IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 4, Issue 5 (May 2014), PP. 47-53

Dissolution and Bioavailability Enhancement of Efavirenz by Hot Melt Extrusion Technique

Smita Kolhe[1], Dr. P.D.Chaudhari[2], Mr. Dhananjay More[3]
[1]*Modern College of Pharmacy (For Ladies), Moshi,MS, India*
[2] *Modern College of Pharmacy, Nigdi, MS, India*
[3]*Emcure Pharmaceuticals Ltd.,Pune, MS, India*

ABSTRACT: *The aim of this study is to improve dissolution and bioavailability of poorly water soluble Efavirenz (Efv), a potent and selective non-nucleoside inhibitor of HIV-1 reverse transcriptase. Its effectiveness can be attributed to its long half-life ($t_{1/2}$) of 52–76 h after single dose. Formulation of poorly water soluble drug for oral delivery is one of the biggest challenges. Amongst the available approaches, the novel solid dispersion (SD) technique known as hot melt extrusion (HME) has often proved to be the most widely used method in improving dissolution and bioavailability of the drugs because of its various advantages. Dissolution enhancement of Efv was done by HME technology where crystalline form of API is converted to amorphous form using hydrophilic polymer like Kollidon VA64. Surfactants like Polyethylene glycol (PEG 4000), polyoxy 35 castor oil (Chremophor EL) and Sorbiton monolaurate (Montane 20PHA) were used as plasticizer for process feasibility. Physical mixtures of drug, polymer and surfactant were prepared in ratio of 1:1:0.1. These physical mixtures were subjected to melt and the resultant formulations were subjected to physical characterization, dissolution, permeability and in vivo testing. Pharmacokinetics of Efv was studied in rats. Drug efflux pumps like P-glycoprotein (P-gp) were recognized to posses' functional role in determining the pharmacokinetics of drugs. Inhibition of P-gp improves intestinal absorption and tissue distribution while reducing the substrate metabolism and its elimination. Drug release showed comparative similar release for all the HME formulations while the permeability studies showed improved permeability of formulations containing Sorbiton Monolaurate and PEG 4000. In comparison to the Non HME (NHME) formulation, the SD prepared with KollidonVA64 (drug: polymer 1:1) by HME process showed a significant enhancement in permeability with all the three surfactants. SD prepared by PEG 4000 resulted in 106.98% enhancement in extent of absorption. In conclusion, solid dispersion prepared using certain polymer could serve as a promising formulation approach to enhance the dissolution and bioavailability of Efv.*

KEYWORDS: *Efv, HME, Invivo, Kollidon VA64, Permeability.*

I. INTRODUCTION

Efv is a potent and selective non-nucleoside inhibitor of HIV-1 reverse transcriptase [1, 2], but its aqueous solubility is relatively poor (<10 μg/mL in water) [1]. For such hydrophobic compound, poor solubility would result in a slow dissolution and hence low and erratic oral bioavailability, which may limit its further clinical application. Majority of the drugs are administered by preoral route. Many hurdles starting from drug dissolution in gastrointestinal fluid to first pass metabolism due to various physicochemical and biopharmaceutical problems occur. It was recently identified that drug efflux pumps like P-gp are playing major role in altering the pharmacokinetics of various drugs and particularly associated with poor bioavailability in co-ordination with gut wall metabolism. Thus, a deep insight and thorough understanding of P-gp, its physiological and biochemical role in effluxing drugs is worthwhile, in order to have an opportunity to improve the bioavailability of drugs restricted by P-gp. [3]Improved clinical efficacy of various drugs observed by P-gp inhibition, P-gp inhibitors are gaining recognition to improve bioavailability by inhibiting P-gp in intestine, brain, liver and kidneys. P-gp can be inhibited (i) by blocking drug binding site either competitively, non-competitive or allosterically; (ii) by interfering ATP hydrolysis ; and (iii) by altering integrity of cell membrane lipids [3].

