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Preparation and statistical optimization of self nanoemulsifying tablets of Efavirenz using 2^3 factorial designs

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Abstract

The objective of the present research was to enhance the solubility of poorly water soluble antiretroviral drug i.e. efavirenz, by self nanoemulsifying drug delivery system (SNEDDS) and formulating it as tablets using 2^3 factorial designs. The SNEDDS were prepared using labrafac PG (15%) as oil, Tween 80 (19%) as surfactant and PEG 200 (38%) as co surfactant that yields the globule size of 142.7nm. The liquid SNEDDS were adsorbed onto aerosil which acts as carrier. The SEM of S-SNEDDS appeared as smooth-surfaced particles without any crystalline shape, indicating complete adsorption of SNEDDS. The absence of drug peak in S-SNEDDS thermogram was attributable to presence of drug in molecularly dissolved state in the vicinity of the lipid excipients. The 2^3 factorial designs were employed to optimize the concentration of Micro crystalline cellulose, PVP and sodium starch glycollate. The observed values were in close agreement with the predicted values thereby validating the feasibility of the optimization procedure in developing self nanoemulsifying tablets. The relative bioavailability of the S SNEDDS and pure drug were 388.49% and 95.39%, respectively. This confirms that the solubility of the drug has been increased leading to increase in the bioavailability of efavirenz.

Keywords: Self nanoemulsifying drug delivery system, 2^3 factorial designs and bioavailability.

Introduction

Human Immunodeficiency Virus (HIV) a causative agent for Acquired Immuno deficiency Syndrome (AIDS) an immuno compromised condition that continues to be a frightening exterminator till date and more than 33 million people were affected worldwide [1]. Prevention of HIV infection to reduce the number of newly infected individuals is an international priority [2]. The main hindrances for highly compliant pharmacotherapy were less number of ARVs approved by the regulatory agencies and poor solubility profile [3]. Efavirenz belongs to the class of non-nucleoside reverse transcriptase inhibitors that inhibits non-competitively human HIV-1 reverse transcriptase [4]. Efavirenz belongs to BCS class 2 drug that has poor solubility and high permeability. The dissolution rate of the drug depends on aqueous solubility of a drug. The poor dissolution rate soaring from low solubility often results in the low bioavailability of orally administered drugs[5]. In order to achieve required therapeutic plasma concentrations after oral administration, poorly water soluble drugs usually requires high doses [6]. To improve the aqueous solubility various formulation strategies were reported in the literature including the use of surfactants, micronization, cyclodextrins, solid dispersions, permeation enhancers and lipids [7]. Self nanoemulsifying drug delivery systems (SNEDDS) are mixtures oil, surfactants, cosurfactants that form fine oil in water nanoemulsions when introduced into aqueous phases under gentle agitation [8]. After the successful development of Neoral Sandimmun the development of lipid formulations, especially SNEDDS, has received increased attention [9]. Due to high patient compliance, relatively easy to produce, easy to market, accurate dosing, good physical and chemical stability makes solid dosage forms like tablets and capsules were the most popular and preferred drug delivery forms. The prepared SNEDDS were formulated as tablets to get the advantage of improved solubility as well as to add the advantages of tablet [10]. Traditional experimentation approach on new pharmaceutical formulations development involves significant amount of time, materials and efforts to get valid results for a complex system. The major drawback of traditional experimentation also consequently associated with high cost, due to the existence of multiple factors that affects the formulation performance and manufacturing process. Recently, design of experiment (DOE) supported by statistical software has been reported as an efficient and powerful tool in the development and optimization of pharmaceutical dosage forms [11]. Factorial design is an efficient method of finding the relative significance of multiple variables and their interaction on the response or outcome of the study [12]. In the current research work, a three factor, two levels (2^3) full factorial design was used to

optimize the concentrations of diluent, binder and disintegrant on the responses hardness, disintegration time and dissolution.

Materials and Methods

Materials

Efavirenz was provided as a gift sample from Shasun labs (Pondicherry, India). Labrafac PG was generous gift from Gattefosse, France (through Bombay College of Pharmacy, Mumbai). Porous Polystyrene beads were obtained as gift sample from Thermax Limited (Pune, India). Accurel was obtained from Membrana (Obernburg, Germany). Tween 80, PEG 200, MCC, sodium starch glycolate and polyvinylpyrollidone purchased from Loba chemie pvt ltd, Mumbai. All other chemicals and buffers used were of analytical grade.

