0.913

The calibration curve of SPM in 0.01 M HCl is shown in the figure. The straight line was obtained after plotting the curve between the concentration of SPM in µg/ml (x-axis) against absorbance (y-axis). The equation of straight line and regression coefficient (r^2) were found to be y = 0.0183x + 0.0158 and 0.9983 respectively. The value of the regression coefficient was closer to 1 indicating the best-fitted line.

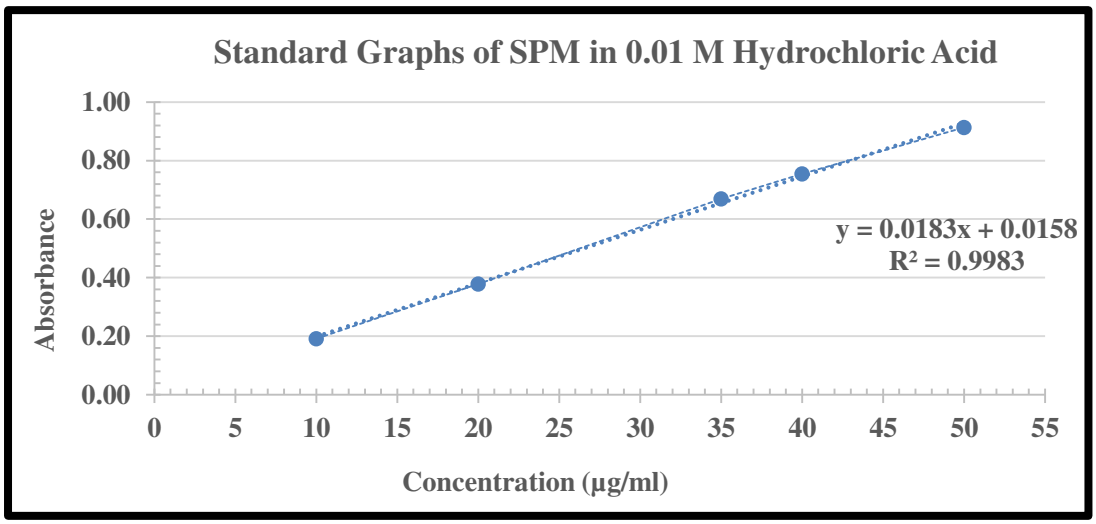

Fig. 7.12 : Standard Graph of SPM in 0.01M HCl

7.9.4.4 Standard Graph of SPM in Phosphate Buffer pH 4.5

The working solutions of 10, 20, 35, 40, and 50 µg/ml concentration of SPM were prepared in phosphate buffer pH 4.5. They were scanned at 267 nm using UV-Visible spectrophotometer using phosphate buffer pH 4.5 as blank. The absorbances of working solutions were recorded and tabulated against the concentration (µg/ml). A

standard graph was plotted between the concentration of SPM in µg/ml (x-axis) against absorbance (y-axis).

Table 7.31: Standard Graph of SPM in Phosphate Buffer pH 4.5

Sr. No.	Concentration of SPM(µg/ml)	Absorbance
1.	10	0.194
2.	20	0.385
3.	35	0.573
4.	40	0.749
5.	50	0.921

The calibration curve of SPM in phosphate buffer pH 4.5 is shown in below figure. The straight line was obtained after plotting the curve between the concentration of SPM in µg/ml (x-axis) against absorbance (y-axis). The equation of straight line and regression coefficient (r^2) were found to be y = 0.0183x + 0.0184 and 0.9988 respectively. The value of the regression coefficient was closer to 1 indicating the best fitted line.