The bioavailability enhancement of poorly water soluble drugs by use of P-gp inhibitors finds to be improving by use of various surfactants. Out of all the surfactants Chremophor EL [4] have been used as P-gp inhibitors for this study. SD technique namely the HME technique was used for the preparation of formulations which were further subjected to pharmacokinetic study. Plasticisers are included to the polymers to facilitate thermal processing, to modify drug release from polymeric system and enhance mechanical properties and surface appearance of dosage form. When plasticisers are added to the polymers the flexibility of the polymers is increased by increasing the intermolecular separation of polymer molecules which results in reduction in glass

transition temperature (Tg), polymer melt viscosity and tensile strength. [5] Various approaches are available to improve dissolution rate of poorly water-soluble drug, including the use of surfactants, inclusion complexation, drug micronization into an amorphous form and SD. [6] In the SD, the drug may be dispersed or solubilized within a polymeric carrier at molecular levels or in the amorphous state, and provide a large surface which leads to significant enhancement in the dissolution process. The improvement in dissolution is mainly attributed to the reduction in particle size, increase in surface area and reduction in crystallinity. Furthermore, no energy is required to breakup the crystal lattice of a drug during the dissolution process, and drug solubility and wettability may be improved by surrounding hydrophilic polymers used in SD. In comparison with traditional methods for preparation of SD, HME is a promising novel technology [7] for improving the bioavailability of water insoluble drugs, and presents many advantages for pharmaceutical applications. It can be used as a continuous process with the absence of organic solvents and subsequent drying steps, which makes scaling up easier. In addition, intense blending and agitation during process prevent the aggregation of drug particles suspending in the molten polymer, leading to a more homogeneous dispersion of fine particles. However, not all the thermal plastic polymer carriers are compatible with the drugs and suitable carriers as well the drug/polymer ratio for a specific drug need to be optimized. In the present studies, we attempted to improve dissolution and bioavailability of Efv by preparation of SD with HME technique. Hydrophilic polymer with certain Tg and backbones will be used to prepare SD. Further pharmacokinetics of Efv in rats was investigated to evaluate the in vivo performance of prepared SD.

II. MATERIALS AND METHODS:

MATERIALS:

Efv was obtained as a gift sample from Emcure Pharmaceuticals ltd (Pune) and all other chemicals were obtained from the college source.

Animals:

Wistar Rats ranging to about 230-290g body weight approximately were used in the study.

METHODS:

2.1 Optimization of Drug: Polymer Ratio [8]

Solubility of Efv and polymer was checked in different solvents such as methanol, ethanol and water. Both the drug and polymer were soluble in ethanol, hence selected as a solvent for optimization of drug: polymer ratio. Drug and polymer (1:1 to 1:5) were solubilised in ethanol. The obtained solution was then poured into the petri plates and films were cast by solvent evaporation method and were observed after 24 hrs at room temperature for their appearance.

2.2 Preparation of HME and NHME formulation

Different concentrations of surfactants in the range of 10%w/w -30%w/w of polymer quantity were studied. Physical mixture of drug, polymer and surfactant were subjected to melt extrusion process. The HME process was carried out using Thermo Scientific Prism Lab Model co-rotating intermeshing twin screw extruder with L/D of 40/1 .The screw speed was adjusted to 100 rpm resulting in residence time in the extruder of less than 1min along with the barrel(melting zone) temperature of 140ºC as melting point of Efv is 139-141 ºC. [2]
Physical mixtures of various formulations were passed through the hot melt extruder. Melt extrudates were cooled at room temperature and were milled using hammer mill, involving coarse milling, fine milling and screening. Milled HME granules were than lubricated and subsequently compressed into tablets. NHME formulation was prepared by direct compression method.

2.3 Dissolution [9]

Tablets were evaluated for dissolution testing using:

Medium: 2% sodium lauryl sulfate in water; 900 mL, degassed
Apparatus 2: 50 rpm
Time: 30 min

2.4 Permeability studies

The prepared tablets were subjected to In vitro permeability test using dialysis membrane LA401. [10] Where the tablets were placed in the dialysis membrane along with dissolution medium. This bag was then introduced into the vessel just above the paddle and below the upper level of dissolution medium.
Medium: 2% sodium lauryl sulfate in water; 900 mL, degassed
Apparatus 2: 50 rpm
Time: up to 7 hrs

2.5 Invivo studies:

Pharmacokinetic evaluation of Efv+ Kollidon VA64+PEG4000 (1:1:0.1), Efv+ Kollidon VA64+ Chremophor EL (1:1:0.1) and Efv+ Kollidon VA64+ sorbiton monolaurate (1:1:0.1) optimised SD were used for in vivo studies in rats weighing 230-290 gm (n=4) of either sex at a dose equivalent to 100 mg/kg of Efv in comparison to Efv pure drug. *In vivo* study protocols were approved by the Institutional Animal Ethics Committee (Regd. No RCPIPER/IAEC/07-2013-14).

