5.0. RESULTS AND DISCUSSION

5.1. Preliminary solubility studies of Efavirenz

Efavirenz was found increase in solubility with PVP, HPC and HPMC. **Table 3** shows the solubility of Efavirenz in various polymers in water at 25^0C. The enhancement of the absolute amounts of the dissolved drug was approximately increased with increase in concentration of polymer. These results are in accordance with the well established formation of soluble complexes between water soluble polymeric carriers and Efavirenz. However, in comparison with hydroxy propyl methyl cellulose, Efavirenz was more soluble in polyvinyl pyrrolidone and hydroxy propyl cellulose.

Table 3: Solubility studies of Efavirenz in different polymers

Sample (Physical mixtures)	Drug & Polymer ratios	Solubility (mg/ml)
Drug	-	2.56
Drug: PVP	1:1	3.62
Drug: PVP	1:2	3.92
Drug: HPC	1:1	3.12
Drug: HPC	1:2	3.45
Drug :HPMC	1:1	2.77
Drug :HPMC	1:2	2.91

5.2. % Practical Yield with and without antisticking agent:

Practical yield of solid dispersions were very low because of sticky nature of material. Almost all polymers were very sticky in nature which influences the stickiness of solid dispersion. When polymers dissolved in solvents, after drying the particle size of polymers were decreased and

surface of particle size increases and which increases the sticky nature. The solid dispersion formulations were sticked to the bottom of vessel and which decreased the practical yield of formulation. In solid dispersion formulation using anti sticking agent like Neusilin US2, it is insoluble in nature and settled at the bottom of the vessel and which prevented the polymers to stick to the vessel and which increased in practical yield of formulation. The results were shown in **Table 4**. Practical yield of solid dispersion using anti sticking agent was more in comparison with solid dispersion without antisticking agent.

Table 4: % Practical yield of Physical mixtures and Solid dispersions, with and without anti sticking Agent

S. No	Sample (ratio)	% Yield	
		Without	With
1	Physical mixture of drug, Kollidon (1:1)	86%	96%
2	Solid dispersion of drug, Kollidon (1:1)	84%	95%
3	Physical mixture of drug, Kollidon (1:2)	87%	95%
4	Solid dispersion of drug, Kollidon (1:2)	83%	93%
5	Physical mixture of drug, HPC (1:1)	86%	97%
6	Solid dispersion of drug, HPC (1:1)	81%	96%
7	Physical mixture of drug, HPC (1:2)	88%	96%
8	Solid dispersion of drug, HPC (1:2)	81%	94%

Note: The Neusilin US2 concentration maintained similar to polymer concentration

5.3. Solubility studies for solid dispersions with antisticking agent:

The solubility of Efavirenz was increased with solid dispersions. Moreover, an increased solubility of Efavirenz in solid dispersion formulation using anti sticking agent was observed in **Table 5**, because this anti sticking agent prevents the agglomeration of drug and polymer complex.

Table 5: Solubility studies of solid dispersion containing Efavirenz in different polymers

Sample (physical mixtures)	Ratio	Solubility (mg/ml)
Drug	-	2.56
Drug: PVP: Neusilin US2	1:1:1	3.62
Drug: PVP: Neusilin US2	1:2:2	4.21
Drug: HPC: Neusilin US2	1:1:1	3.01
Drug: HPC: Neusilin US2	1:2:2	3.52

5.4. Flow properties

The formulations were evaluated for their flow properties and results were summarized in **Table 6**. Angle of repose was in the range of Around 48 for physical mixtures. Carr index was found to be around 28 and Hausner ratio around 1.37 for physical mixtures and angle of repose was around 30, Carr index was around 16 and Hausner ratio around 1.1 for solid dispersion prepared by using anti sticking agent. These values indicate that the solid dispersions prepared by using Neusilin having the good flow properties, which suggest neusilin increases the flow properties of solid dispersion by reducing the stickiness of mixture.

Table 6: Flow properties of physical mixtures and solid dispersions with and without anti-sticking agent

S.No	Sample (ratio)	Angle of repose	Carr Index (IC)	Hausner Ratio (HR)
1	Physical mixture of drug and Kollidon (1:1)	48	28	1. 37
2	Solid dispersion of drug and Kollidon (1:1)	57	33	1.48
3	Physical mixture of drug and Kollidon (1:2)	47	28	1.39
4	Solid dispersion of drug and Kollidon (1:2)	56	32	1.49
5	Physical mixture of drug and HPC(1:1)	49	30	1.35
6	Solid dispersion of drug and HPC(1:1)	46	27	1.39
7	Physical mixture of drug and HPC(1:2)	51	31	1.38
8	Solid dispersion of drug and HPC(1:2)	50	29	1.41
9	Physical mixture of drug, Kollidon&Neusilin US2 (1:1:1)	40	19	1.24
10	Solid dispersion of drug, Kollidon&Neusilin and Neusilin US2 (1:1:1)	34	14	1.16
11	Physical mixture of drug, Kollidon&Neusilin and Neusilin US2 (1:2:2)	39	18	1.23
12	Solid dispersion of drug, Kollidon&Neusilin and Neusilin US2 (1:2:2)	31	16	1.16
13	Physical mixture of drug, HPC and Neusilin US2 (1:1:1)	39	17	1.22
14	Solid dispersion of drug, HPC and Neusilin US2 (1:1:1)	33	15	1.15
15	Physical mixture of drug, HPC and Neusilin US2 (1:2:1)	40	14	1.19
16	Solid dispersion of drug, HPC and Neusilin US2 (1:2:1)	30	15	1.15

5.5. Drug content

The drug content was found in the range around 100%, indicating the acceptability of solvent heat method for preparation of solid dispersions **Table 7**. Low values of standard deviation in drug content of physical mixtures and solid dispersions indicated uniform drug distribution in all the prepared batches.

