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Enhancement of Solubility and Bioavailability of Etravirine Solid Dispersions by Solvent Evaporation Technique with Novel Carriers

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Abstract: *Among the different solubility enhancement techniques, solid dispersion is the most efficient technique in improving the solubility and rate of in-vitro / and in-vivo dissolution of poorly soluble drug substance (s). Etravirine is a new non-nucleoside reverse transcriptase inhibitor of human immune deficiency virus type 1, which is belongs to BCS class IV molecule. In the present study, immediate release solid dispersion of antiretroviral Etravirine was formulated by solvent evaporation technique. Twelve solid dispersions were prepared with 1:1:1 and 1:2:1 ratios of drug: carrier: surfactant. There was significant improvement in the rate of drug release from all 12 solid dispersions and found to be comparable to the dissolution profiles of Innovator product (Intelence® 200 mg Tablets). The solid dispersion formulation (ESE6) comprising Etravirine: Kolliphor P407: surfactant (1:2:1) by solvent evaporation process has shown enhanced solubility about 9 folds and significant improvement in rate of drug release. Polymorphic form of Etravirine has been converted into an amorphous form from crystalline within the solid dispersion formulation. Formulation (ESE6) has shown marked increase in rate of dissolution and bioavailability. $AUC_{0\text{-}inf}$ was increased by 2.1 folds, C_{max} increased by 2.3 folds and t_{max} reduced by 1 hr as compared to the Etravirine.*

Key words: *Bioavailability, Etravirine, Solubility, Solvent evaporation.*

I. Introduction

The solubility behavior of drugs remains one of the most challenging aspects in formulation development. With the advent of combinatorial chemistry and high throughput screening, the number of poorly water soluble compounds has dramatically increased [1]. There are several pharmaceutical strategies available to improve the aqueous solubility of poorly soluble drugs: solid dispersion, solubilization using surfactant, the use of co-solvent, reduction of particle size, hydrotropy and the use of aqueous soluble derivatives or salts. Among all technique solid dispersion, is the most efficient technique from the dispersion in carrier more specially define the system has the dispersion of the one or more active ingredient in an inert matrix at solid state perform by melting method, solvent evaporation method and melting solvent [2]. Drug release is a crucial and limiting step for oral drug bioavailability, particularly for drugs with low gastrointestinal solubility and high permeability. By improving the drug release profile of these drugs, it is possible to enhance their bioavailability and reduce their side effects. Solid dispersions are one of the most successful strategies to improve the drug release of poorly soluble drugs [3]. . Solid dispersion of drug in a water soluble polymer has been shown to be one of the most promising strategies to improve solubility [4].

Solid dispersion is well established as a formulation system for enhancing the bioavailability of poor water soluble active pharmaceutical ingredients. Most poorly water-soluble APIs exist in an amorphous form within the solid dispersion, thereby enhancing their dissolution and oral absorption by attaining a highly supersaturated state above their equilibrium solubility [5]. Although there was a great interest in solid dispersion systems during the past four decades to increase dissolution rate and bioavailability of poorly water-soluble drugs, their commercial use has been very limited, primarily because of manufacturing difficulties and stability problems. Solid dispersions of drugs were generally produced by melt or solvent evaporation methods. The

materials, which were usually semisolid and waxy in nature, were hardened by cooling to very low temperatures [6]. The mechanisms for the enhancement of the dissolution rate of solid dispersions have been proposed by several investigators. Drugs molecularly dispersed in polymeric carriers may achieve the highest levels of particle size reduction and surface area enhancement, which result in improved dissolution rates. Furthermore, no energy is required to break up the crystal lattice of a drug during dissolution process, and drug solubility and wettability may be increased by surrounding hydrophilic carriers [7].

Etravirine is a new non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immune deficiency virus type 1 (HIV-1), chemically 4-[6-Amino-5- bromo-2-[(4-cyanophenyl) amino] pyrimidin-4-yl] oxy- 3, 5-dimethylbenzonitrile. Etravirine is a highly potent inhibitor of HIV-1 replication, with activity in the nanomolar range comparable to that of the commonly prescribed NNRTI Etravirine [8].

II. Materials and Methods

Materials

INTELENCE® (Etravirine) 200 mg conventional tablets were obtained from Tibotec Pharmaceuticals Ltd, manufactured by Janssen Cilag S.p.A., Latina, Italy. Etravirine was generous gift from Hetero drugs limited, Hyderabad, India. Kolliphor P 407 and Kolliphor P188 were obtained from BASF, US. Kolliwax GMS II, Kolliphor RH-40, Kolliphor EL, Kolliphor HS-15, Kolliphor TPGS, Kollidon 30 and Soluplus were gifted from BASF, Germany. Kleptose ® HPB was obtained from Roquette Pharma, France. HPMC AS and HPMC 2.5 cPs were gifted by Dow Chemicals, USA. All other chemicals used were of analytical grade.

Methods

Preliminary solubility studies of Etravirine

A solubility measurement of etravirine was performed according to a published method [9]. Initially 1 part of Etravirine was added to 25ml of aqueous solution of water soluble carriers like Kolliphor RH-40 / Kolliphor EL / Kolliphor TPGS/ Kolliphor HS-15 / Kolliphor P 188 / Kolliphor P 407 / Kolliwax GMS II/, Soluplus / Docusate sodium (DSS 100%) / Kleptose HPB / HPMC AS / Kollidon 30 / HPMC 2.5 cPs / mixture of Kolliphor P407 and P188 in 1:1 ratio with equal proportion of Sodium lauryl sulphate (SLS) and were taken in screw capped bottles. Samples were shaken for the 48 hours at room temperature. Subsequently, the suspensions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Etravirine in UV/Visible spectrophotometer at 235 nm.