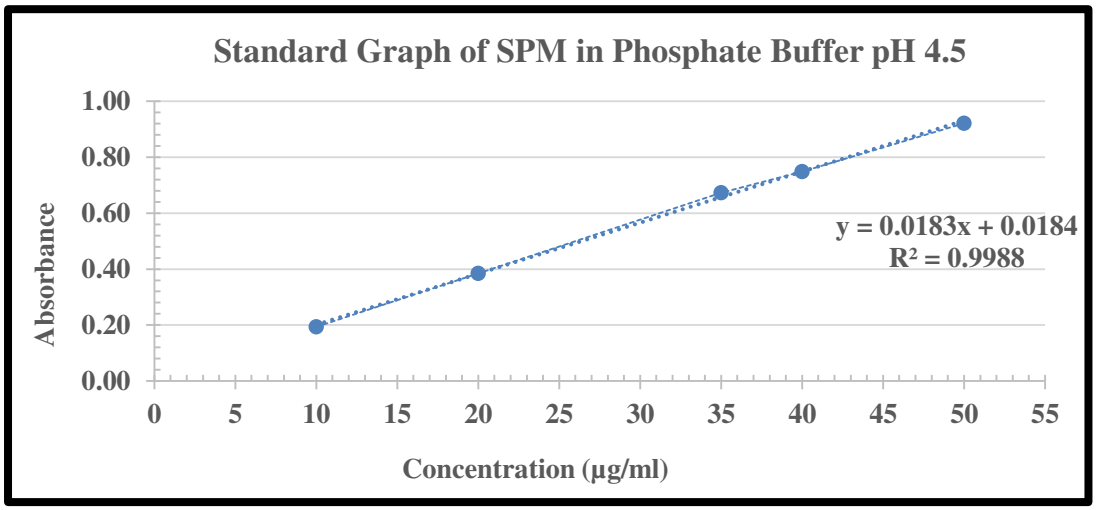

Fig. 7.13 : Standard Graph of SPM in Phosphate Buffer pH 4.5

7.9.4.5 Standard Graph of SPM in PB pH 8.0

The working solutions of 10, 20, 35, 40, and 50 µg/ml concentration of SPM were prepared in phosphate buffer pH 8.0. They were scanned at 267 nm using UV-Visible spectrophotometer using phosphate buffer pH 8.0 as blank. The absorbances of

working solutions were recorded and tabulated against the concentration (µg/ml). A standard graph was plotted between concentration of SPM in µg/ml (x-axis) against absorbance (y-axis).

Table 7.32: Standard Graph of SPM in Phosphate Buffer pH 8.0

Sr. No.	Concentration of SPM(µg/ml)	Absorbance
1.	10	0.191
2.	20	0.382
3.	35	0.620
4.	40	0.699
5.	50	0.899

The calibration curve of SPM in phosphate buffer pH 8.0 is shown in below figure. The straight line was obtained after plotting the curve between the concentration of SPM in µg/ml (x-axis) against absorbance (y-axis). The equation of straight line and regression coefficient (r^2) were found to be y = 0.0173x + 0.0221 and 0.9979 respectively. The value of regression coefficient was closer to 1 indicates best fitted line.