Table 7: Assay of physical mixture and solid dispersions with different polymers

Codes	Sample	Assay (%)
PM1	Physical mixture of Drug: PVP: Neusilin (1:1:1)	97 ±0.52
FSD1	Free flowing Solid dispersion of Drug: PVP: Neusilin (1:1:1)	98 ±0.62
PM2	Physical mixture of drug: PVP: Neusilin (1:2:2)	98 ±0.47
FSD2	Free flowing Solid dispersion of drug: PVP: Neusilin (1:2:2)	99 ±0.19
PM3	Physical mixture of Drug: HPC: Neusilin (1:1:1)	96 ±0.61
FSD3	Free flowing Solid dispersion of Drug: HPC: Neusilin (1:1:1)	97 ±0.56
PM4	Physical mixture of Drug: HPC: Neusilin (1:2:2)	99 ±0.48
FSD4	Free flowing Solid dispersion of Drug: HPC: Neusilin (1:2:2)	99 ±0.45

Note: All the values are Mean ± SD.

5.6. *In vitro* release studies

The dissolution data showed, faster dissolution rate observed for free flowing solid dispersion formulation using anti sticking agent in comparison with pure drug **Table 8 & Figure 1**. The enhanced dissolution rate of Efavirenz from the free flowing solid dispersions formulation might be due to the increase in the drug wettability and the Efavirenz, PVP interactions. Our experimental approach has taken into account that the data from our previous physic-chemical investigations demonstrating that Efavirenz exists as crystalline in nature and in solid dispersion formulation shows amorphous and particle size of particles are very small. The improvement of dissolution of solid dispersion must be due to the increased surface area of the smaller particles.

Table 8: *In vitro* dissolution profile

Time in Min	Pure Drug	PM1	FSD1	PM2	FSD2	PM3	FSD3	PM4	FSD4
0	0	0	0	0	0	0	0	0	0
5	6	12	37	14	56	9	22	14	29
10	9	19	52	22	66	12	41	19	44
15	10	24	64	29	73	16	49	21	59
30	14	26	80	31	89	19	59	24	71
45	19	29	94	34	99	21	71	28	80

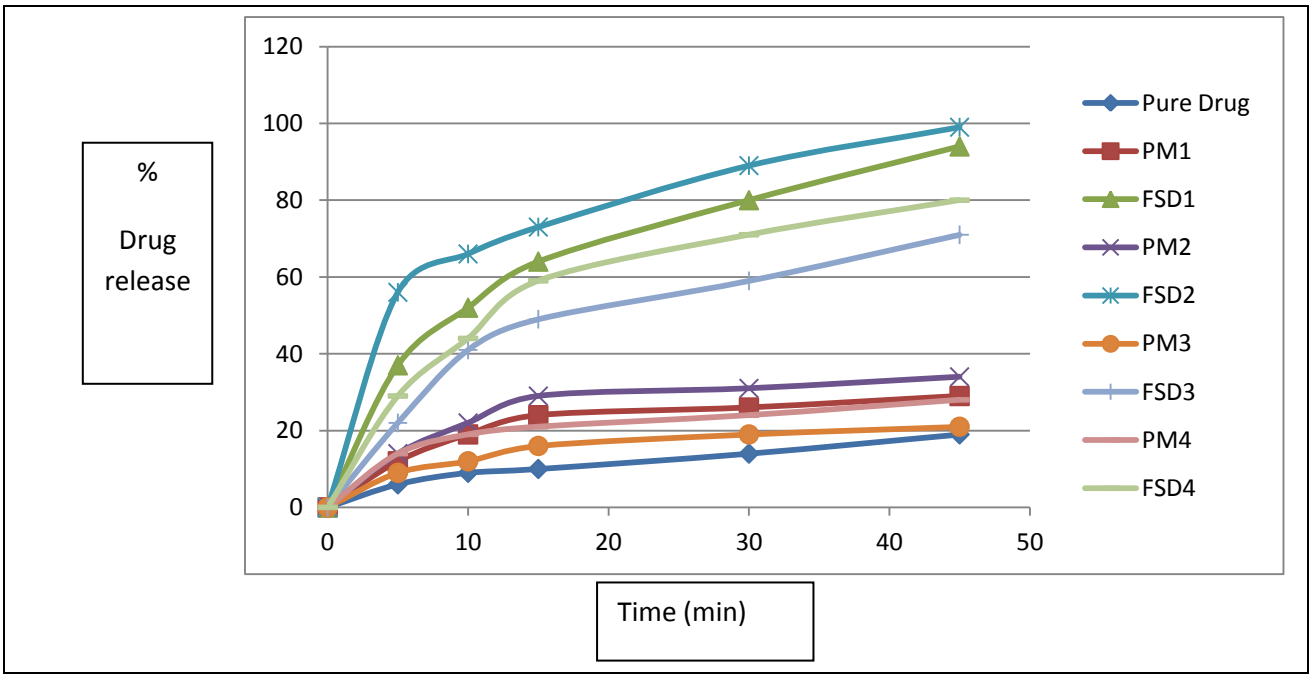


Figure 1: Comparative dissolution graphs of as such API, Physical mixtures and free flowing solid Dispersions