Preparation of Liquid SNEDDS

Based on the preliminary studies (solubility studies, pseudoternary phase diagram and thermodynamic stability study) a self nano emulsifying system of efavirenz was prepared using labrafac PG as oil, Tween 80 as surfactant and PEG 200 as co surfactant [13].
The preparation of the SNEDDS contains the following steps:
1. 50mg of efavirenz was selected as the dose for incorporation into the oil phase.
2. The surfactant and co surfactant was added to the oil phase and mixed with magnetic stirrer.
3. Equilibrating in room temperature for 24hr before use.

Preparation of Solid SNEDDS

Aerosil, accurel MP 1000 and porous polystyrene spheres were used as the solid adsorbents to load efavirenz SNEDDS. The lipid formulation was added in increments and blended with the adsorbent at the following fixed efavirenz SNEDDS to adsorbent ratios by weight. Briefly, a constant aliquot of efavirenz SNEDDS was initially added to and mixed with the adsorbent in a mortar. The addition of lipid was suspended when non flowing mass is formed. The obtained mass was passed through 250 μm mesh to get uniformity in particle size. The flow behavior and loading efficiency of the adsorbed blend was then analyzed. The powder samples were stored in a desiccator until further evaluation. [14]

Characterization of solid SNEDDS

Scanning Electron Microscope

The surface morphology of Solid SNEDDS was examined by scanning electron microscope (Hitachi S3400, Tokyo, Japan). The powder samples were glued and mounted on metal sample plates. After this the samples were gold coated with a sputter coater using an electrical potential of 2.0 kV at 25 mA for 10 min[15].

Differential Scanning Calorimetry

The physical state of drug in solid SNEDDS was characterized by the differential scanning calorimetry thermogram analysis. The samples (about 3.00 mg) were placed in standard aluminum pans, and dry nitrogen was used as effluent gas. All samples were scanned at a temperature ramp speed of 5 C /min and the heat flow from 0 to 200 C [16].

Reconstitution properties of solid SNEDDS

The mean globule size and polydispersity index (P.I.) of the resulting nanoemulsions were determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to Brownian motion of the particles) using a Zetasizer 3000 (Malvern Instruments Worcestershire, UK) Light scattering was monitored at 25ºC at a 90ºangle.

Experimental design

The 2^3 full factorial designs were carried out systematically with three factors at two levels to prepare the efavirenz loaded SNEDDS tablets. A total of eight experimental trials were done at all possible combinations. Venturing taken during optimization of excipient the amount of MCC (A), PVP (B), and the amount of sodium starch glycolate (C) were selected as the independent variables that were varied at two levels (low and high). The levels of the variables studied were chosen so that their relative divergence was capable to have a quantifiable effect on the response, along with the information that the selected levels are within practical use [17, 18]. The responses (dependent variables) selected for the study were hardness, disintegration and cumulative drug release at 40 min. the statistical experimental design was generated and evaluated using Design-Expert 8.0.6.1 software (Stat-Ease Inc., USA). The independent variables with its levels and the responses were shown in Table 1.

Table 1: Variables in 2^3 factorial design

Independent variable	Levels	
	Low	High
A: MCC	200.00	300.00
B: PVP	12.00	20.00
C: SSG	8.00	16.00
Dependent variable		
Y1 Hardness(kg)		
Y2 Disintegration Time(sec)		
Y3 % Cumulative drug release(%)		

Preparation of tablets

The archetypal formula for preparation of efavirenz loaded SNEDDS tablet used in this study was given in Table 2. Tablets were punched by direct compression of mixtures on a B2 rotary tablet press (Remak, Ahmedabad) with flat plane face punches (punch diameter = 12mm) at 50 rpm. The excipients were screened through a #20 mesh. The final mixture to be compressed was prepared by mixing in a polyethylene bag manually for 10min. finally lubricant was added to this blend and motley properly for 2 min. [19, 20]

Table 2: Typical Efavirenz loaded SNEDDS tablet composition

Ingredient	Qty (mg)
Efavirenz loaded SNEDDS	100
MCC	200/300
PVP	12/20
SSG	8/16
Magnesium stearate	5
Talc	5
Lactose q.s	500

Evaluation of tablets [21, 22]

Hardness test

Monsanto hardness tester (Tab-Machines Ltd., India) was used to determine tablet hardness. Ten tablets were chosen randomly from the composite samples for each of the tableting runs and the average value was determined.