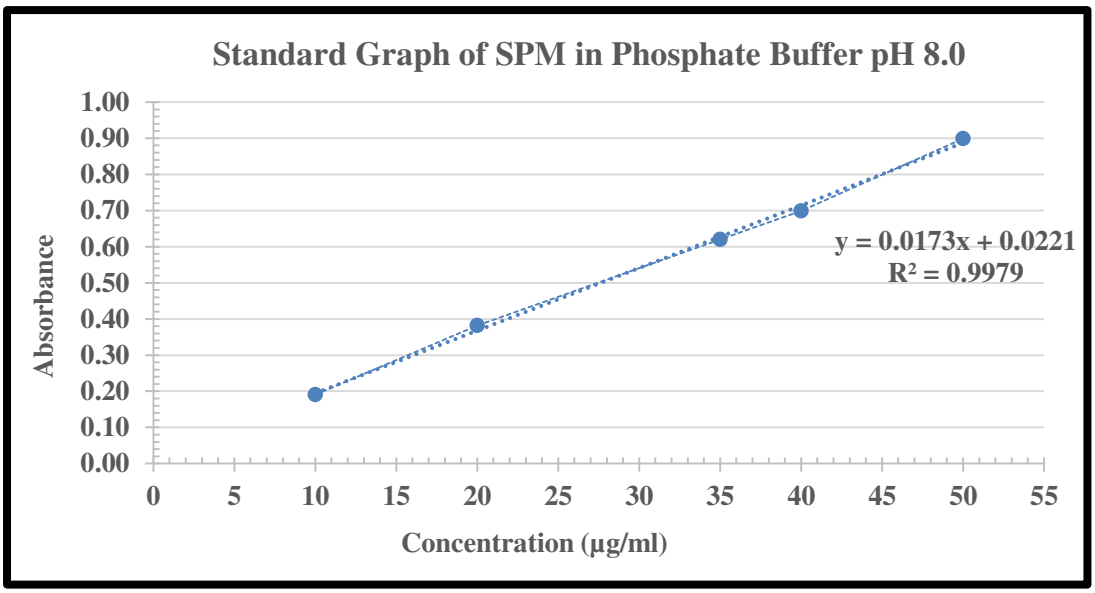

Fig. 7.14 : Standard Graph of SPM in Phosphate Buffer in pH 8.0

7.9.5 Solubility

Solubility is one of the most important parameters in relation to achieving the desired concentration of the drug in systemic circulation in order to obtain the required pharmacological response. Poorly water-soluble drugs with a slow absorption, for instance, may show inadequate bioavailability. The solubility of sitagliptin phosphate monohydrate in various solvent was determined at 25°C.

Table 7.33 : Solubility Study of SPM

Sr. No.	Solvent	Solubility (mg/ml)
1	Distilled water	69.53±0.02
2	Hydrochloric acid (0.01M)	68.19 ±0.01
3	Sodium citrate solution (0.1M)	67.07 ±0.009
4	Sodium carbonate (0.1M)	41.77±0.03
5	Phosphate buffer pH 4.5	49.6 ± 0.01
6	Phosphate buffer pH 8.0	72.631 ±0.006
7	Dimethyl Sulfoxide	90.33 ±0.025
8	Ethanol	0.01 ±0.002
9	Methanol	0.04 ±0.001
10	Acetonitrile	0.003±0.0002

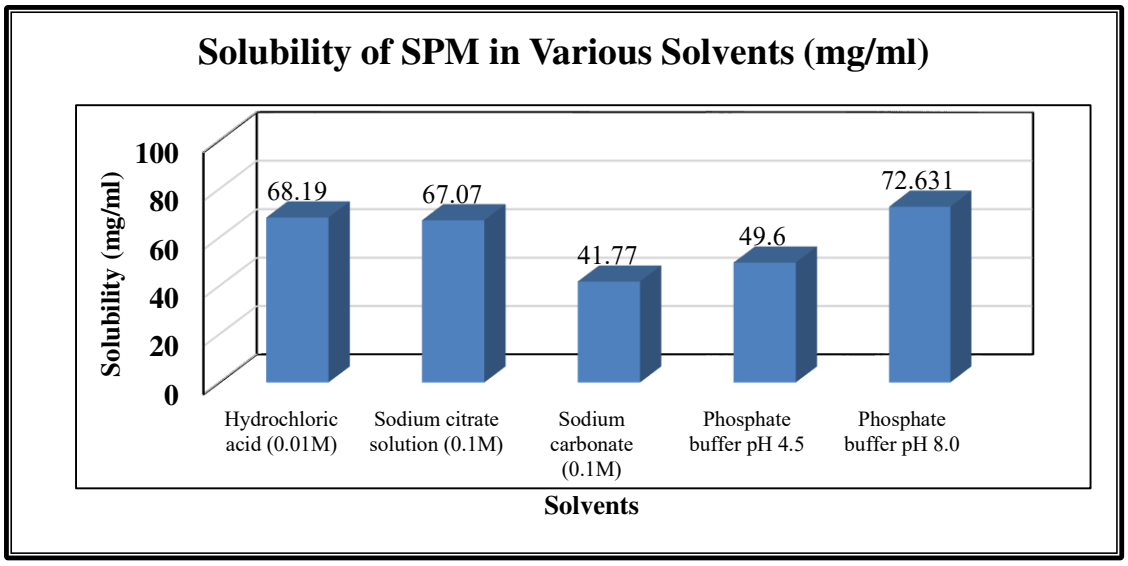

Fig. 7.15: Solubility of SPM in Various Solvents

The saturation solubility values of SPM in various solvents shows that SPM shows pH dependent solubility. As solubility was pH dependent the partition coefficient was also found to be pH dependent.

It was confirmed that Sitagliptin phosphate monohydrate had relatively higher solubility in distilled water compared with solutions of lower pH. Overall, sitagliptin phosphate monohydrate was confirmed as a soluble material at higher pH.

Sitagliptin phosphate monohydrate was found to be soluble in organic solvents like dimethyl sulfoxide and N, N-dimethyl formamide. Sitagliptin phosphate monohydrate was practically insoluble in ethanol, methanol and acetonitrile according to USP <1296>.