Preparation of solid dispersions of Etravirine by solvent evaporation method

Etravirine solid dispersions of twelve formulations were prepared by using various carriers shown in Table 1 like Kleptose HPB, Kolliwax GMS II, Kolliphor P407, HPMC AS, Soluplus and mixture of Kolliphor P 407 and P188 in 1:1 ratio etc., with surfactant, i.e., Sodium laury sulphate (SLS) in proportions viz. 1:1:1, 1:2:1 (Drug: Carrier: Surfactant). The drug and carrier along with SLS was dissolved in Methanol and triturated in dry mortar until the solvent is evaporated and a clear film of drug and carrier was obtained. Then the dispersion was subjected to Methanol solvent evaporation by placing in vacuum dryer at 50°C chamber for 30 min period. The resultant solid dispersion was scraped out with a spatula. Solid dispersions were pulverized in a mortar and pestle and passed through a 420 µm (ASTM #40 mesh) mesh before packing in an airtight container [10].

Table 1: Composition of Etravirine solid dispersions by Solvent evaporation method

Ingredients	ESE1	ESE2	ESE3	ESE4	ESE5	ESE6	ESE7	ESE8	ESE9	ESE10	ESE11	ESE12
Etravirine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Kleptose HPB	2.0	4.0	-	-	-	-	-	-	-	-	-	-
Kolliwax GMS – II	-	-	2.0	4.0	-	-	-	-	-	-	-	-
Kolliphor P407	-	-	-	-	2.0	4.0	-	-	-	-	-	-
HPMC AS	-	-	-	-	-	-	2.0	4.0	-	-	-	-
Soluplus	-	-	-	-	-	-	-	-	2.0	4.0	-	-
Mixture of Kolliphor P 407 and P188 in 1:1 ratio	-	-	-	-	-	-	-	-	-	-	2.0	4.0
SLS	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Methanol (mL)	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

Solubility studies of Etravirine solid dispersions by solvent evaporation method

Solubility measurements of Etravirine were performed according to a published method [9]. Samples were shaken for the 48 hours at room temperature. Subsequently, the suspensions were filtered through a Whatman filter paper no 1. Filtered solutions were analyzed for the Etravirine in UV/Visible spectrophotometer at 235 nm.

Evaluation of Etravirine solid dispersions

Solid dispersions obtained from the above method were tested for their % Practical yield, % Assay and *in vitro* release studies (30 mg of Avicel PH102 was added and filled in hard gelatine capsule shells).

% Practical Yield

Percentage practical yield was calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. SDs were collected and weighed to determine practical yield (PY) from the following equation.

$$\% \text{ Practical Yield} = \frac{\text{Practical weight (Solid dispersion)}}{\text{Theoretical weight (Drug+Polymer+Surfactant)}} \times 100$$

% Assay

10 units of Solid dispersions were taken in a mortar and mixed well using pestle, from which weight equivalent to 200mg of Etravirine taken and dissolved in 100 ml of methanol. The solution was filtered, diluted suitable and drug assay was analyzed at λ_{max} 235 nm against blank by UV/Visible spectrophotometer.

***In vitro* drug release studies**

The dissolution test was performed using USP type 2 dissolution apparatus (paddle method) with 900 ml of 1.0 % SLS in 0.01 M HCl in two phases: Phase 1: 500 ml of degassed 0.01 M HCl (First 10 min) and Phase 2: Add 400 ml of 2.25% SLS in 0.01 M HCl (After 10 min) at an temperature of 37 ± 0.5^0C with a paddle speed of 50 rpm. The solid dispersion equivalent to 200 mg of Etravirine was added and the sample of 10ml were withdrawn and replaced with the same volume of the dissolution medium at 5, 10, 15, 30, 45, 60 and 90 minutes time intervals. The obtained samples were analyzed by using UV-Visible spectrophotometer at 235nm. The cumulative percentage release was calculated.

Characterization

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra for Etravirine (pure drug), physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The IR spectrum of the samples was prepared using KBr (spectroscopic grade) disks by means of hydraulic pellet press at pressure of seven to ten tons [11, 12].

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5ºC/min, over a temperature range of 0 to 250ºC [12] .

Powder X-ray diffraction (p XRD)

A Bruker D8 diffractometer was used to perform powder X-ray diffraction (PXRD) of all samples. A Cu K-α 1 tube was the source, set at 40 KV and 50mA. A scan from 2 to 60^0 2 θ was carried out at a rate of 0.01220^0 2θ/s. The diffractometer was calibrated using powdered α-alumina. Hot-melt extruded samples were ground before analysis [12, 13].

Scanning electron microscopy (SEM)

The shape and surface morphology of the Etravirine and optimized formulation of solid dispersion prepared by solvent evaporation was examined using XL 30 model JEOL 6800 scanning electron microscope (Japan) [12, 14].

Stability studies

Prepared solid dispersions were placed inside sealed 40cc HDPE container with child resistant cap under controlled temperature environment inside stability chamber (Thermo Lab, India) with relative humidity of 75%±5%RH and temperature of $40\ ^0C\pm2^0C$ for stability studies. Samples were removed after 1, 2, 4 and 6 months, evaluated for % drug assay and *in vitro* dissolution study and compared with those SD tested immediately after preparation [15].

***In vivo* studies**

Animal preparation

Healthy male Wistar rats were (weighing approximately 250±25 g) selected for this study, all the animals were healthy during the period of the experiment. The study was conducted with prior approval of Institutional Animal Ethical Committee (IAECNO: P28/ VCP/ IAEC/ 2014/ 03/ DBP/ AE12). All efforts were made to maintain the animals under controlled environmental conditions (Temperature $25^0C \pm 2^0C$, Relative Humidity 45%±5%RH and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply. Rats were fed with standard diet and water ad libitum.

Pharmacokinetic study [16]

The pharmacokinetic characteristics for Etravirine pure drug suspension and optimized preparation of solid dispersions were evaluated using twelve healthy Male Wister rats weighing 250±25g. Rats were divided in to two groups at random, each group containing six animals. First group was administered Etravirine (as such) suspension was prepared in 0.5% w/w of HPMC 2.5cPs, second group was administered optimized preparation of solid dispersion suspension was prepared in 0.5% w/w of HPMC 2.5cPs by oral route at an equivalent dose of 200 mg/kg body weight. About 500 µl of blood was withdrawn from retro orbital plexus at different time intervals such as 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00 and 24.00h. Blood samples were transferred into eppendorf tubes containing heparin in order to prevent blood clotting. The samples were centrifuged immediately at 4000 rpm and the plasma was stored in light-protected container at -20 ^{0}C till analysis.