Disintegration test

The tablet disintegration test was performed employing disintegration tester (Disintegration Tester ED-2AL, Electrolab, Mumbai) using demineralized water at 37±2 C. The time required for tablet disintegration was determined by visual observation. Each value reported is the maximum time of 6 independent measurements

Friability

The friability of the tablets was determined by weighing 20 tablets dedusted prior testing in an analytical balance and moved for 4 min in an friability tester (Electrolab, EF2 Friabilator USP), set at speed of 25 revolutions per minute. After 4 min all loose dust was removed from the tablets, then they were reweighed and the percentage friability was calculated.

Drug content estimation

The drug was extracted from S SNEDDS using methanol, suitably diluted and analyzed using UV-VIS spectrophotometer (Shimadzu UV 1800, Japan) at a λmax of 247nm. The experimental studies were performed in triplicate.

Drug release

In vitro drug dissolution tests were performed using the USP 24 method with a dissolution apparatus 2 (Electrolab TDT-08L, Mumbai). The dissolution tests were carried out at 37±0.5 C in 900 ml of 0.1 N HCl at 100 rpm. Results are averaged from three-replicated experiments. During the release studies, 1 ml of SIF sample was withdrawn and quantification was performed using UV/Vis spectrophotometry. The withdrawn volume was replaced each time with fresh thermostated 0.1 N HCl [23]

Pharmacokinetic studies

The oral pharmacokinetics of drug was assessed in Wistar rats (220-250 g) of either sex at a dose equivalent to 10 mg/kg of drug. Two types of systems were systematically compared: (i) drug-loaded S-SNEDDS, (ii) extemporaneous suspensions. *In vivo* study protocols were approved by the Institutional Animal Ethics Committee (Regd. No 107/2012). A wash out period of one month was given between testing of two products.

After collecting the zero hour blood sample (blank), the product in the study was administered orally with 10 ml of water. No food or liquid other than water was permitted until 4 hours following the administration of the product. Blood samples (0.5 ml) were collected from tail vein at 0.25, 0.5, 1, 2, 4, 8 and 12 hours after administration. The blood samples were collected in heparinized tubes and were centrifuged at 10000 rpm for 10 min and the plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay on the same day. Plasma concentrations of drug were determined by a known HPLC method after revalidation [15].

Stability studies

The OPT S-SNEDDS formulation was subjected to stability studies, carried out at 40±2°C/70% ± 5% RH as per the ICH guidelines. The formulation was kept in air-tight glass vials and assayed periodically, at the time points of 0, 1, 3 and 6 months, for drug content and dissolution performance.

Results and Discussion

Preparation of liquid SNEDDS

Efavirenz showed maximum solubility in Labrafac PG (Oil), tween 80 (Surfactant) and PEG 200 (Co surfactant) hence these excipients were selected for the preparation of SNEDDS. The characteristic peaks of efavirenz (3319cm^{-1}, 1060cm^{-1}, 750 cm^{-1}, 1039 cm^{-1}, 820 cm^{-1}) were not affected and prominently observed in IR spectra of efavirenz along with other excipients(figure not given). This clearly shows there is no interaction between drug and excipients.

Pseudo-ternary phase diagrams were constructed to identify the nanoemulsion regions and to optimize the concentration of the selected vehicles. The optimized formulation labrafac PG(15%) as oil, Tween 80(19%) as surfactant and PEG 200(38%) as co surfactant were robust to all dilutions and did not show any phase separation or precipitation. The globule size of the formulation was found to be 142.7nm.