7.9.6 Partition Coefficient (Ko/w)

The octanol/water distribution coefficient (Ko/w) of sitagliptin is dependent on pH as the SPM shows pH dependent solubility. The Ko/w values at pH 4.5 (PB), 8.0 (PB) and 7.0 (water) were found to be -1.07 ±0.002, -0.02 ±0.001and 1.14±0.002 respectively for triplicate determination. A partition coefficient of SPM for octanol and water system reported in the literature is 1.8. The experimental value matches with the reference value.

7.9.7 pH of 2% Solution

The pH of 2% solution of Sitagliptin Phosphate Monohydrate was determined for triplicate and found to be 8.72 ±0.02.

7.9.8 Loss on Drying (%)

Accurately weighed 2 g of SPM previously screened through sieve number 80 was placed in dry weighing bottle and transfer the bottle in hot air oven maintained below 10°C temperature than the melting point of SPM for 1-2 hours hot air oven till constant weight was observed. The loss on drying value for the SPM sample was found to be 2.71±0.032 for triplicate determination. The acceptance criteria as per United States Pharmacopoeia (USP) is not more than 3.3 to 3.7%. It indicates that the sample meets the criteria for Loss on Drying.

7.10 Excipient Compatibility Studies

The samples were evaluated for physical characteristics (physical appearance) and results of the compatibility study are reported in the following tables.

Table 7.34 : Excipient Compatibility Outcome for SPM

Binary mixture	Observation	
	Initial (0 days)	After 7 days
Sitagliptin Phosphate Monohydrate (API)	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Stearic acid	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Palmitic acid	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Polyvinyl Pyrrolidone (K-30)	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Dicalcium phosphate	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Titanium dioxide	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Polyethylene glycol-4000	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Colloidal Silicon Dioxide (Aerosil)	Off white powder	No discoloration.
Sitagliptin Phosphate Monohydrate + Talc	Off white powder	No discoloration.
Mixture of Sitagliptin Phosphate Monohydrate + All above excipients	Off-white wet mass	No discoloration.

Inference: There was no change in the physical observations of the binary mixture when exposed at 50°C±2°C for 7 days. Further, the finalized formulation will be evaluated for accelerated stability study using all these excipients. Hence, it can be concluded that the above excipients are compatible with the drug substance.

7.11 Formulation Development

7.11.1 Selection of Filler

Three different fillers were evaluated for suitability. Blending was performed using Microcrystalline Cellulose (Avicel PH 101), Spray Dried Lactose (DCL-11), and Dicalcium Phosphate as filler. All three final blends were compressed.

Table 7.35 : Formulation of Sitagliptin Batches- Selection of Filler

Study Outcome	Formulation code		
	B1	**B2**	**B3**
Physical appearance	Free from defects	Free from defects	Minor sticking
Hardness (kg/cm2)	4.8±0.10	5.3±0.15	5.2±0.11
Disintegration time (min-sec)	11’22”	5’45”	5’30”

Inference:

- The compression activity for the above trials was found satisfactory, except for formulation code B3 (Spray Dried Lactose as filler), where minor sticking was observed.
- The hardness for all three trials was found comparable, but for the same hardness higher disintegration time was noted for formulation code B1 (Dicalcium phosphate as filler).
- Hence based on the overall observation of the above trials, Microcrystalline Cellulose (Avicel PH 101) was selected as a suitable filler for further optimization.

7.11.2 Selection of Disintegrant:

Two different Disintegrants (Sodium starch glycolate and Croscarmellose sodium) were evaluated for suitability. Optimization was performed using Microcrystalline Cellulose (Avicel PH 101), as filler. Both the blends were compressed.

Table 7.36 : Formulation of Sitagliptin Batches- Selection of Disintegrant

Study Outcome	Formulation code	
	B4	B5
Physical appearance	Free from defects	Free from defects
Hardness (kg/cm2)	5.5±0.14	5.4±0.15
Disintegration time (min-sec)	7'22"	5'40"

Inference:

- The disintegration time for both trials was almost comparable, however being an immediate release dosage form, Croscarmellose sodium with lesser disintegration time was selected as a disintegrant.

7.11.3 Selection of Glidant and Lubricant:

The outcome of above trials (selection of filler and disintegrants) indicates suitability for the use of Colloidal Silicon Dioxide (Aerosil) and Talc as glidant and lubricant in the finalized formulation.