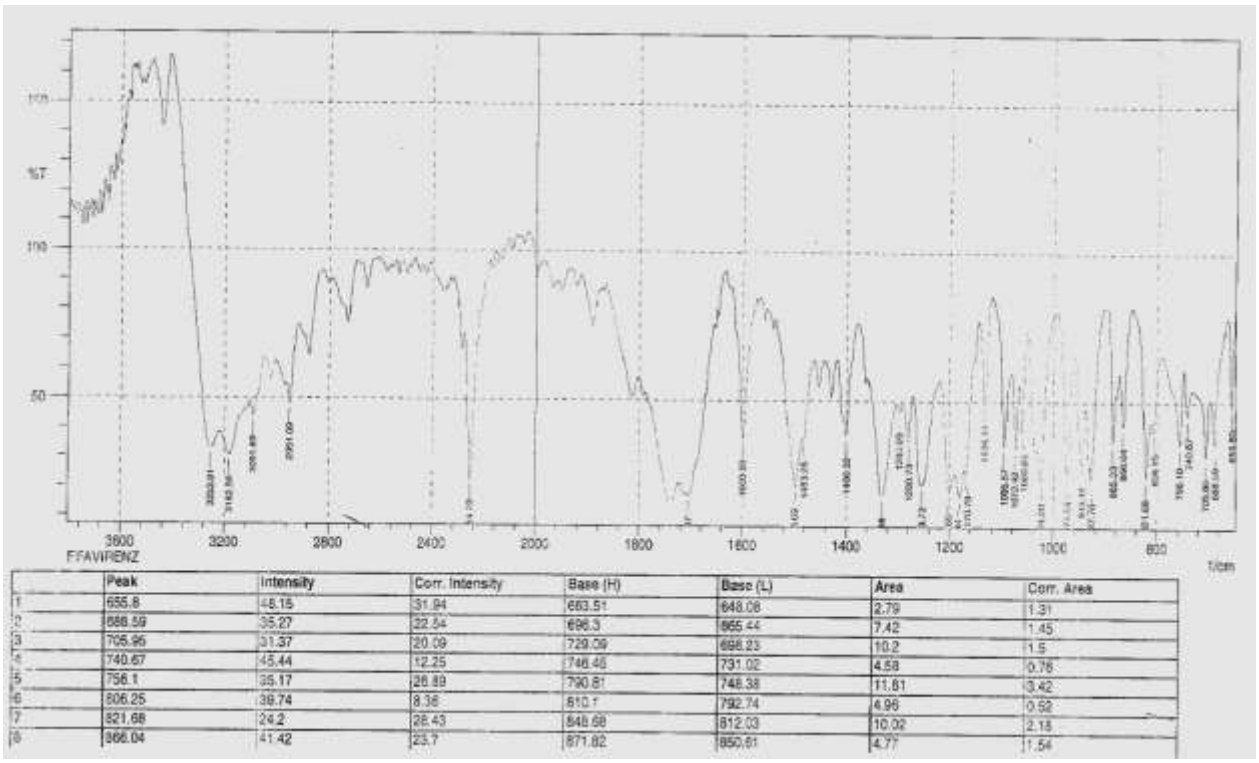
5.7. FT-IR studies

FT-IR spectrums are mainly used to determine if there is any interaction between the drug and any of the excipient used.

In order to get evidence on the possible interactions of drug with the carrier, FTIR analysis was used. **Figures 2-6** shows the IR spectra of Efavirenz, neusilin US2, Kollidon 30, placebo and the optimized formulation. **Figure 7** shows the overlay of the spectra of Efavirenz, Solid dispersion formulation and Placebo.

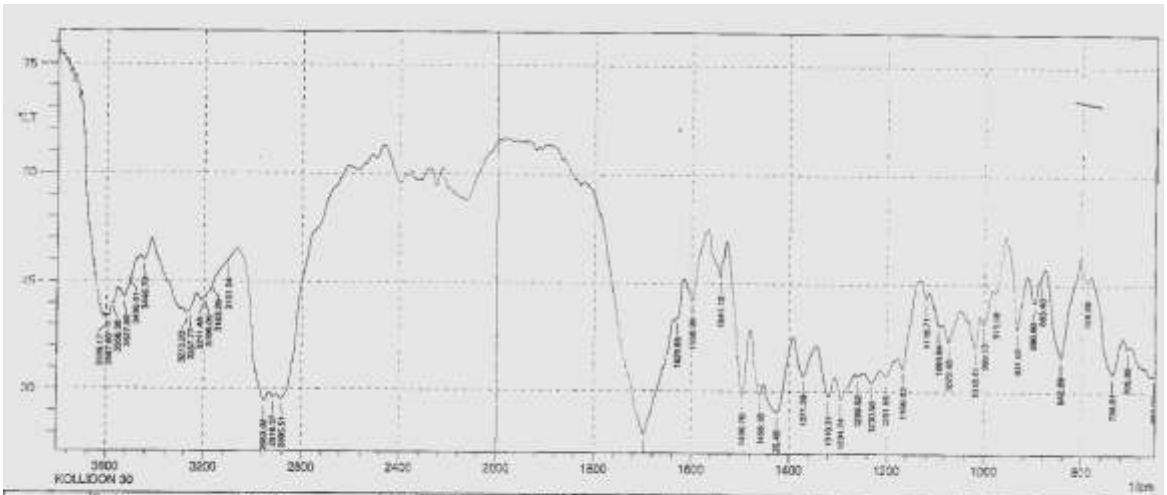
The optimized formulation displayed the characteristic peaks at wave numbers nearer to that of pure Efavirenz. The IR spectral analysis of pure drug showed characteristic peaks at wave numbers, 655.8, 688.59, 705.95, 740.67, 758.1 and 806.25. The optimized formulation displayed the characteristic peaks at wave numbers, 651.94, 688.59, 705.95 and 742.59.

Overall there was no alteration in the characteristic peaks of Efavirenz suggesting that there was no interaction between the drug and polymers.