Preparation of S-SNEDDS

The S-SNEDDS were prepared using carriers like aerosil, accurel and porous polystyrene beads. The mean particle size of aerosil was 12nm and has specific surface area (BET) 380±30 m^2/g. Because of its small particle size and large specific surface area the S-SNEDDS prepared with aerosil had high loading efficiency and desirable flow characteristics (table 3). So it was selected as a suitable excipient for the preparation of S-SNEEDS.

Table 3: Flow rate and Loading efficiency of drug loaded adsorbents

SNEDDS	Adsorbent	Mean particle size	Angle of repose	Loading efficiency %
Efavirenz	Aerosil	12nm	18.7	138
	PPB	0.2 µm	23.2	112
	Accurel	0.3-1 µm	22.6	120

Scanning electron microscopy

The scanning electron micrographs of S-SNEDDS formulations were shown in fig. 1. The S-SNEDDS appeared as smooth-surfaced particles without any crystalline shape, indicating complete adsorption of SNEDDS. The SNEDDS prepared with aerosil appeared as rough-surfaced particles, indicating that the liquid SNEDDS was absorbed or coated inside the pores of silicon dioxide. The surface area of porous polystyrene beads and accurel also shows absence of crystalline particles indicating complete adsorption of SNEDDS.

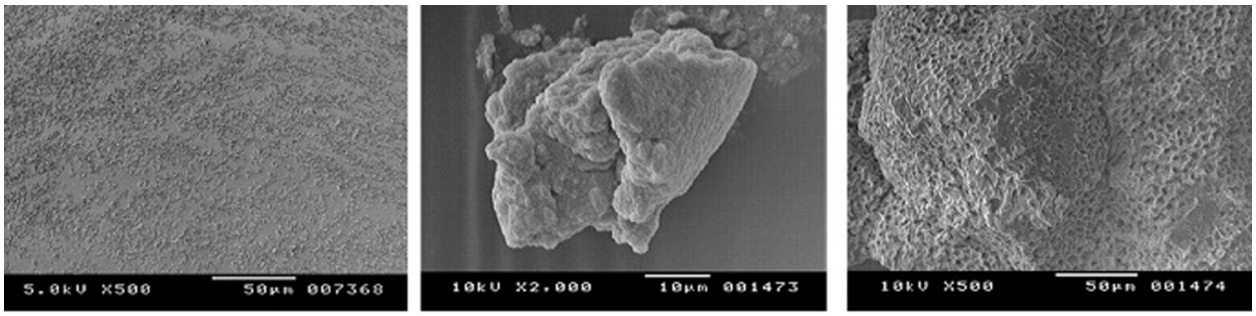

Figure. 1. Scanning electron micrograps of SNEDDS loaded with Porous polystyrene beads(A), aerosil (B) and accurel (C)

Differential Scanning Calorimetry

The DSC thermograms of pure drug and S-SNEDDS were shown in fig. 2. The DSC thermograms showed pronounced melting peak for pure Efavirenz at 137.5 C correlating to its melting point. The absence of drug peak in S-SNEDDS was due to presence of drug in molecularly dissolved state in the vicinity of the lipid excipients. [14]. The SNEDDS loaded accurel shows melting at 152.25 C correlating to the melting point of accurel.PPB loaded SNEDDS shows melting peak at 82.05 C correlating to the melting point of PPB.

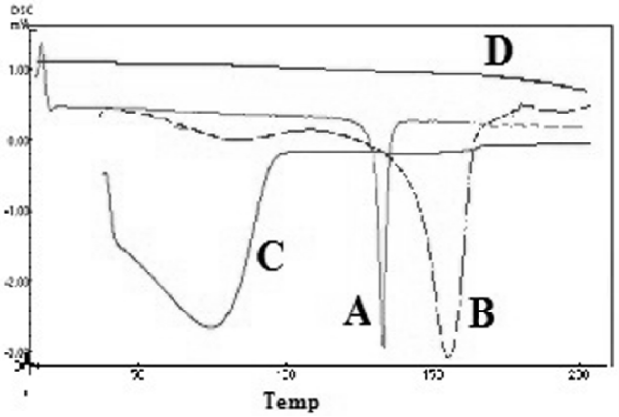

Figure. 2. DSC curves of pure drug (A), SNEDDS loaded accurel (B), SNEDDS loaded PPB (C) and SNEDDS loaded Aerosil (D)

Reconstitution properties of solid SEDDS

The z-average diameter and polydispersity index of the solid and liquid SNEDDS are presented in Table 4. As shown, the z-average droplet sizes of both systems were less than 150 nm. The droplet size of the nanoemulsion from the solid SNEDDS was slightly increased, but the difference is not statistically significant compared to the liquid SNEDDS.