Table 7.37 : Formulation of Sitagliptin Batches- Selection of Glidant and Lubricant

Study Outcome	Formulation code	
	B2	B5
Physical appearance	Free from defects	Free from defects
Hardness (kg/cm2)	5.3±0.15	5.4±0.15
Disintegration time (min-sec)	5'45"	5'40"

Inference:

- The compression process and outcome of formulation codes B2 and B5 were found to be defect free and smooth, thus Talc and Colloidal Silicon Dioxide (Aerosil) were selected in the final formula.

7.11.4 Preparation of SPM Tablets

The tablets were prepared by direct compression method using a 10-station rotatory tablet compression machine using 6 mm standard biconcave circular punches. The

hardness was adjusted to 5 kg/cm^2. The prepared tablet cores were evaluated for quality control tests and results were recorded.

7.11.5 Preparation of Coated SPM Tablets

The tablet cores were coated using hot melt coating agents as per the coating composition and using coating parameters described in the material and method section. The coated tablets were evaluated for quality control tests.

7.12 Evaluation of SPM Coated Tablets

The different experiments were conducted with varying concentration of hot melt coating agents, stearic acid or Palmitic acid. Accordingly, pore former Polyethylene Glycol 4000 (PEG 4000) concentration was varied. The hot melt-coated tablets in comparison with core tablets were analysed for weight variation, Thickness, Hardness, Weight gain, Disintegration time, Friability, Drug content and Moisture uptake.

Table 7.38: Evaluation of SPM Uncoated and Coated Tablets

Study Outcome	Formulation Code								
	Core	F1	F2	F3	F4	F5	F6	F7	F8
Weight variation (mg)*	130.12±0.18	135.45±0.19	135.36±1.04	134.27±0.43	135.42±2.29	134.34±1.18	133.87±2.35	134.04±1.08	134.43±0.18
Thickness (mm)@	2.83±0.011	2.91±0.013	2.89 ±0.008	2.90 ±0.011	2.84 ±0.016	2.89 ±0.009	2.84 ±0.012	2.87 ±0.018	2.91±0.021
Hardness (kg/cm^2) @	5.0±0.2	5.2±0.10	5.1±0.15	5.0±0.2	4.8±0.15	5.1±0.2	5.0±0.10	5.0±0.2	4.9±0.15
Disintegration time (min) @	5	13	10	7	5	14	10	7	4
Friability (%)$	0.32	0.15	0.17	0.20	0.18	0.13	0.14	0.13	0.16
Drug content (%)*	99.29 ±1.39	99.87±2.91	100.21±2.15	98.93 ±3.11	99.06 ±0.73	98.76±2.65	100.11±1.13	99.57±2.68	98.32 ±2.62
Moisture uptake (%)@	0.38 ±0.09	0.09 ±0.01	0.10 ±0.01	0.12 ±0.02	0.13 ±0.03	0.05 ±0.01	0.07 ±0.02	0.08±0.03	0.11 ±0.03

Where, *,@ and $ indicates sample size (n) 20, 06 and 10 respectively.

Inference:

- The weight variation, hardness, and thickness data of all experiments for the hot melt coated tablets indicate that the coating was uniform across individual tablets.
- The moisture uptake for all hot melt coated tablets was comparatively better than that of the core tablets.
- The disintegration time decreases as the level of hot melt coating and the quantity of pore former PEG 4000 increases in coating composition.

- The formulation F1 (Stearic acid -HMC agent) and F5 (Palmitic acid -HMC agent) having higher disintegration time (>10 mins), moreover formulation F4 and F8 has lower disintegration (≤ 5 mins).
- **In-vitro Dissolution Test of Prepared SPM Tablets:** An in-vitro dissolution test was conducted on the SPM tablet core, and the SPM tablets coated with stearic acid and palmitic acid. As stearic acid and palmitic acid level in the coating composition increases drug release decreases proportionally.
- Formulation F4 and F8 follows the acceptance criteria as per USP and hence selected as suitable formulations. These formulations (F4 and F8) releases more than 80% drug in 30 min and more than 90% drug in 45 min. The SPM core tablets release more than 90% drug within 15 min.