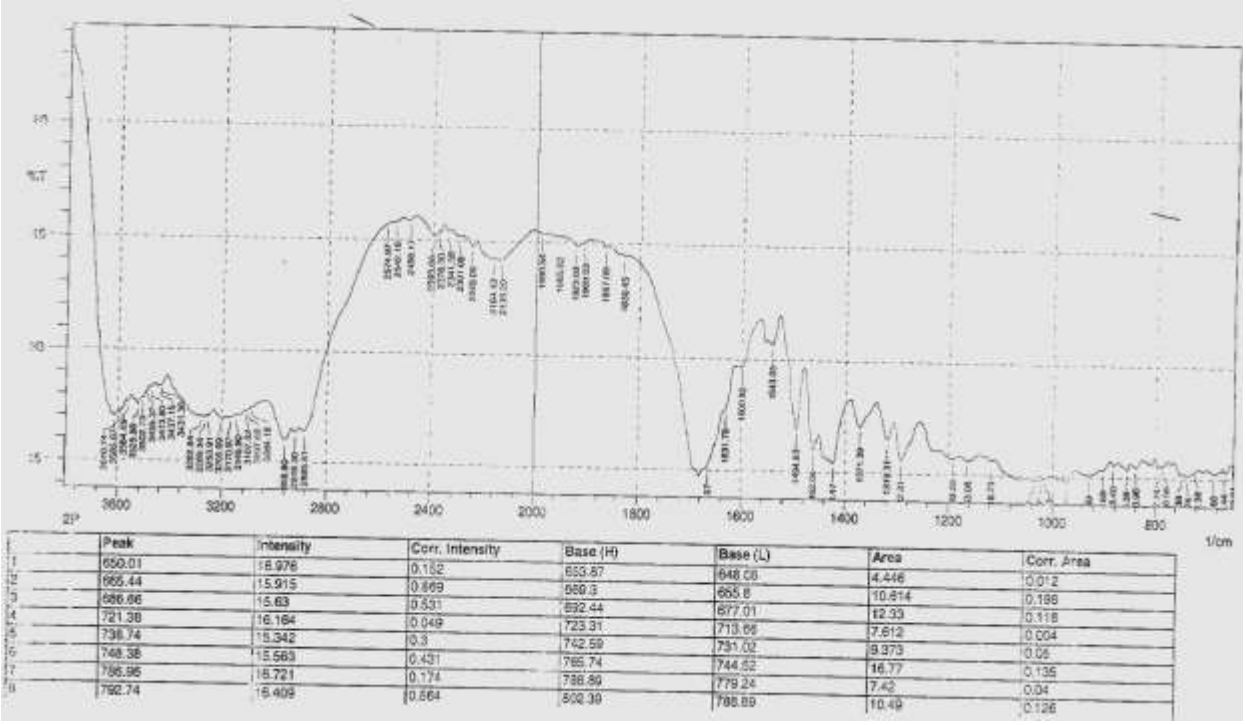
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	655.8	48.15	31.94	663.51	648.08	2.79	1.31
2	688.59	35.27	22.54	696.3	665.44	7.42	1.45
3	705.96	31.37	20.09	729.09	696.23	10.2	1.5
4	740.67	45.44	12.25	746.46	731.02	4.58	0.76
5	756.1	35.17	26.89	790.81	748.38	11.81	3.42
6	806.25	39.74	8.36	810.1	792.74	4.96	0.52
7	821.68	24.2	26.43	848.68	812.03	10.02	2.18
8	866.04	41.42	23.7	871.82	850.61	4.77	1.54

Figure 2: EFAVIRENZ



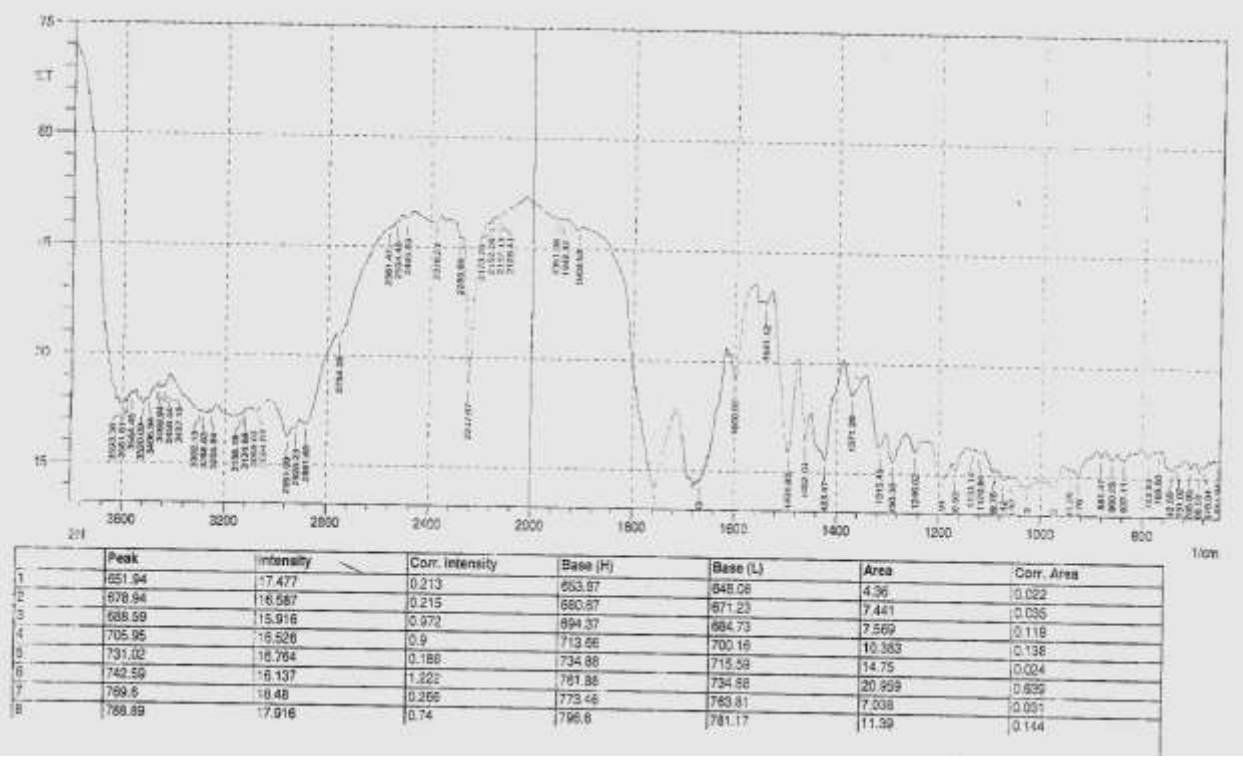
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	650.01	32.323	0.142	657.72	648.08	4.721	0.009
2	705.95	34.22	0.835	713.66	700.15	5.881	0.059
3	736.81	32.588	7.98	779.24	715.59	27.231	3.023
4	788.89	45.426	1.62	802.39	781.17	7.115	0.205
5	842.89	34.78	13.097	873.75	804.32	36.925	4.738
6	883.4	45.465	0.357	885.33	875.65	3.198	0.001
7	896.9	42.473	3.176	910.4	889.18	7.556	0.348
8	931.62	36.764	9.936	954.76	912.33	14.954	1.713