Table 4: Droplet size with polydispersity index of the reconstituted nanoemulsions

Formulation	z-Average diameter (nm)	Polydispersity index (PDI)
Liquid SNEDDS	142.8	0.581
Solid SNEDDS	145	0.725

Preparation of Efavirenz loaded SNEDDS tablet

The experimental design methodology could be a very economic way to obtain maximum information, which can save a significant amount of time. Moreover, it can reduce the materials used for analyses and the personal costs as well. To identify the most important factors among all the factors screening is done at beginning of the experimental procedure. From the experimental results (Table 5), the effects of all studied variables and the variable interactions were graphically and statistically interpreted for all responses.

Table 5: Observed Response in 2^3 Factorial Design for Efavirenz loaded SNEDDS tablet

Std	Run	Factor 1 *A:MCC* mg	Factor 2 *B:PVP* mg	Factor 3 *C:SSG* mg	Response 1 *Hardness* kg	Response 2 *Disintegration Time* (Sec)	Response 3 *% Cumulative drug release*
2	1	300.00	12.00	8.00	5.9	125	76.4
8	2	300.00	20.00	16.00	4.6	98	87.3
1	3	200.00	12.00	8.00	5.2	112	82.6
5	4	200.00	12.00	16.00	3.4	72	99.2
7	5	200.00	20.00	16.00	4.3	92	90.6
4	6	300.00	20.00	8.00	7.1	152	68.5
3	7	200.00	20.00	8.00	6.3	138	71.2
6	8	300.00	12.00	16.00	4	85	95.4

The results of ANOVA indicated that all models were significant ($p < 0.05$) for all response parameters investigated. Model simplification was carried out by eliminating non-significant terms ($p > 0.05$) in polynomial equations. Values of "Prob > F" less than 0.0500 in all the cases indicates model terms are significant. The Pred R-Squared is in reasonable agreement with the Adj R-Squared. The signal to noise ratio is measured by Adeq Precision and ratio greater than 4 is desirable. The value shows much higher than 4 confirms an adequate signal (Table 6).

Table 6: Summary of results of regression analysis for responses

	Value	F-value	p-value
Hardness			
R-Square	0.9865		
Adj R-Squared	0.9763	97.16	0.0003
Pred R-Squared	0.9458		
Adeq Precision	26.291		
Disintegration Time			
R-Square	0.9867		
Adj R-Squared	0.9768	99.08	0.0003
Pred R-Squared	0.9469		
Adeq Precision	26.275		
% Cumulative Drug Release			
R-Square	0.9950		
Adj R-Squared	0.9913	266.61	< 0.0001
Pred R-Squared	0.9801		
Adeq Precision	42.528		

Figure 3 shows the effect plots for hardness, disintegration time and friability, where negative values indicate a negative effect of a specific variable on the response factors. The application of factorial design yielded the following regression equations.

Hardness =+4.775+6.0E-003 * MCC+0.118 * PVP-0.256* SSG

Disintegration Time=+105.0+0.115* MCC+2.68 * PVP-5.62 * SSG

% Cumulative drug release =+84.22-0.04 * MCC-1.12 * PVP+2.30 * SSG

Hardness, Disintegration time and *% Cumulative drug release* values for all the eight formulations varied from 3.4 to 7.1 kg, 72-152s and 68.5-99.2% respectively. These outcomes depicts that the variables chosen have strong influence on the selected responses.