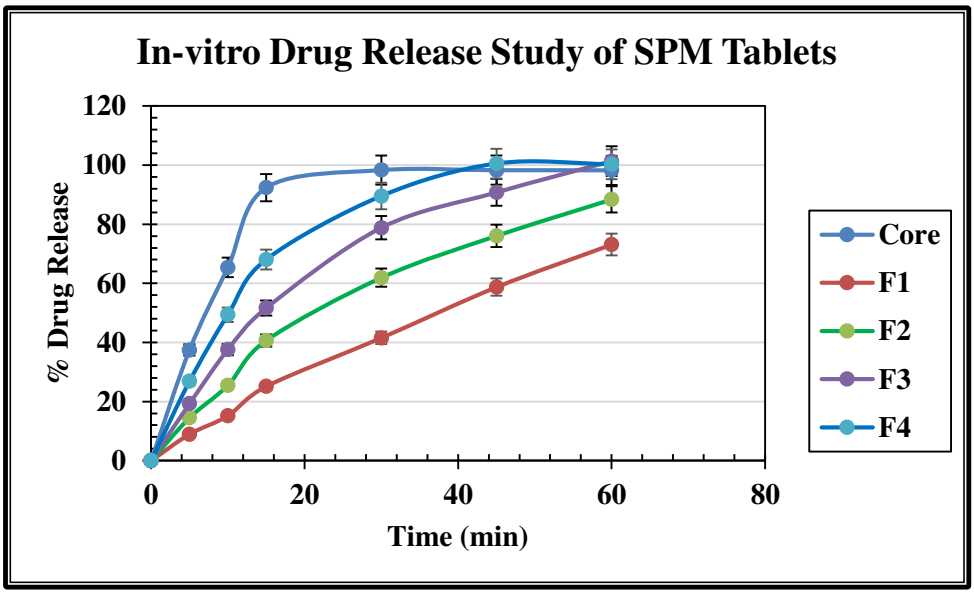

Fig. 7.16 : In-vitro Drug Release Study of Uncoated Tablet and Stearic Acid Coated Tablets

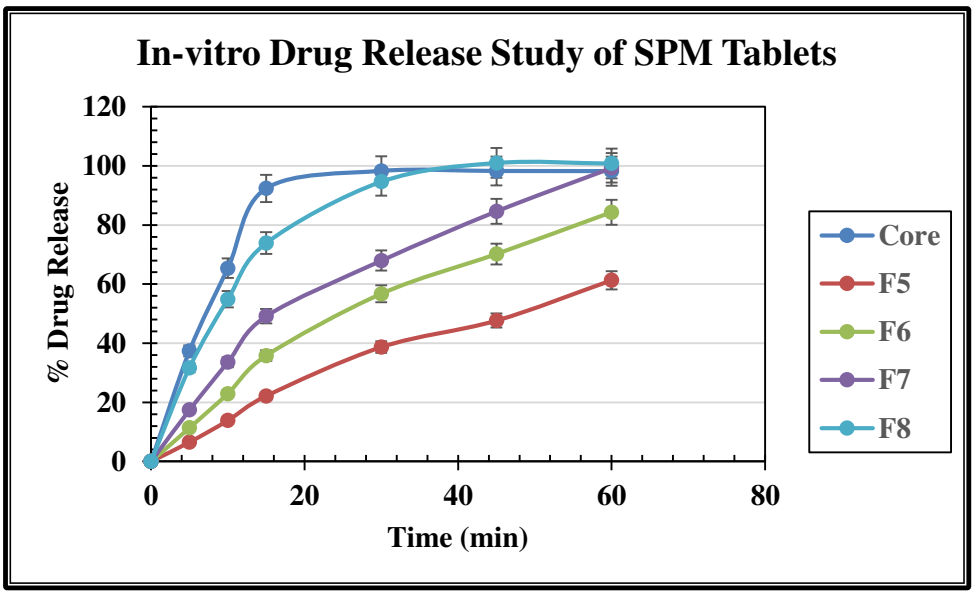

Fig. 7.17: In-vitro Drug Release Study of Uncoated Tablet and Palmitic Acid Coated Tablets