Determination of Etravirine in Rat plasma by HPLC method [17]

Determination of Etravirine by high performance liquid chromatography using a RP-C18 chromatographic column, Phenomenex Kinetex (150 mm × 4.6 mm i.d) and the mobile phase consisted of 20mM potassium dihydrogen phosphate aqueous solution (40%) and Acetonitrile (60%). The pH was adjusted to 3.2 using phosphoric acid. At a flow rate 1 ml /min and the wavelength detection was 304 nm. Retention times of Etravirine and internal standard Itraconazole was 2.4 and 5.32min respectively.

Pharmacokinetic analysis

The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (C_{max}), time to attain C_{max} i.e., T_{max} and $t_{½}$ values, area under plasma concentration–time curve from zero to the last sampling time (AUC_{0-t}), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\ inf}$). AUC_{0-t} was calculated by the linear trapezoidal rule and $AUC_{0-\infty}$ from the following formula.

$AUC_{0-\ inf} = AUC_{0-t} + C_t / K_E$

III. Results and Discussion

Preliminary solubility studies of Etravirine

In case of solid dispersions, initially preliminary solubility analysis were carried out to select the appropriate water soluble carriers for the preparation of solid dispersion in which Etravirine pure drug solubility was found to be 0.07±0.03 mg/ml. From this physical mixture of Drug: Kolliphor P 407: SLS in the ratio of 1:1:1 shown highest drug solubility i.e. 0.20 ±0.01mg/ml when compared with other physical mixtures. For all the water soluble carriers used in preliminary solubility studies, except Kolliphor P407, Kleptose HPB, Kolliwax GMS II, HPMC 2.5 cPs, mixture of Kolliphor P407 and P188, and Soluplus gave turbid solutions. The results are tabulated in Table 2 and graphical representation was shown in (fig. 1).

Table 2: Preliminary solubility studies of Etravirine with different polymer and Surfactant

S. No	Sample (Physical mixtures)	Ratio	Solubility(mg/ml)*
1.	Pure drug	-	0.07±0.03
2.	Drug: Kolliphor RH-40:SLS	1:1:1	0.09 ±0.04
3.	Drug: Kolliphor EL:SLS	1:1:1	0.10 ±0.02
4.	Drug: Kolliphor TPGS:SLS	1:1:1	0.12 ±0.01
5.	Drug: Kolliphor HS-15:SLS	1:1:1	0.13 ±0.04
6.	Drug : Kolliphor P188:SLS	1:1:1	0.16±0.03
7.	Drug : Kolliphor P 407:SLS	1:1:1	0.20±0.01
8.	Drug : Kolliwax GMS II :SLS	1:1:1	0.10±0.02
9.	Drug : Soluplus:SLS	1:1:1	0.12 ±0.01
10.	Drug : DSS 100%:SLS	1:1:1	0.10±0.01
11.	Drug : Kleptose HPB:SLS	1:1:1	0.12±0.04
12.	Drug : HPMC AS:SLS	1:1:1	0.11±0.02
13.	Drug: Kollidon 30:SLS	1:1:1	0.09±0.02
14.	Drug: HPMC 2.5 cPs:SLS	1:1:1	0.10±0.01
15.	Drug: mixture of Kolliphor P407 and Kolliphor P188:SLS	1:1:1	0.11±0.01

***** Range, n=3

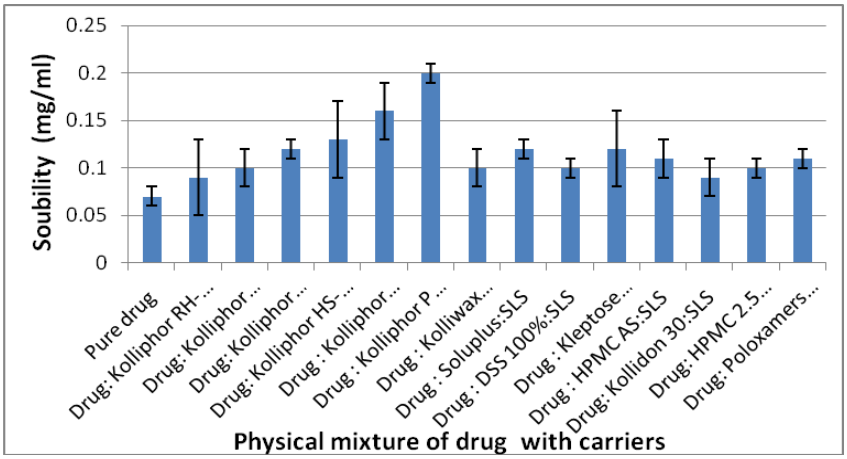


Fig. 1: Solubility studies of Etravirine physical mixture

Preparation of Etravirine solid dispersions

Solid dispersions of Etravirine were prepared by using Kolliphor P407, Kleptose HPB, Kolliwax GMS II, HPMC 2.5 cPs, mixture of Kolliphor P188 and Kolliphor P407 in 1:1 ratio, and Soluplus. In the present investigation 12 formulations were prepared and their complete composition was shown in Table 1. All the solid dispersions prepared were found to be fine and free flowing powders.

Solubility studies of Etravirine solid dispersions

Different formulations of solid dispersions were prepared by solvent evaporation method with their respective carrier along with surfactant. After preparation of solid dispersion, solubility of drug substance was carried out. The formulation (ESE6) with Kolliphor P407 in the ratio of 1:2:1 (drug: carrier: surfactant) shown highest solubility i.e. 0.62±0.04 mg/ml, better improvement was found in the solubility when compared to that of the pure drug (Pure drug solubility is 0.07±0.03 mg/ml). The results are tabulated in Table 3 and graphical representation was shown in fig. 2.