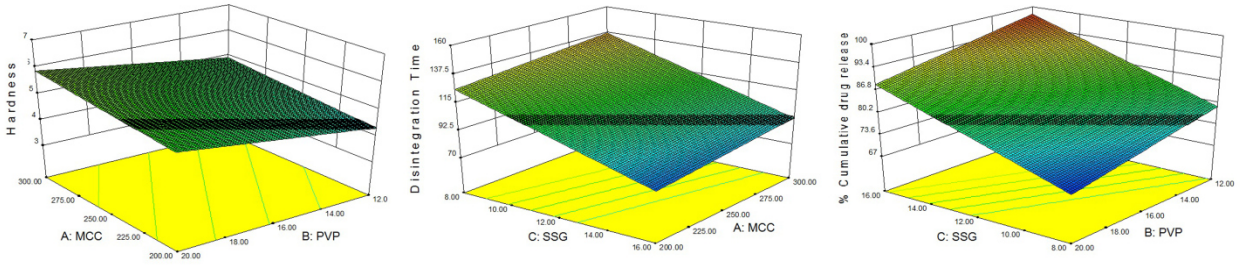

Figure. 3. Three-dimensional response surface plot depicting the impact of MCC:PVP, MCC:SSG and SSG:PVP on hardness, disintegration time and % drug release respectively

The MCC PH 101 having the particle size of 50µm that absorbs the any squeezing of lipid occurs while punching of tablets. MCC has good wicking and absorbing capacities. PVP K 30 is used because of its directly compressible property that acts as binder. SSG acts

as super disintegrant. [MCC accelerates water penetration into tablets can cause easily swelling of SSG, and this reveals readily superdisintegrant property of SSG [22]. The excellent batch flowability and compressibility properties were attributed to the presence of microcrystalline cellulose, MCC, (Avicel 102), which is an excellent filler/flow-aid for direct compression. [24]

The figure 3. exhibits, as the concentration of PVP increases, the hardness of the tablets increases due to the increased bonding between the particles. Tablets of MCC disintegrated rapidly due to the rapid passage of water into the tablets resulting in the instantaneous rupture of the hydrogen bonds. Disintegration occurs by rapid uptake of water followed by rapid and enormous swelling by SSG. It was essential to use suitable inert compression diluents to improve the compactibility of tablets. In all the formulations, the hardness test indicated good mechanical strength and friability was less than 1%, which indicated that the tablets had a good mechanical resistance. The extent of dissolution, however, is dependent on the reversible attraction and surface adsorption of efavirenz and the oily formulation onto the adsorbents. Therefore, physical properties of the ingredients used to prepare the solid compacts have a profound effect on the emulsion release rate. This relationship between the formulation ingredients (independent variables) and emulsion release rates (dependent variables) was elucidated using 3 D graphs. [25]. Drug content was found to be 98-102% which was within the limits.

To optimize the final tablet formulation, the required limits of the response values were clearly defined, and the combinations of variables which resulted in tablets meeting the required specifications were calculated using the software to obtain tablets with the following properties: a hardness of 4, disintegration time of less than 80 sec and % cumulative release of more than 95%.

To verify the reproducibility, a new formulation as shown in Table 7 was prepared according to the predicted levels and evaluated. The overlapping of the obtained result over the predicted values confirms the practicability and validation of the model

Table 7: Optimisation of final tablet

Number	MCC (mg)	PVP (mg)	SSG (mg)	Hardness (kg)	Disintegration Time(sec)	% Cumulative drug release
Predicted	278.75	12.00	15.88	4	79.9	96.1
Actual	278.75	12.00	15.88	4	78	97
Relative error(%)				0	2.37	0.9

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Pharmacokinetic studies

Plasma samples were analyzed for efavirenz employing reverse phase HPLC (RP-HPLC). The mobile phase consisted of acetonitrile:water (70:30). Each of the plasma samples was thawed to room temperature. One milliliter of diethyl-ether was added to it and mixed using a vortex mixer for 30 s. The upper organic phase was transferred and evaporated to dryness on a water bath at 50°C. To each dry residue, 0.5 ml of mobile phase was added, and mixed using the vo\ rtex mixer for 10 s to reconstitute the same. The reconstituted sample was transferred to the HPLC vials and injected into the integrated liquid chromatographic system Shimadzu LC-2010 HT equipped with a UV detector and a C18 column. The flow rate was kept at 1 ml min^{-1} and the UV detector was set at an absorption max of 247 nm. From the chromatogram efavirenz was observed at retention time of 5.6 min [3, 26].