- Formulation F8 shows smoother surface and low water uptake value than formulation F4. The in-vitro drug release profile from F8 was found to be faster than F4 formulation. Therefore, dissolution profile of F8 formulation was compared with innovator product.
- **In-vitro Dissolution Study of Marketed Product:** In-vitro dissolution of marketed film coated tablets of sitagliptin phosphate monohydrate (Jankey® by Cadila Pharmaceutical Ltd., India) was conducted on twelve marketed tablets. As per United States Pharmacopoeia (USP) chapter <711> USP dissolution apparatus II (Paddle type) using 900 ml phosphate buffer pH 6.8 at 37± 0.5°C operated at 50 rpm. The samples were collected at 5, 10, 15, 30, 45 and 60 min and diluted suitably and analyzed using UV-Visible spectrophotometer. The sample solutions were filtered through Whatman filter paper (0.45 µm), from this filtered solution, 0.5 mL solution was taken into 10 mL volumetric flask and volume was made up with 6.8 pH buffer and solutions were analyzed at 267 nm by UV Spectrophotometer. The release in the dissolution medium was determined by software (PCP Disso v 2.08).
- **Acceptance criteria reported in USP-** Not less than 80% of labelled amount should release in 30 min and not less than 90% of labelled amount should release

in 45 min. The in-vitro dissolution test marketed sample, Jankey® were conducted and as per acceptance criteria given in USP reference tablets passes the test. Hence the same conditions were used for the in-vitro dissolution of prepared formulations.

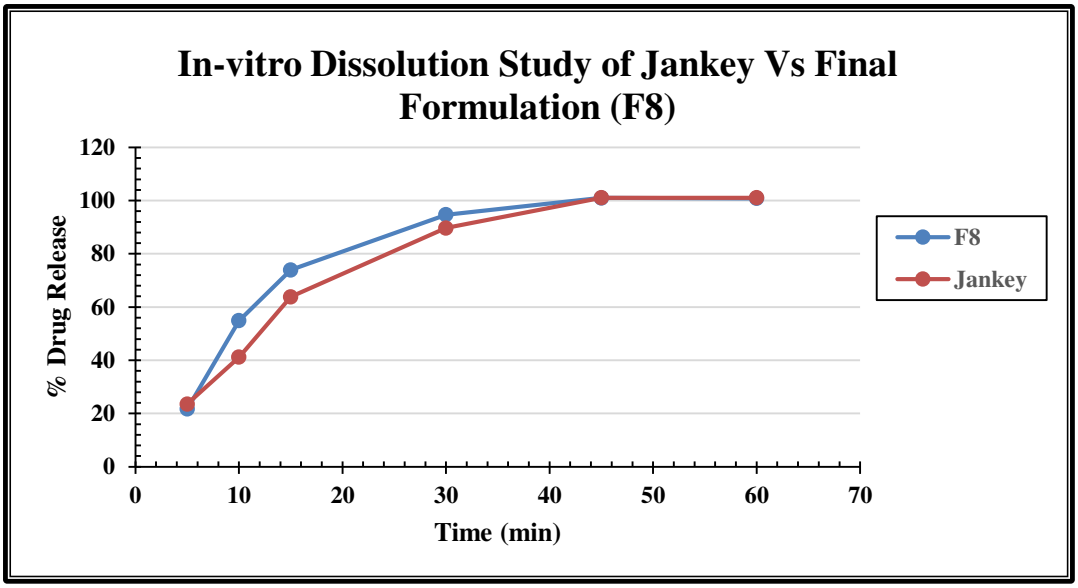

Fig. 7.18: In-vitro Dissolution Study of Marketed Product and Final Formulation

- **Similarity Factor (f_2) :** The dissolution profile of F8 formulation was compared with both innovator sample, where Similarity factor (f_2) was found to be 55. Hence F8 was considered as suitable formulation for stability study.

7.13 Stability Evaluation

- Stability-charged experimental Batch: F8
- Pack: Alu-Alu Blister pack.
- Stability Condition: At temperature 25±2°C & 60±5% RH and 40 ± 2°C & 75 ± 5% RH for 6 months in the stability chamber (Remi Laboratory Instrument, CHM-6).
- Frequency:
    - For 40 ± 2°C & 75 ± 5% RH: Initial, 1month, 2 Month, 3 month and 6 months
    - For 25±2°C & 60±5% RH : Initial, 1month, 2 Month, 3 month , 6 months.

- Testing: Physical appearance, drug content, moisture uptake and in-vitro drug release.