Table 3: Solubility studies of solid dispersions prepared by solvent evaporation method

S. No.	Formulation code	Solubility (mg/ml)*
1.	Etravirine	0.07±0.03
2.	ESE1	0.41±0.02
3.	ESE2	0.43±0.02
4.	ESE3	0.46±0.03
5.	ESE4	0.34±0.01
6.	ESE5	0.51±0.01
7.	ESE6	0.62±0.04
8.	ESE7	0.43±0.04
9.	ESE8	0.39±0.02
10.	ESE9	0.45±0.01
11.	ESE10	0.37±0.02
12.	ESE11	0.46±0.04
13.	ESE12	0.42±0.01

***** Range, n= 5

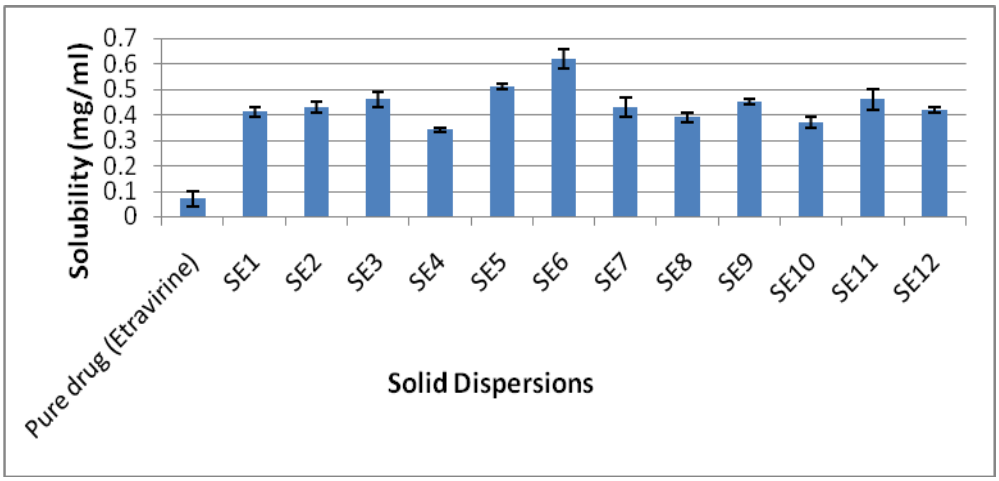


Fig. 2: Solubility studies of Etravirine solid dispersion

% Practical yield and % Assay

The results of % practical yield for all formulations of solid dispersions found to be 92.87% - 98.68%. Maximum yield was found to be 98.68% in formulation ESE6. The Assay of the prepared solid dispersions was found to be in the range of 86.64 - 96.05 %. Maximum % assay i.e. 96.05% was found in the formulation ESE6. The results of % practical yield studies and % Assay are shown in Table 4.

Table 4: % Practical yield and % Assay of Etravirine solid dispersions

S. No	Formulation	% Yield	% Assay*
1	ESE1	94.24	91.47±1.24
2	ESE2	93.45	93.47±1.43
3	ESE3	94.65	87.62±1.72
4	ESE4	94.06	86.6±1.21
5	ESE5	97.15	92.45±1.56
6	ESE6	98.68	96.05±2.20
7	ESE7	93.72	93.50±3.15
8	ESE8	94.22	94.52±2.30
9	ESE9	92.87	91.53±3.45
10	ESE10	94.26	92.57±3.70
11	ESE11	94.68	93.50±2.85
12	ESE12	93.18	94.52±2.40

*Mean ± SD, n= 3

***In vitro* dissolution studies**

The % cumulative drug release in FDA recommended dissolution media for formulations ESE1-ESE12, as such drug and corresponding Innovator product are tabulated in Table 5 and 6. It shows the cumulative percent drug released as a function of time for all formulations. The cumulative percent drug released after 90 min was 56.8%, 60.2%, 88.6%, 89.6%, 98.8%, 99.2%, 72.4%, 79.5%, 80.2%, 84.2%, 82.1% and 87.6 % for ESE1-ESE12 respectively and was 38.9 % for pure drug, where as Innovator product showed 92.8% in 90 min. *In vitro* studies revealed that there is marked increase in the dissolution rate of Etravirine from all the solid dispersions when compared to pure Etravirine itself. The rate of drug release form the solid dispersion ESE5 and ESE6 was found to be on higher side and complete drug release in 90 minutes as compared to the drug release profiles of Innovator product in the same dissolution media. The solid dispersion formulation ESE6 comprising Etravirine, Kolliphor P407 and SLS in 1:2:1 ratio has shown complete drug release and at faster rate as compared with rest of solid dispersion formulations and dissolution profiles of Innovator product. This may be attributed to the increase in drug wettability, conversion to amorphous form and solubilization of the drug due to hydrophilic carrier. The increase in dissolution rate is in the order of Kolliphor P407> Kolliwax GMS II> mixture of Kolliphor P 407 and 188 > Soluplus > HPMC AS> Kleptose HPB. The graphical representation of solid dispersions of ESE1 – ESE6 and ESE7 – ESE12 is depicted in (fig. 3 and 4) respectively.

Table 5: I*n vitro* dissolution profiles of pure drug, Innovator product and different formulations of Etravirine solid dispersions (ESE1-ESE6)

Time in Min	Cumulative % drug release*							
	Etravirine	Intelence® 200 mg	ESE1	ESE2	ESE3	ESE4	ESE5	ESE6
0	0	0	0	0	0	0	0	0
5	14.9±2.5	22.4±2.9	21.5±1.3	23.3±3.5	26.9±2.5	26.9±3.7	23.3±3.4	40.1±2.3
10	20.0±2.7	29.4±1.4	31.0±2.4	28.8±2.3	41.1±1.5	37.1±2.4	36.2±1.4	59.2±2.8
20	27.4±2.2	42.8±1.8	32.5±3.3	31.8±1.5	59.0±3.2	58.0±1.2	46.3±2.3	68.5±2.2
30	29.2±1.4	52±1.3	33.1±2.6	32.5±1.6	68.5±3.3	63.5±3.8	72.1±2.9	77.2±2.3
45	30.3±1.5	66.8±1.6	36.0±2.4	37.5±1.3	76.2±1.4	78.2±2.5	83.2±1.4	89.4±3.0
60	34.9±1.2	78.2±1.7	41.2±4.3	41.9±1.8	82.2±4.4	84.2±1.6	91.5±3.6	96.6±1.6
90	38.9±0.9	92.8±2.2	56.8±3.4	60.2±1.2	88.6±1.3	89.6±1.8	95.8±3.3	99.2±2.3