Collection of blood sample: [11]

200 µL blood samples were collected (into ependroff tubes containing 20 µL of heparin solution) through tail vein from rats under light ether anesthesia at 30 min, 1 hour, 2 hour, 5 hour, 8 hour and 12 hours after oral administration.

Drug solution: Efv 10 mg/mL suspension in 0.5 % CMC prepared immediately before administration Polymeric formulations were prepared as 20 mg/mL suspensions in 0.5 % CMC prepared immediately before administration

Dose: 100 mg/kg Efv oral

Calibration curve:

Preparation of Standard solutions in plasma as in Table 1.

TABLE 1: PREPARATION OF STANDARD SOLUTION IN PLASMA

Standard drug solution (prepared in Methanol) (µg/mL)	Amount of plasma (µL)	Amount added (µL)	Final concentration in Plasma (µg/mL)
200	995	5	1
200	990	10	2
200	975	25	5
200	963.5	37.5	7.5
2000	995	05	10
2000	990	10	20

Extraction procedure for the plasma samples and calibration curve related samples was as follows

Extraction procedure:

- Blood samples were centrifuged at 1500 rpm for 10 minutes for separation of serum
- To 75 µL of serum sample, 10 µl of internal standard (100 ppm propyl paraben solution in methanol) and 30 µL of 1.0 N NaOH solution was added and this mixture was extracted twice with 2 mL dichloromethane.
- After addition of dichloromethane, each time the samples were vortexed for 5 minutes.
- The vortexed samples were centrifuged at 2000 rpm for 10 minutes.
- Dichloromethane layers (lower layers) were pooled and dried at room temperature.
- The dried dichloromethane extracts were reconstituted in 200 µl mobile phase, filtered through 0.2 µ and processed for HPLC analysis as stated below.

HPLC analysis:

- The HPLC system Agilent 1200 HPLC, a gradient quaternary system (having four ports/reservoirs for mobile phase), a manual rheodyne injector of 20 µm loop with DAD detector (diode array detector system) was used.
- The HPLC column used in this study was Luna C 18 (2) stationary phase with 25 cm length, 4.6 mm internal diameter and 5 µm particle size.

- **HPLC analysis of standard mixture of Efv and propyl paraben**: The different methods were tried to obtain Efv peak well resolved from the peak of internal standard propylparaben. HPLC chromatograph of mixture of Efv and propyl paraben were well resolved by optimized chromatographic conditions and is provided below. It is necessary that the standard mixture of Efv and propyl paraben should passes the system suitability parameters like capacity factor, number of theoretical plates, tailing factor and resolution. With the optimized chromatographic parameters (stationary phase, mobile phase, flow rate, detection wavelength, volume of injection applied) the standard mixture of Efv and propyl paraben is well resolved.

- Initially, the sample of Efv and propylparaben were dissolved in methanol (as all the compounds are soluble in methanol).
- Then, dilution of all the samples made with mobile phase [Efv (5 ppm) + Propyl paraben (5 ppm)] was performed.
- The column was saturated with mobile phase with 1 mL/min flow rate.

Mobile Phase: Methanol: Water (80:20, v/v)
Wavelength of detection: 254 nm
The HPLC method revalidated was found suitable for the estimation of Efv in plasma samples. The mobile phase consists of a mixture of Mobile Phase: Methanol: Water (80:20 v/v).

III. RESULTS AND DISCUSSION

Based on film casting method, 1:1 ratio of Drug: Polymer was finalized as no recrystallization of API was observed in the film after 48 hours of storage. Recrystallization was observed in sample of pure drug only. This indicated that 1:1 drug: polymer ratio was sufficient to hold the amorphous form of API in solid solution. Use of 10%w/w concentration of surfactants gives same solubility enhancement as compared to use of 20%w/w and 30%w/w concentrations. So 10%w/w concentration of surfactants was finalized in formulations and only these formulations were subjected to further study like dissolution, permeability and in vivo study.