Figure 3: KOLLIDON 30



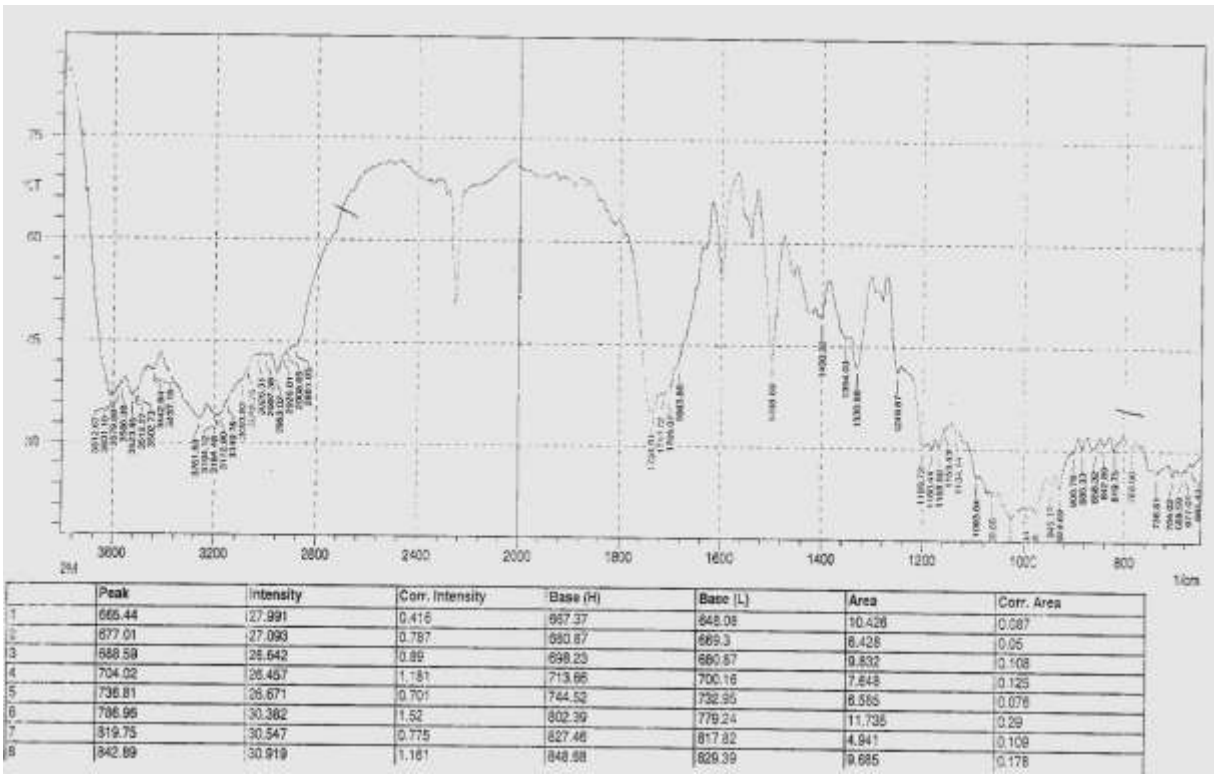
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	650.01	16.976	0.132	653.87	648.08	4.446	0.012
2	665.44	15.915	0.869	669.3	665.6	10.614	0.198
3	686.66	15.63	0.531	692.44	677.01	12.33	0.118
4	721.38	16.164	0.049	723.31	713.66	7.612	0.004
5	738.74	15.342	0.3	742.59	731.02	9.373	0.05
6	748.38	15.563	0.431	765.74	744.52	16.77	0.135
7	786.96	16.721	0.174	788.89	779.24	7.42	0.04
8	792.74	16.409	0.864	802.39	788.89	10.49	0.126

Figure 4: PLACEBO



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	651.94	17.477	0.213	653.87	648.08	4.36	0.022
2	678.94	16.587	0.215	680.87	671.23	7.441	0.035
3	688.59	15.916	0.972	694.37	684.73	7.569	0.118
4	705.95	16.526	0.9	713.66	700.16	10.383	0.138
5	731.02	16.764	0.188	734.88	715.59	14.75	0.024
6	742.59	16.137	1.222	761.88	734.88	20.959	0.639
7	769.6	18.48	0.266	773.46	763.81	7.038	0.091
8	788.89	17.916	0.74	796.8	781.17	11.39	0.144

Figure 5: PHYSICAL MIXTURE



	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	665.44	27.991	0.416	667.37	648.08	10.426	0.087
2	677.01	27.099	0.787	680.87	669.3	6.428	0.05
3	688.59	26.642	0.89	698.23	680.87	9.832	0.108
4	704.02	26.457	1.181	713.66	700.16	7.648	0.123
5	736.81	26.671	0.701	744.52	732.95	6.565	0.076
6	786.96	30.362	1.52	802.39	779.24	11.736	0.29
7	819.75	30.547	0.775	827.46	817.82	4.941	0.109
8	842.89	30.919	1.161	848.68	829.39	9.685	0.178