*****Mean±SD, n=3

Table 6: In vitro dissolution profile of pure drug, Innovator product and different formulations of Etravirine solid dispersions (ESE7-ESE12)

Time in Min	Cumulative % drug release*							
	Etravirine	Intelence® 200 mg	ESE7	ESE8	ESE9	ESE10	ESE11	ESE12
0	0	0	0	0	0	0	0	0
5	14.9±2.5	22.4±2.9	26.8±2.0	30.3±2.5	28.2±2.9	29.1±1.9	25.2±3.7	26.6±2.9
10	20.0±2.7	29.4±1.4	30.3±2.9	36.9±1.5	36.8±3.0	35.6±2.5	32.6±1.9	36.7±3.9
20	27.4±2.2	42.8±1.8	46.5±3.3	46.5±2.7	48.2±2.6	47.8±2.7	44.6±2.5	58.8±2.0
30	29.2±1.4	52±1.3	59.5±3.8	58.2±2.6	59.4±2.3	58.2±2.4	56.8±1.4	63.4±1.4
45	30.3±1.5	66.8±1.6	64.5±1.9	64.5±2.2	66.2±2.8	67.6±3.4	68.5±2.7	77.7±3.8
60	34.9±1.2	78.2±1.7	70.9±3.3	77.3±2.9	75.2±2.4	78.2±2.0	79.9±2.9	84.4±2.2
90	38.9±0.9	92.8±2.2	72.4±3.1	79.5±2.8	80.2±2.8	84.2±2.2	82.1±3.8	87.6±1.7

*****Mean±SD, n=3

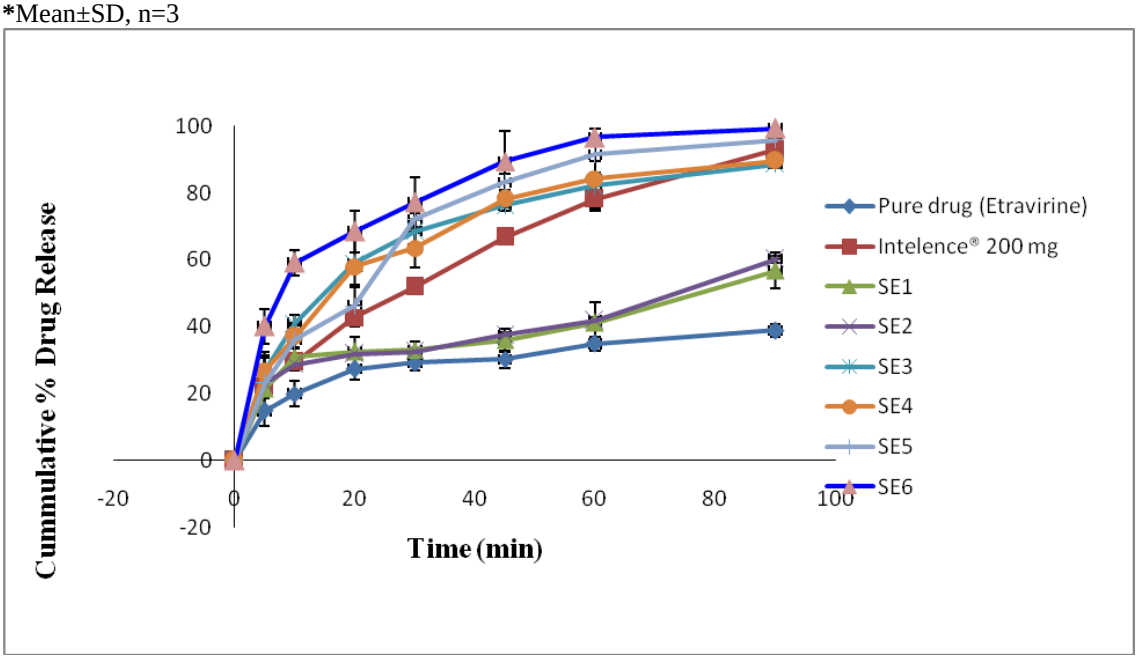


Fig. 3: Comparative In vitro dissolution profiles of Etravirine, Innovator and Etravirine solid dispersions (ESE1-ESE6).

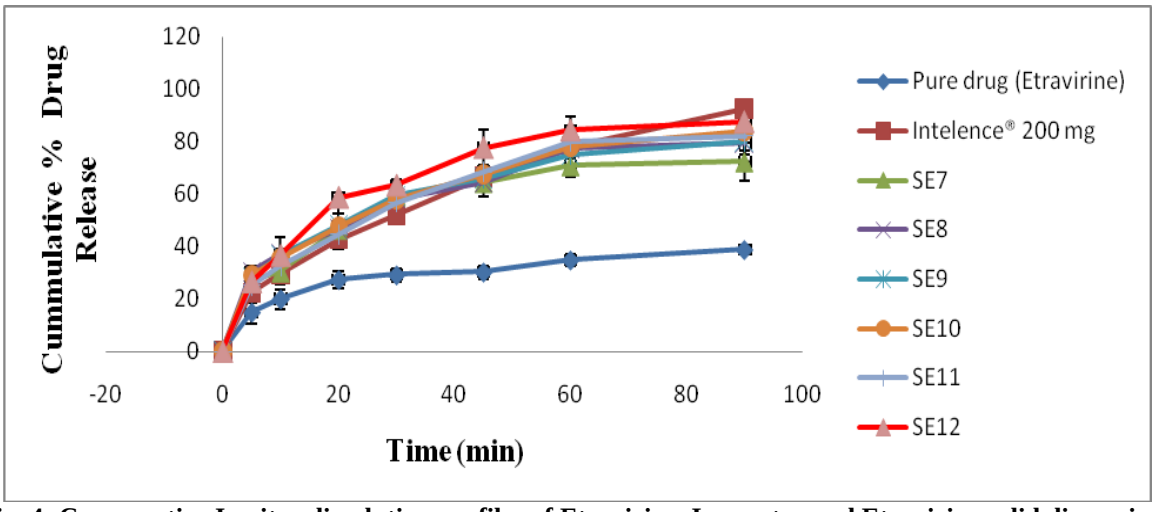


Fig. 4: Comparative In vitro dissolution profiles of Etravirine, Innovator and Etravirine solid dispersions (ESE7-ESE12).