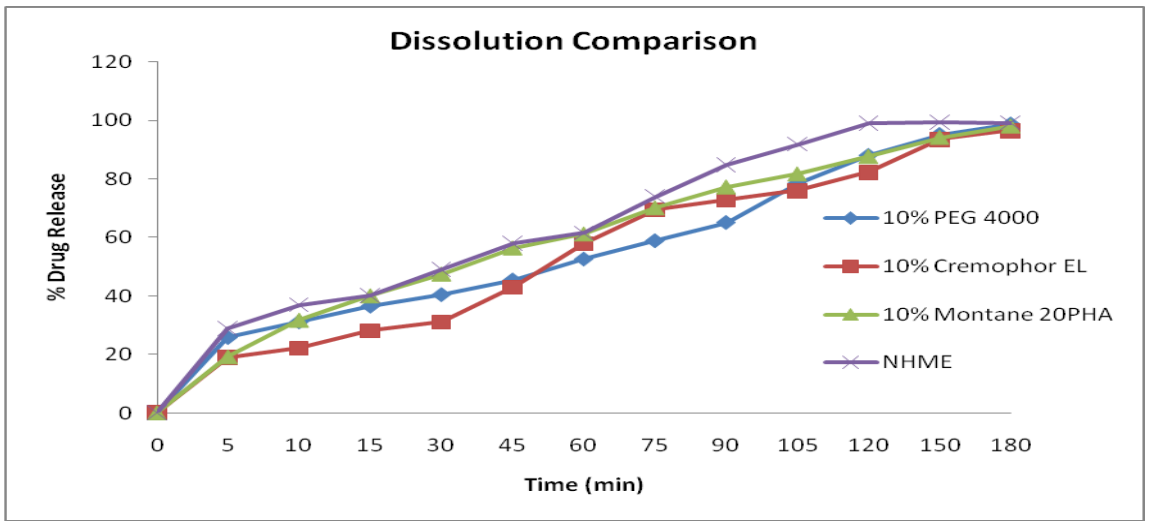

FIGURE 1: DISSOLUTION COMPARISON HME AND NHME FORMULATIONS

Kollidon VA 64 is a hydrophilic polymer and form a gel on the surface of tablet when comes in contact with dissolution media. These tablets shows disintegration pattern by erosion mechanism. The disintegration time (DT) of tablets of HME formulations was about three times more than DT of NHME formulation. Higher DT affected the complete dissolution of HME formulations in 30 min. Office of Generic Drugs (OGD) of US FDA suggested the dissolution time points upto 180 min for Norvir tablets (Ritonavir) [12] may be due to prolong disintegration time of the dosage form. Norvir tablets are also manufactured by using HME technology. [13] So, it was decided to perform dissolution of Efv upto 180min to achieve complete drug release. As expected the complete release was observed at 180 min but, the dissolution rate of HME formulations is quite slower than NHME formulation may be due to higher DT of HME formulations. (as in fig. 1) Different types of surfactants did not show any improvement in dissolution rate or may not be reflected by present dissolution method. Official dissolution method of Efv was unable to discriminate the formulation changes in dissolution profile.