Figure 6: SOLID DISPERSION (OPTIMISED) COMPOSITION

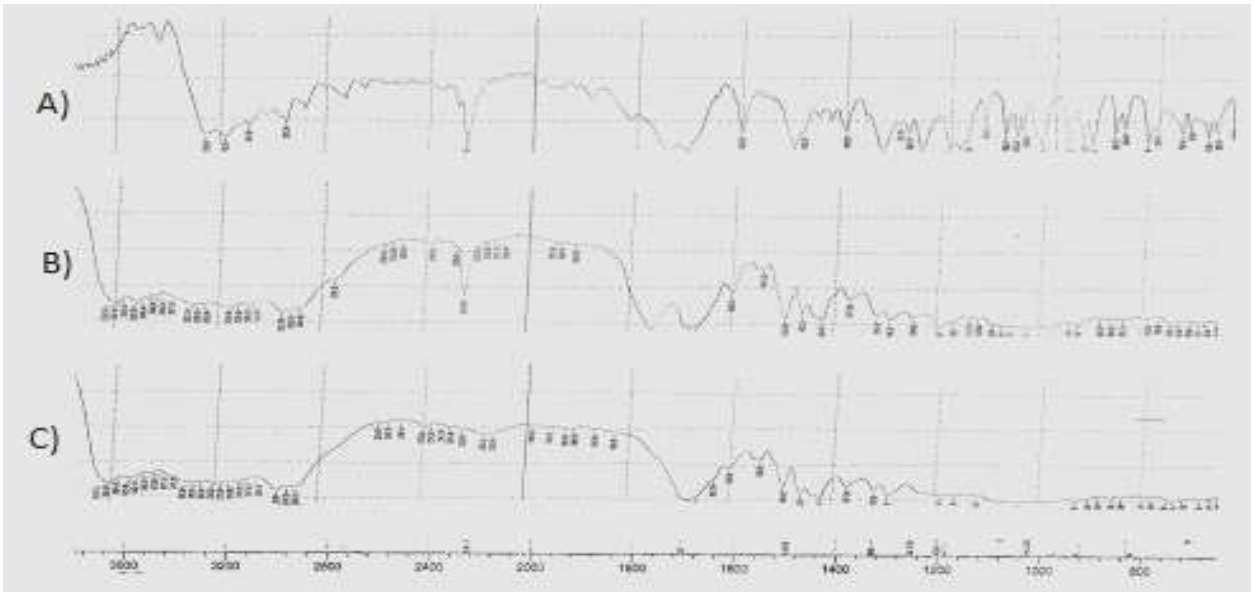


Figure 7: Overlay of FT-IR spectra of A) Efavirenz, B)Solid dispersion formulation C) Placebo

5.8. DSC (Differential Scanning calorimetry) studies

The DSC thermo grams of Pure Efavirenz showed in F**igure 8,** sharp endothermic peaks at melting point, indicating that the drug is highly crystalline. The absence of drug peak in the free flowing solid dispersion formulation (FSD2) indicating the drug was in amorphous form.

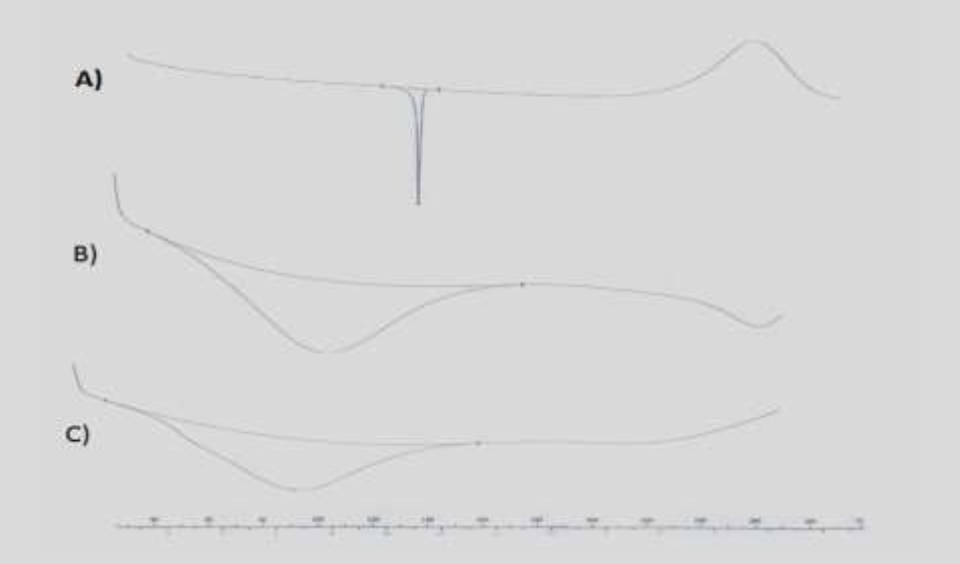
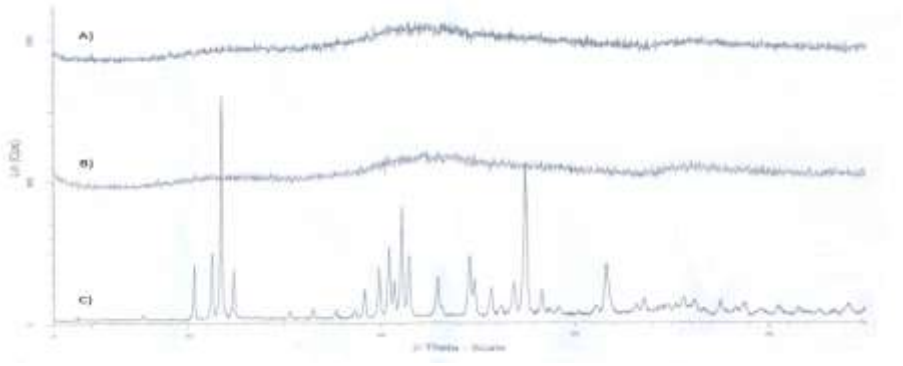


Figure 8: DSC thermo grams of pure drug, formulation and placebo.