FTIR studies

The prominent peaks of pure drug Etravirine was observed (Fig. 5) in the region of 3410.26 cm^{-1} due to the (Aromatic primary amine stretching), a peak at 2368.86 cm^{-1} due to aryl C=N stretching and a peak at 2978.19 cm^{-1} due to Aromatic C-H stretching. At the lower frequencies 650 cm^{-1} (C-Br), 1365.65 cm^{-1} (primary and tertiary amine), 1188.19 cm^{-1} (ether C-O-C stretching) observed. Kolliphor P407 (fig. 6) shows the prominent peak at 3410.26 cm^{-1} due to polymeric OH stretching, a peak at 2978.19 cm^{-1} due to the (aliphatic CH_3 stretching) and a peak at 1188.19 cm^{-1} due to (C-O-C stretching). Physical mixture (fig. 7) of the drug and Kolliphor P407 shows summation of the spectra of the drug and Kolliphor P407 equivalent to the addition of the spectrum of polymer and drug. This indicates that interaction has occurred with simple physical mixture of drug and polymer. In case of optimized solid dispersion preparation (ESE6) (Fig. 8) shows overlapping of O-H and N-H group and broadening of peak was observed. However other peaks related to C-O-C, C-H stretching remains unchanged. This indicates that overall symmetry of the molecule might not be significantly changed.

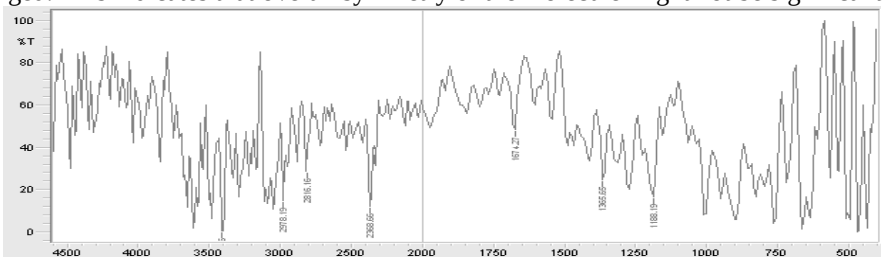


Fig. 5: FTIR spectra of pure drug

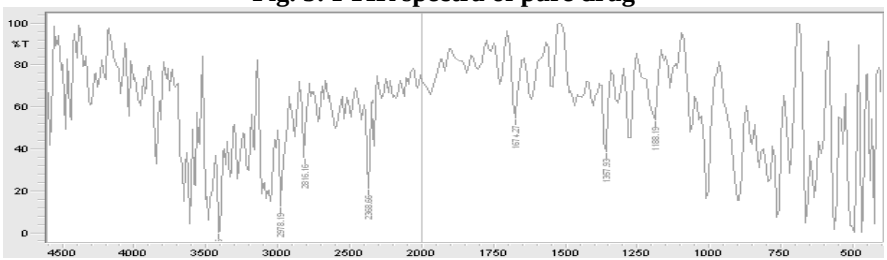


Fig. 6: FTIR spectra of Kolliphor P407

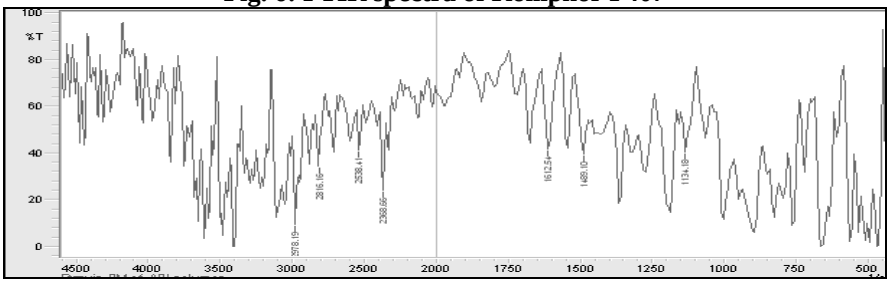


Fig. 7: FTIR spectra of Physical mixture of Etravirine+Kolliphor P407 + SLS

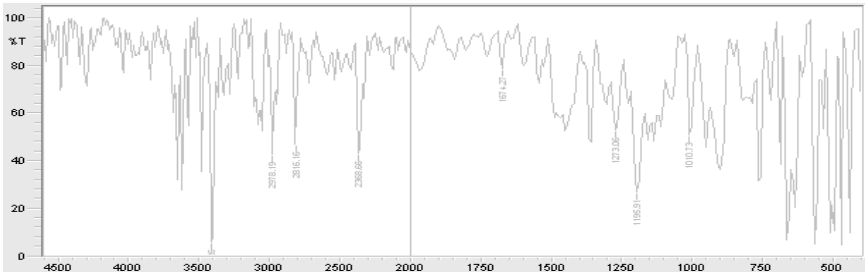


Fig. 8: FTIR spectra of formulation ESE6 solid dispersion

Differential Scanning Calorimetry

The DSC thermo grams of Pure Etravirine showed in (Fig. 9), sharp endothermic peak at melting point 265 ^{0}C, indicating that the drug is highly crystalline. The absence of drug peak in the solid dispersion formulation ESE6 indicating the drug was converted into an amorphous form. As the intensity of the endotherm was markedly decreased in the drug - Kolliphor P407 with SLS solid dispersion, the faster dissolution rate of the drug from the solid dispersion is attributed to the reduction in the crystallinity of the drug. Crystallization inhibition is attributed to the entrapment of the drug molecules in the polymer matrix during solvent evaporation.