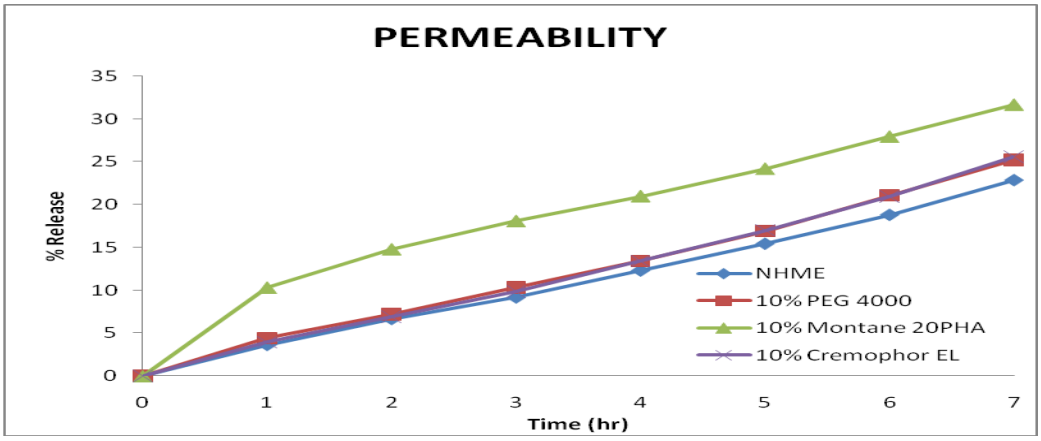

FIGURE 2: PERMEABILITY COMPARISON HME AND NHME FORMULATIONS

Invitro permeability method using dialysis membrane was able to discriminate formulation changes. Formulation with 10%w/w PEG 4000 and Sorbiton monolaurate shows improvement in dissolution rate may be due to higher HLB values of 12 and 18.5 respectively than HLB [14, 15] value 8 of Chremophor EL [16]. Higher is the HLB value of surfactant higher will be the depression in Tg [17] of polymer which leads to lowering of melt viscosity and increases intermolecular separation of polymer. Lower melt viscosity of polymer increases the efficiency of mixing of API and polymer. Increased intermolecular separation in polymer chains improves the molecular level dispersion of API in polymer bed. NHME tablets shows lower in vitro permeability compared to all Efv HME formulations (fig. 2), even though have higher dissolution rate. Increase in, in vitro solubility and permeability may increase the bioavailability which leads to reduction in some fold of Efv dose.