A) Efavirenz, B) Solid dispersion formulation C) Placebo

5.9. XRD (X-Ray diffraction) studies

In X-ray diffraction study, the absence of drug peak in the free flowing solid dispersion formulation (FSD2) indicating that the drug was in amorphous state showed in **figure 9**.



A)Formulation B)Placebo c)pure efavirenz

Figure 9: Powder X-ray diffraction patterns of pure drug, placebo and formulation

5.10. Microscopic studies:

The results of the microscopic examination of solid preconcentrates are shown in Figure 3. Microscopic studies revealed that the morphology of formed particles is spherical in shape with the size of 2-3 microns. It reveals that the surface of the solid dispersion particles is rather smooth and transparent.

Table 9: Composition of solid dispersion, physical mixture and placebo formulations

FORMULATION				
COMPONENTS(%Wt/Wt)	**API**	**Solid dispersion**	**Physical mixture**	**Placebo**
Efavirenz	50	50	50	
PVP k29/32		100	100	100
Neusilin US2		100	100	100
Total		250	250	200
Solvent		Ethanol		

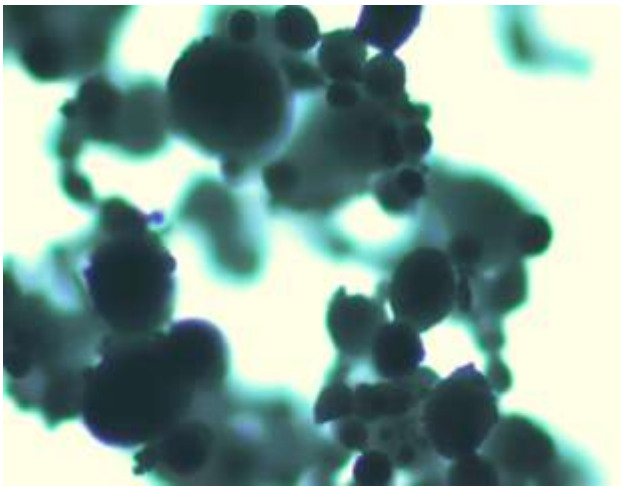


Figure 10: Microscopic examination of Solid dispersions

5.11. Pharmacokinetic parameters comparison for pure drug suspension and free flowing solid dispersion formulation:

Figure 11 shows the plasma concentration–time curve in Wistar rats after a single oral dose of Efavirenz free flowing solid dispersion formulation as compared to Efavirenz pure suspension. At all the indicated time points, the Efavirenz plasma concentrations in rats treated with free flowing solid dispersion formulation were significantly higher than those treated with pure drug suspension. Plasma pharmacokinetic parameters of Efavirenz after oral administration of the two formulations to Wistar rats are shown in **Table 10**.

As can be seen from the above table, the test preparation shows the increased AUC and C_{max} values, which are about 3.04 and 1.25 times, respectively, as high as those in the reference formulation. Accordingly, it can be identified that the Efavirenz of the free flowing formulation is significantly increased in comparison with that of the pure drug (Efavirenz suspension). Cmax of the free flowing solid dispersion formulation 1.16 µg mL−1 was significant ($p<0.05$) as compared to the pure drug suspension formulation 0.52 µg mL−1. Tmax of both free flowing solid dispersion formulation and pure drug suspension was 1.88 and 1.50 h, respectively. AUC is an important parameter in evaluating bioavailability of drug from dosage form, as it represents

the total integrated area under the blood concentration time profile and represents the total amount of drug reaching the systemic circulation after oral administration. $AUC_{0-\infty}$inf for free flowing solid dispersion formulation was higher (8.05 µg hmL−1) than the pure drug suspension formulation 2.65 µg hmL−1. Statistically, $AUC_{0-\infty}$t of the free flowing solid dispersion formulation was significantly higher ($p<0.05$) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of Efavirenz from free flowing solid dispersion formulation as compared to the pure drug suspension formulation**. Figure 5** and **Figure 6** showed the difference of LC-MS chromatograms between pure drug suspension and solid dispersion formulations of Efavirenz at maximum concentrations. Calculated concentration was found to be more for solid dispersion formulations compared with pure drug of Efavirenz.

Table 10: Pharmacokinetic Parameters of Efavirenz Free flowing solid dispersion formulation and pure drug

Pharmacokinetic parameters	**Efavirenz**	**Efavirenz-SD**	**Ratio (Free flowing solid dispersion/Suspension)**
Dose (mg/kg)	50	50	1.00
C max (µg/ml)	0.52	1.16	2.24
AUC 0-t (µg.hr/ml)	2.62	7.94	3.04
AUC 0-inf (µg.hr/ml)	2.65	8.05	3.04
T max (hr)	1.50	1.88	1.25
t 1/2 (hr)	4.22	8.72	2.07
K el (hr^{-1})	0.164	0.081	0.49