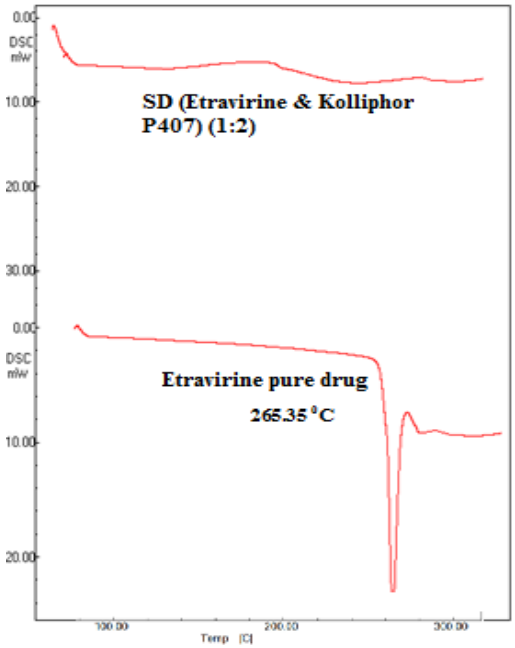


Fig. 9: DSC thermograms of Etravirine pure drug and optimized formulation ESE6.

X-Ray Diffraction patterns

The Etravirine solid dispersions were analyzed in Bruker A6 advanced PXRD instrument to find out whether the solid dispersions of various drug polymer ratios are crystalline or amorphous. The presence of numerous distinct peaks in the XRD spectrum indicates that etravirine was present as a crystalline material. The XRD pattern depicted by physical mixture reveals a decrease in the number of peaks which probably represents decrease in crystallinity. On the other hand, the spectrum of optimized formulation ESE6 of solid dispersion was characterized by the complete absence of any diffraction peak, which is characteristic of an amorphous compound (fig. 10). The enhancement in the dissolution rate of the drug from the drug-Kolliphor P407 solid dispersion is ascribed to the marked reduction in the crystallinity of the drug.

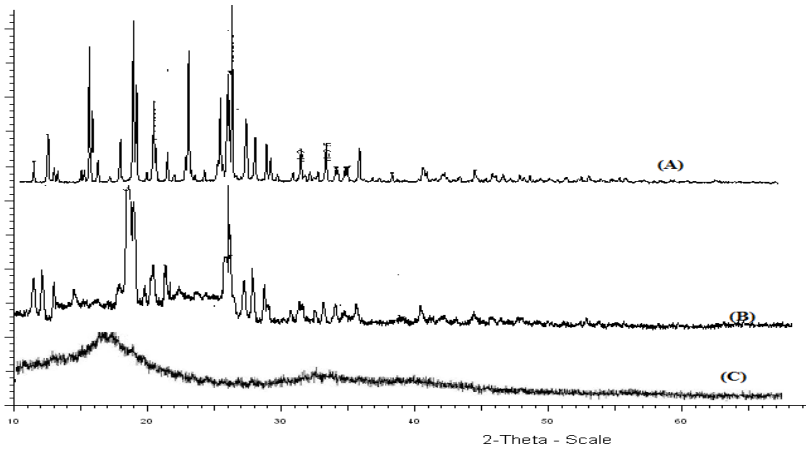
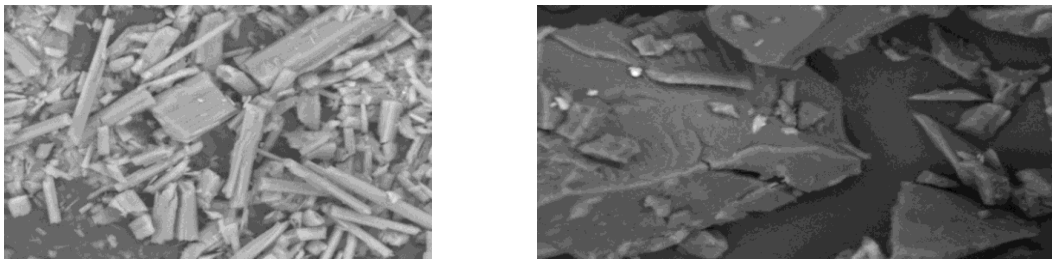


Fig. 10: X-Ray powder diffractograms of Etravirine pure drug (A), Physical mixture (B) and optimized formulation ESE6 (C)

SEM Studies

SEM photographs for pure drug (a) and optimized formulation ESE 6 (b) are shown in fig. 11. The drug crystals seemed to be smooth-surfaced, irregular in shape and size. In case of Solid dispersions, it was difficult to distinguish the presence of drug crystals. The drug surface in solid dispersion seems to be more porous in nature. Solid dispersions appeared as uniform and homogeneously mixed mass with wrinkled surface. Drug crystals appeared to be incorporated into the particles of the polymers. The solid dispersion looked like a matrix particle. The results could be attributed to dispersion of the drug in the molten mass of the polymer.



a. Pure drug (Etravirine) **b. Optimized Solid dispersion**

Fig. 11: SEM pictures of drug and optimized Solid dispersion formulation ESE6

Stability studies

Optimized formulation (ESE6) was selected for stability studies on the basis of faster rate of drug release and complete cumulative % drug release. The resulting solid dispersion of ESE6 eq. to 200 mg of Etravirine was filled in empty hard gelatin capsules, placed in 40 CC (Low weight) High Density Poly Ethylene (HDPE) of 30's count and sealed properly. Stability studies were conducted for 6 months at Accelerated stability conditions according to ICH guidelines. The physical state of the drug was characterized by XRD after charging for 6 months (fig. 12). The Assay and drug release (at 90 minutes) was evaluated at initial 1 month, 2 months, 3 months and 6 months of stability loading. Based on the results it was concluded that the test product ESE6 was found to be stable during a 6-month period. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences which is depicted in Table 7.

Table 7: Stability Evaluation parameters of Etravirine solid dispersion formulation (ESE6) charged at 40 ±2^{0}C /75 ±5%RH

Solid dispersion formulation (ESE6)	% Assay	*In-vitro* drug release (%)
Initial	96.05	99.20
1 Month	95.50	98.60
2 Months	95.15	97.95
3 Months	95.05	96.50
6 Months	94.20	96.05

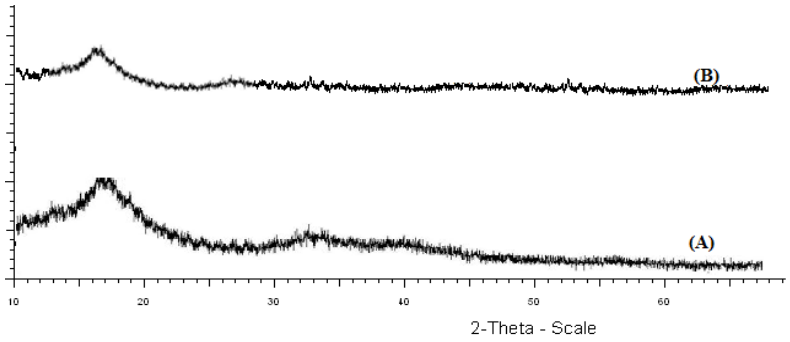


Fig. 12: X-Ray powder diffractograms of Etravirine solid dispersion formulation ESE6 (A), after 6 months of stability studies (B)