Table 7.39: ACC Stability Data for SPM Tablets

40 ± 2°C & 75 ± 5% RH					
Formulation Code	**Station**	**Appearance**	**Drug Content (%) (90.0 -110.0)**	**Moisture Uptake (%)**	**Drug Release (%) (NLT 80% (Q) in 30 minutes)**
Core Tablets	Initial	White	99.2	0.38	98
	1 M	Slightly off white	97.8	0.47	97
	2M	Off white	95.3	0.69	98
	3M	Off white to yellow	94.7	1.16	94
	6M	Light Yellow	91.2	2.13	92
F8	Initial	Yellowish white	98.3	0.11	93
	1 M	Yellowish white	98.2	0.15	94
	2M	Yellowish white	98.0	0.13	92
	3M	Yellowish white	97.7	0.22	92
	6M	Yellowish white	98.3	0.43	93

Table 7.40: Long-Term Stability Data for SPM Tablets

25±2°C & 60±5% RH					
Formulation Code	Station	Appearance (White tablets)	Drug Content (%) (90.0 - 110.0)	Moisture Uptake (%)	Drug Release (%) (NLT 80% (Q) in 30 minutes)
Core Tablets	Initial	White	99.2	0.38	98
	1 M	Slightly off white	98.8	0.39	98
	2M	Off white	97.9	0.44	96
	3M	Off white	96.5	0.55	95
	6M	Off white	94.6	1.10	94
F8	Initial	Yellowish white	98.3	0.11	93
	1 M	Yellowish white	97.9	0.19	92
	2M	Yellowish white	97.3	0.13	95
	3M	Yellowish white	97.0	0.23	92
	6M	Yellowish white	98.0	0.38	94

The appearance of the uncoated tablet was found to be change from white to off-white to yellowish in uncoated tablets. In case of coated tablets, the color of coated tablet was retained as yellowish white due to palmitic acid in coating composition.

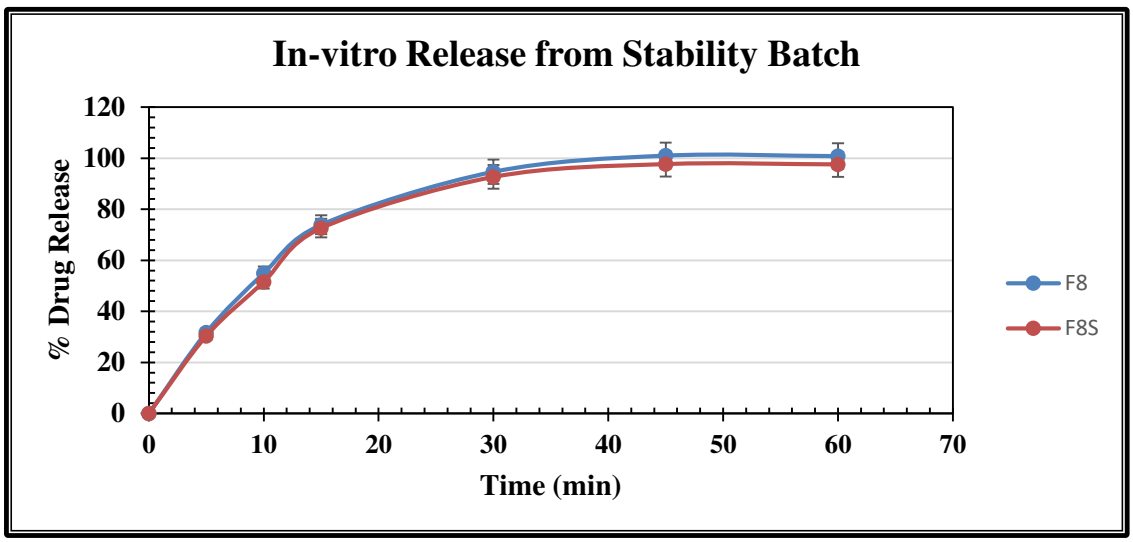

Fig. 7.19: In-vitro Release from Stability Batch

The in-vitro drug release from F8 formulation on the next day of preparation was compared with stability batch stored at accelerated conditions (40 ± 2°C & 75 ± 5% RH) after 6 months. No significant change in drug release was observed indicates F8 formulation was found to be stable. And after application of hot melt coating with hydrophobic coating agent like palmitic acid can protect core from moisture and resist the absorption of moisture by sitagliptin phosphate monohydrate and hence improve the stability.