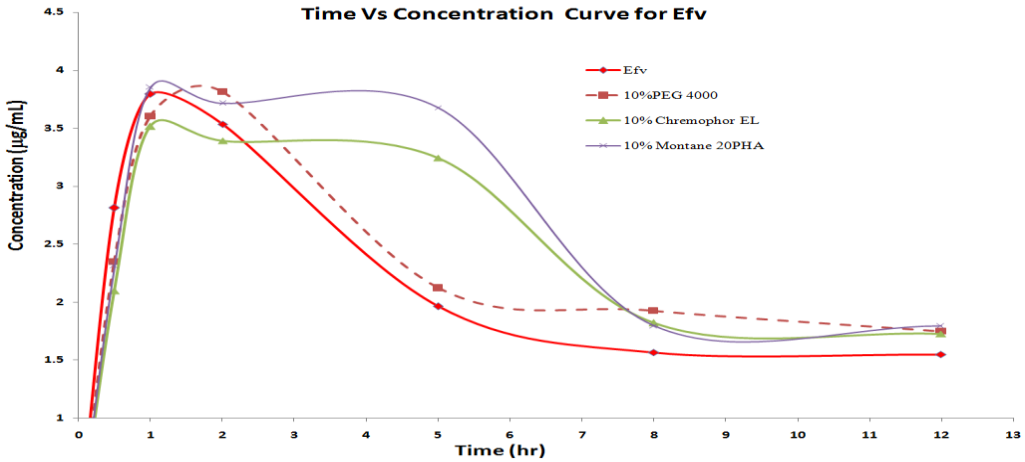

FIGURE 3: ANIMAL STUDY

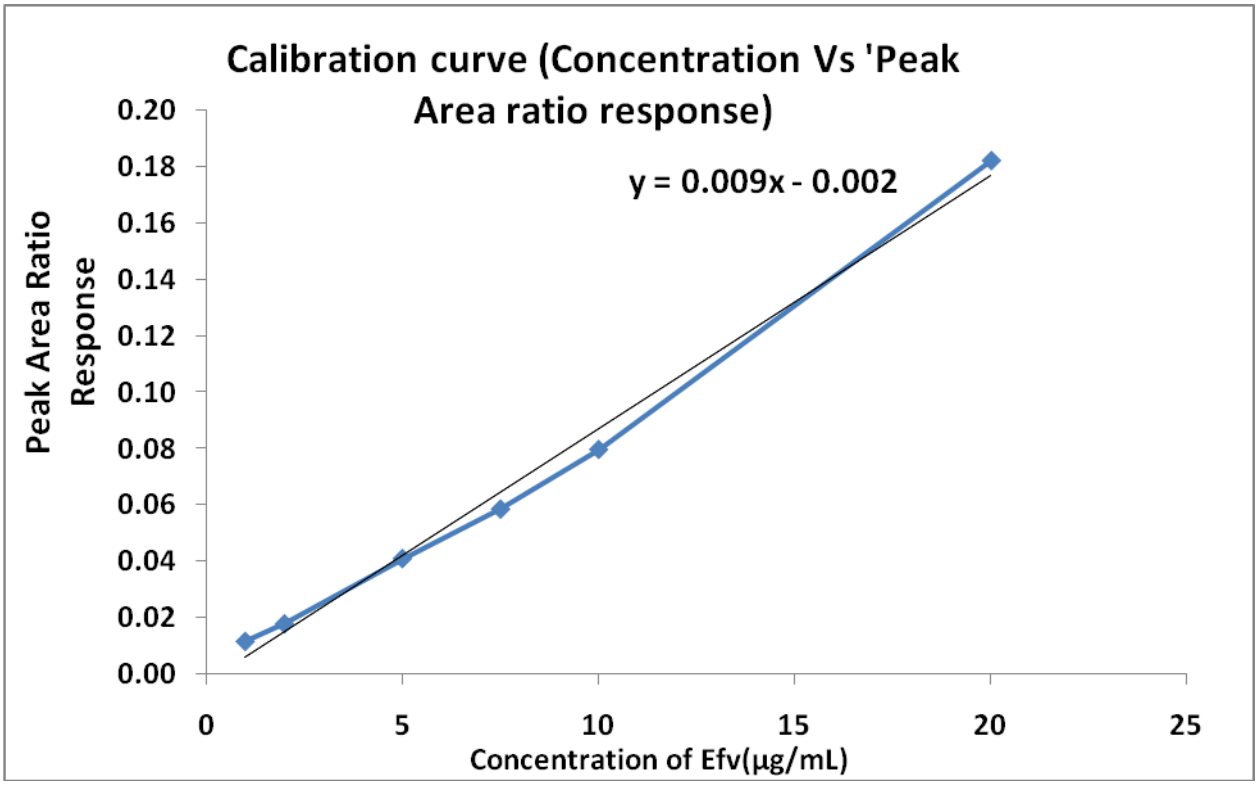

FIGURE 4: CALIBRATION CURVE

Linearity of the method was in the concentration range 0.5- 10 µg/0.5 mL of Plasma (Fig. 4). Plasma concentrations of Efv following the oral administration of API and its SD is shown in Fig. 3. Pharmacokinetic parameters estimated are summarized in Table 2.

Efv was found to be absorbed rapidly when given orally, and a highest peak plasma concentration (Cmax) 4.1 µg/mL was observed at 2.25 hrs after administration of E3 formulation (Montane 20PHA).
All the pharmacokinetic parameters (Table 2) namely Cmax, Tmax, (AUC) ∞ indicated rapid and higher absorption of Efv when administered as SD (E1) prepared using PEG 4000. Similar Cmax value and higher Tmax value was observed with the SD E1 (PEG 4000) compared to those of Efv in NHME form. Higher Cmax and lower Tmax values were observed with E3 formulation and plain API respectively. $AUC_{0-\infty}$ was also much higher in case of SD prepared with PEG 4000 when compared to plain API and other HME formulations. $AUC_{0-\infty}$ was increased from 43 for Efv API to 89, 52 and 52 for E1, E2, E3 formulations respectively. Extent of absorption was increased by 106.98%, 20.93%, and 20.93% in E1, E2, and E3 formulations respectively. Noticeable boost in $AUC_{0-\infty}$ of E1 formulation was observed may be due to high HLB value of PEG 4000 (HLB- 18.5). HLB values of Montane 20PHA and Chremophor EL are 12 and 8 respectively so comparatively less improvement in absorption was observed in E2 and E3 formulations. Higher the HLB value of surfactant higher will be the drop in T_g of polymer during HME process which reduces melt viscosity of polymer and allows maximum and efficient mixing of drug and polymer to form ideal solid solution. Efv is not a substrate [18] of P-gp activity so P-gp inhibitor like Chremophor EL failed to enhance its bioavailability. Montane 20PHA does not have P-gp inhibition activity and has lower HLB value, hence comparatively less improvement in absorption was observed.