Table 8: Pharmacokinetic parameters

Product	C_{max} (mcg/ml)	T_{max} (h)	K_{el}	$T_{1/2}$	$(AUC)_0^t$
Efavirenz	10.46	3.7	0.1776	3.9	95.39
Efavirenz S SNEDDS	42.6	1	0.2038	3.4	388.49

Table 8 shows significant improvement of drug absorption for the S SNEDDS than the pure drug. The results showed that Cmax of S SNEDDS was higher than that of pure drug. Additionally, Tmax of the S SNEDDS was all shorter than that of the pure drug, suggesting that SE technique could improve drug release and absorption in GIT. The relative bioavailability of the S SNEDDS and pure drug were 388.49% and 95.39%, respectively. It indicated that the absorption of efavirenz was evidently improved after it was dispersed in solid SE formulations as a dissolved state.

Stability studies

The optimized formulation was stable throughout the study period. There is no significant change in drug content and dissolution performance.

Conclusion

These current research evidenced the successful loading of efavirenz SNEDDS in aerosil by adsorption technique. Self emulsifying properties were still retained after loading and no significant changes in the particle size were observed. The S SNEDDS tablets were prepared by 2^3 factorial design using design

expert software. The results of ANOVA indicated that all models were significant. The model was used to predict the levels of the factors A, B and C required obtaining an optimum formulation with moderate hardness, minimum disintegration time and maximum % drug release. The observed values were in close agreement with the predicted values thereby validating the feasibility of the optimization procedure in developing self nanoemulsifying tablets. The *in vivo* study in wistar rats showed significantly enhanced bioavailability from S-SNEDDS compared with pure drug. Considering the significant increment of pharmacokinetic parameters (AUC and Cmax) and the thermodynamically and chemically stable drug delivery system, S SNEDDS may be an effective formulation strategy for the other BCS class II and IV drugs.

Authors' contributions

PS designed the work under the guidance of PKK. All the work starting from designing the experiment, performing, interpreting was done PS. PKK involved in the analyzing the data's and carrying out analytical work. PS drafted the article with the suggestion of PKK.

Conflicts of interest

Authors have no conflicts of interest.

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References

[1]. Kumari G, Singh RK. 2012. Highly Active Antiretroviral Therapy for treatment of HIV/AIDS patients: Current status and future prospects and the Indian scenario. HIV & AIDS Review 11:5-14.

[2]. Date AA, Shibata A, Goede M, Sanford B, Bruzzo KL, Belshan M, Destache CJ. 2012. Development and evaluation of a thermosensitive vaginal gel containing raltegravir + efavirenz loaded nanoparticles for HIV prophylaxis. Antiviral Research 96:430-436.

[3]. Chiappetta DA, Hocht C, Taira C, Sosnik A. 2011. Oral pharmacokinetics of the anti-HIV efavirenz encapsulated within polymeric micelles. Biomaterials 32:2379-2387.

[4]. Veldkamp AI, Heeswijk RV, Meenhorst PL, Mulder JW, Lange JMA, Beijnen JH, Hoetelmans RMW. 1999. Quantitative determination of efavirenz (DMP 266), a novel non-nucleoside reverse transcriptase inhibitor, in human plasma using isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. Journal of Chromatography B 734:55-61.

[5]. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. 2011. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. Int J Pharm 420:1-10.

[6]. Kumar BP, Yunoos M, Chandana N, Habeeb N, Himaja B. 2010. Enhancement of dissolution rate of Efavirenz by solid dispersion technique. J Pharmacy Res 3(12):2840-2842.

[7]. Atef E, Belmonte AA. 2008. Formulation and in vitro and in vivo characterization of a phenytoin self-emulsifying drug delivery system (SEDDS). Eur J Pharm Sci 35:257-263.

[8]. Dixit RP., Nagarsenker MS. 2008. Self-nanoemulsifying granules of ezetimibe: Design, optimization and evaluation. Eur J Pharm Sci 35:183-192.