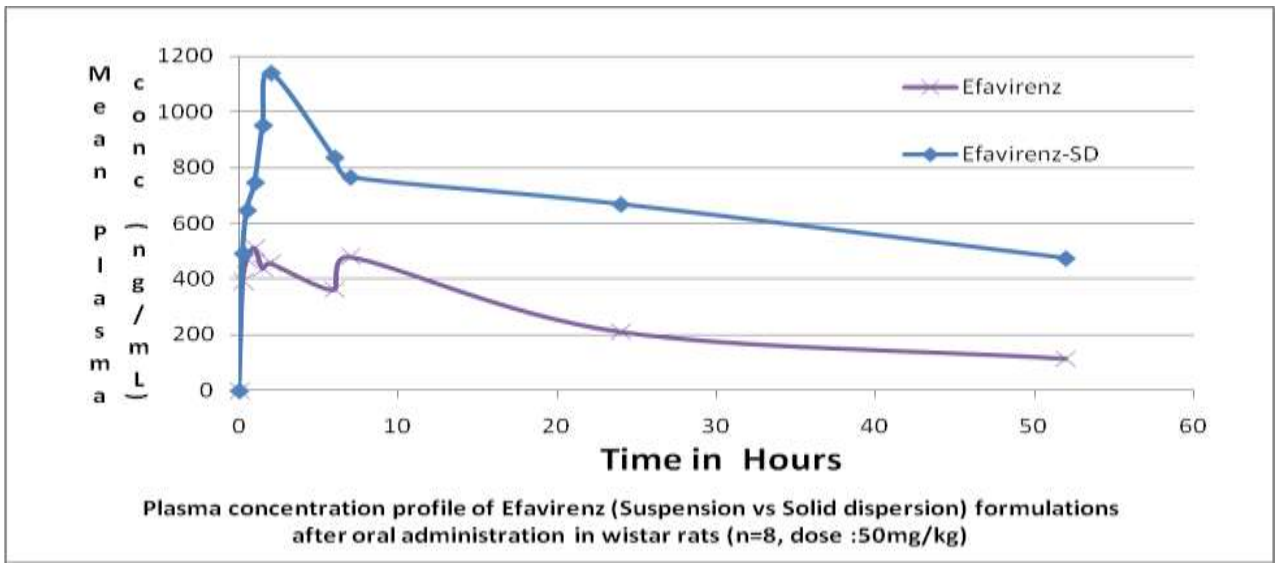
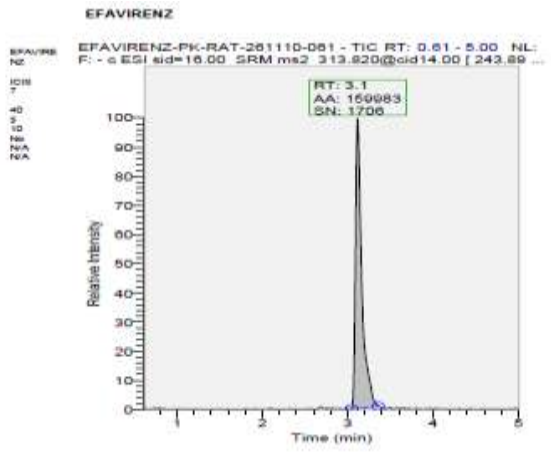
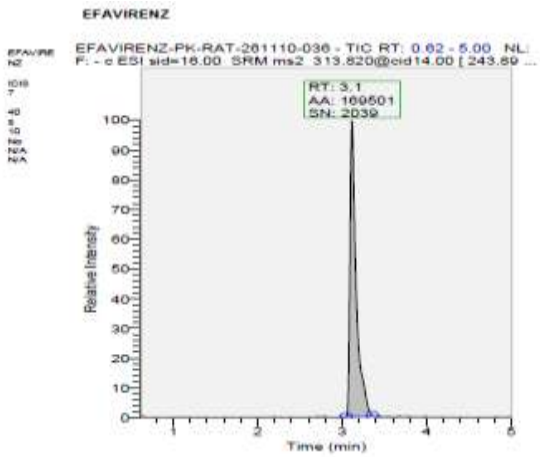


Figure 11: Plasma concentrations of Efavirenz (suspension Vs solid dispersion) formulations after oral administration in Wister rats (n=8, Dose: 50mg/kg)

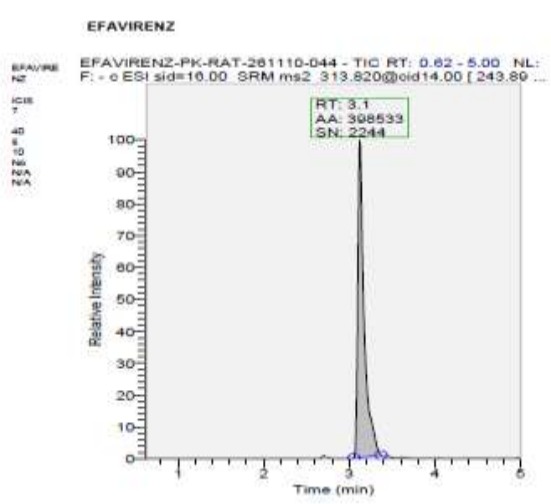


Calculated concentration: 532.52ng/ml

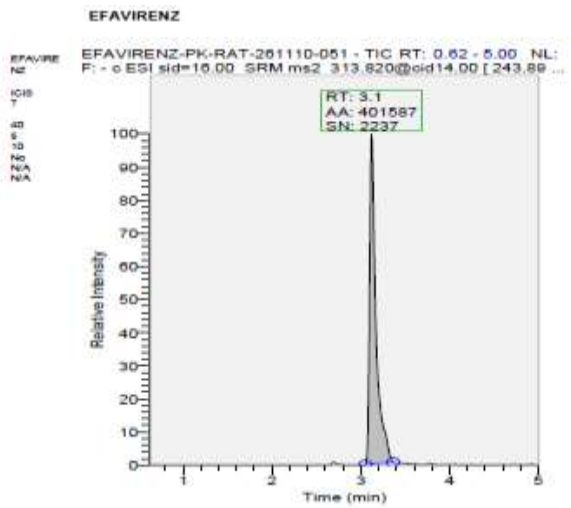


Calculated concentration: 528.85ng/ml

Figure 12: Reference LC-MS Chromatograms for Pure drug suspension at maximum conc.



Calculated concentration: 1002.17ng/ml



Calculated concentration: 1225.68ng/ml

Figure 13: Reference LC-MS Chromatograms for Free flowing solid dispersion formulation at maximum concentration.