TABLE 2: PHARMACOKINETIC PARAMETERS OF EFV

PARAMETERS / SAMPLES	Tmax (hrs)	Cmax (µM)	AUC_{last} (hr*µM)	AUC_{inf} (hr*µM)	MRT_{inf} (hr)
Efv	1.25	3.9	26	43	12
E1	1.50	3.9	28	89	34
E2	1.50	3.6	30	52	14
E3	2.25	4.1	32	52	12

IV. CONCLUSION

SD prepared using PEG 4000 improved the bioavailability where as other SD failed to improve almost none of the pharmacokinetic parameters. This study reveals the solubility enhancement by HME technology and bioavailability enhancement was mainly achieved by surfactant with higher HLB value like PEG 4000. Use of comparatively hydrophobic surfactants like Montane 20PHA not improved the absorption due to its lower HLB value and lack of P-gp inhibition activity. Surfactant like Chremophor EL failed to modify or enhance rate and extent of absorption, as Efv was not a substrate of P-gp activity.

HME technology improved the physical parameters and pharmacokinetic parameters which confirmed the versatile application of this technology in pharmaceutical development of poorly water soluble drugs.

ACKNOWLEDGEMENT

The authors are thankfull to Principal Dr. S. N. Dhole Modern College of Pharmacy (for Ladies), Moshi for providing necessary support and facilities. I am also thankfull to Dr. C. R. Patil for providing his support for carrying out animal study.

REFERENCES

[1] www. Rx list .com
[2] www.drugs.com
[3] Manthena V.S. Varma , Yasvanth Ashokraj ,Chinmoy S. Dey and Ramesh Panchagnula. P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement, *Pharmacological Research 48*, 2003, 347–359.
[4] PMID:10918954 Arzneimittelforschung 2000 Jun
[5] Alazar N. Ghebremeskel , Chandra Vemavarapu and Mayur Lodaya, Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble API: Selection of polymer–surfactant combinations using solubility parameters and testing the processability. *International Journal of Pharmaceutics 328*, 2007, 119–129.
[6] K.P.R. Chowdary and Veeraiah Enturi, Preclinical Pharmacokinetic Evaluation of Efavirenz Solid Dispersions in Two New Modified Starches, *Journal of Applied Pharmaceutical Science Vol. 3 (4 Suppl 1)*, 2013, S89-S92.
[7] Mohammed Maniruzzaman, Joshua S. Boateng, Martin J. Snowden, Dennis Douroumis, A Review of Hot-Melt Extrusion: Process Technology to Pharmaceutical Products, International Scholarly Research Network, ISRN Pharmaceutics, 2012, 1-9.
[8] Kolter K, Karl M, Nalawade S, Rottmann N, Hot Melt Extrusion with BASF Pharma Polymers: Extrusion Compendia, October 2010,pg 80.
[9] www.usp.org
[10] HIMEDIA material safety data sheet
[11] Suresh K. Balani, Laura R. Kauffman, Florencia A. Deluna, and Jiunn H. Lin, Nonlinear Pharmacokinetics Of Efavirenz (Dmp-266), A Potent Hiv-1 Reverse Transcriptase Inhibitor, In Rats And Monkeys, *Drug Metabolism And Disposition Vol. 27, No. 1*, 41-45.
[12] www.fda.gov
[13] www.aidsmeds.com
[14] Shuo Cao, Effect of surfactants on the pretreatment of sugarcane bagasse with dilute ammonia: A thesis by B. S., Henan University, 2006, December 2012, pg 19
[15] Raymond Rowe, Paul Sheskey, Sian Owen, Pharmaceutical Excipients, pg 1546
[16] Raymond Rowe, Paul Sheskey, Sian Owen, Pharmaceutical Excipients, pg 1521
[17] Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble API: Selection of polymer surfactant combinations using solubility parameters and testing the processability. Alazar N. Ghebremeskel , Chandra Vemavarapu, Mayur Lodaya ,International Journal of Pharmaceutics, 328 (2007) 119–129.
[18] Nathalie Berruet, Stephanie Sentenac, Daniel Auchere, François Gimenez, Robert Farinotti, Christine Fernandez, Effect of efavirenz on intestinal p-glycoprotein and hepatic p450 function in rats. Journal of Pharmacy and Pharmaceutical Sciences, August 4, 2005.