Pharmacokinetic parameters comparison for Etravirine pure drug suspension and optimized formulation of solid dispersion (ESE6)

The Etravirine plasma concentrations in rats treated with optimized preparation of solid dispersion was significantly higher than those treated with pure drug suspension. Plasma pharmacokinetic parameters of Etravirine after oral administration of the formulation to Wister rats are shown in Table 8. Based on the results, it was clearly evident that Etravirine from an optimized preparation of solid dispersion ESE6 was significantly increased in comparison with that of the pure drug (Etravirine suspension). C_{max} of the optimized preparation of solid dispersion was 4.84 µg /ml, ($p<0.05$) and was significantly higher as compared to C_{max} of the pure drug suspension, i.e., 2.08 µg/ml. T_{max} of optimized formulation (ESE6) and pure drug suspension was 1.00 and 2.00 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. $AUC_{0\text{-}inf}$ for optimized solid dispersion formulation was 8.05µg h/ml, significantly higher than the pure drug suspension 3.85 µg h/ml. Statistically, $AUC_{0\text{-}t}$ of the optimized preparation of solid dispersion was significantly higher ($p<0.05$) as compared to pure drug suspension. Higher amount of drug concentration in blood indicated better systemic absorption of Etravirine from optimized solid dispersion formulation (ESE6) as compared to the pure drug suspension (fig. 13).

Table 8: Pharmacokinetic parameters of Etravirine from an Optimized formulation of solid dispersion and pure drug

Pharmacokinetic Parameters	Etravirine (API)	Etravirine Capsules (ESE6)
C_{max} (µg/ml)	2.08±1.32	4.84±0.56
$AUC_{0\text{-}t}$ (µg h/ml)	2.62±1.55	7.54±1.74
$AUC_{0\text{-}inf}$ (µg h/ml)	3.85±0.24	8.05±0.45
T_{max} (h)	2.00±0.05	1.00±0.04
$t_{1/2}$ (h)	3.32±0.01	4.52±0.04
K el (h^{-1})	0.194±1.22	0.151±1.42

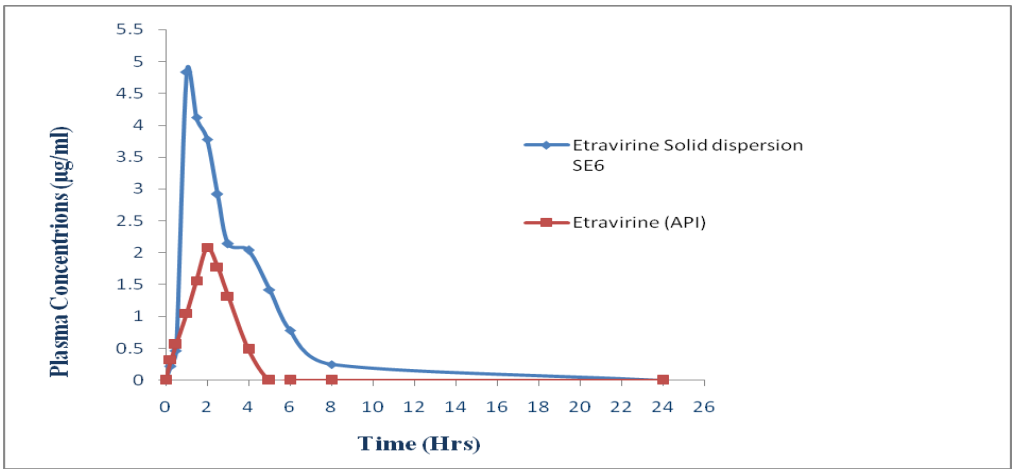


Fig. 13: Plasma concentration profiles of Etravirine solid dispersions (ESE6) and Etravirine

IV. Conclusion

In the present study it was clearly demonstrated that Etravirine solid dispersion formulation can be effectively produced by processing via solvent evaporation method with enhanced solubility and dissolution rate. Novel polymer–surfactant combinations were optimized and stable SD systems were developed successfully. Utilization of Kolliphor P407 along with suitable surfactants offers excellent possibilities to develop stable amorphous solid dispersion. Comparative in vitro dissolution studies for Etravirine (API), marketed Innovator product (Intelence® 200 mg Tablets) and 12 test Solid dispersion formulation were carried out in FDA recommended dissolution media. Based on the in vitro dissolution profiles, it was clearly evident that Solid dispersion formulation (ESE6) comprising Etravirine: Kolliphor P407: SLS in 1:2:1 has shown enhanced solubility nearly 9 fold as compared to pure drug. There was a significant improvement in the rate of drug release from all 12 solid dispersions and found to be comparable and among 12 test formulation the drug release form ESE5 and ESE6 was found to be on higher side and complete as compared to that of dissolution profiles of Innovator product (Intelence® 200 mg Tablets). Analysis by differential scanning calorimetry (DSC) and powder X-ray diffraction (p XRD) showed that Etravirine existed in the amorphous form within the solid dispersion formulation fabricated using the solvent evaporation process. Additionally, scanning electron microscopy (SEM) studies suggested the conversion of crystalline etravirine to an amorphous form. A marked increase in dissolution and bioavailability was exhibited by optimized etravirine solid dispersion (ESE6). AUC (0-t) was increased about 2.9 folds, C_{max} increased about 2.3 folds and t_{max} reduced by 1 hr, when compared to the pure drug. Thus, the study has illustrated the potential use of a solid dispersion system for the delivery of a very poorly soluble drug Etravirine with a better bioavailability. Finally it could be concluded that solid dispersion of Etravirine using novel carriers would improved the aqueous solubility, dissolution rate and thereby enhancing its systemic availability.

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