[9]. Djuris MMJ, Djekic L, Vasiljevic D, Ibric S. 2012. Characterization and evaluation of solid self-microemulsifying drug delivery systems with porous carriers as systems for improved carbamazepine release. Int J Pharm 436:58- 65.

[10]. Late SG, Yu YY, Banga AK. 2009. Effects of disintegration-promoting agent, lubricants and moisture treatment on optimized fast disintegrating tablets. Int J Pharm 365:4-11.

[11]. Husban FA, Perrie Y, Mohammed AR. 2011. Formulation of multiparticulate systems as lyophilised orally disintegrating tablets. Eur J Pharm Biopharm 79:627-634.

[12]. Hiorth M, Liereng L,Reinertsen R, Thob I. Formulation of bioadhesive hexylaminolevulinate pellets intended for photodynamic therapy in the treatment of cervical cancer. 2013. Int J Pharma 441(1-2):544-554.

[13]. Selvam RP, Kulkarni PK, Dixit M. 2013. Preparation and evaluation of self-nanoemulsifying formulation of efavirenz. ind j pharm edu res 47(1):47-54.

[14]. Kang JH, Oh DH, Oh YK, Yong CS, Choi HG. 2012. Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system

(solid SNEDDS). Eur J Pharma Biopharm 80:289-97.

[15]. Zhang J, Gao Y, Qian S, Liu X, Zu H. 2011. Physicochemical and pharmacokinetic characterization of a spray-dried malotilate emulsion. Int J Pharm 414:186-192

[16]. Beg S, Jena SS, Patra SN, Rizwan M, Swaina S, Sruti J, Rao MEB, Singh B. 2013. Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential. Colloids and Surfaces B: Biointerfaces 101:414-23.

[17]. Malakara J, Nayak AK. 2012. Formulation and statistical optimization of multiple-unit ibuprofen-loaded buoyant system using 2^3-factorial design. Chem Eng Res and Design 90(11):1834-1846.

[18]. Singh G, Pai RS, Devi VK. 2012. Response surface methodology and process optimization of sustained release pellets using taguchi orthogonal array design and central composite design. J Adv Pharm Tech Res 3(1):30-40.

[19]. Swamy PV, Shahidulla SM, Shirsand SB, Hiremath SN, Ali MD. 2008. Orodispersible tablets of carbamazepine prepared by direct compression method using 3^2 full factorial design. Dhaka Univ J Pharm Sci 7(1):1-5.

[20]. Madgulkar AR, Bhalekar MR, Kolhe VJ, Kenjale YD. 2009. Formulation and optimization of sustained release tablets of venlafaxine resinates using response surface methodology. Indian J Pharm Sci 71 (4):387-394.

[21]. King'ori LD, Walker RB. 2012. The use of response surface methodology to evaluate the impact of level 2 SUPAC-IR changes on the in vitro release of metronidazole and ranitidine from a fixed-dose combination tablet. Dissolution Technologies 28-36.

[22]. Bayrak Z, Tas C, Tasdemir U, Erol H, Ozkan CK, Savaser A, Ozkan Y. 2011. Formulation of zolmitriptan sublingual tablets prepared by direct compression with different polymers: In vitro and in vivo evaluation. Eur J Pharm Biopharm 78:499-505

[23]. Bhagwat DA, D'Souza DI. 2012. Formulation and evaluation of solid self micro emulsifying drug delivery system using aerosil 200 as solid carrier. Int Curr Pharma J 1(12):414-9.

[24]. Gryczke A, Schminke S, Maniruzzaman M, Beck J, Douroumis D. 2011. Development and evaluation of orally disintegrating tablets (ODTs) containing Ibuprofen granules prepared by hot melt extrusion Colloids and Surfaces B: Biointerfaces 86:275-284.

[25]. Nazzal S, Nutan M, Palamakula A, Shah R, Zaghloul AA, Khan MA. 2002. Optimization of a self-nanoemulsified tablet dosage form of ubiquinone using response surface methodology: effect of formulation ingredients International Journal of Pharmaceutics 240:103-114.

[26]. Chowdary KPR, Devi GSA. 2012. Preclinical pharmacokinetic evaluation of efavirenz -cyclodextrin - pvp inclusion complexes. Int J Pharm Sci Rev Res 15(1):23-6.