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Research Article

Development and *In vivo* Evaluation of Pitavastatin Self-emulsifying Drug Delivery Systems

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ABSTRACT

The current research was intended at formulation and *in vivo* evaluation of pitavastatin self-nano emulsifying drug delivery system (PTN SNEDDS) for enhanced drug dissolution and bioavailability. Solubility studies carried out to construct pseudoternary phase diagram employing the blends of oil (capmulPG8), surfactant (acrysol K140), and cosurfactant (transcutol P). The PNT SNEDDS was prepared and optimized by adopting response surface methodology employing a 3^3 Box-Behnken design. The SNEDDS formulations characterized for % drug content, % entrapment efficiency, *in-vitro* release studies, particle size, zeta potential, Fourier Transform Infrared Spectroscopy (FTIR), and scanning electron microscopy (SEM) studies. The bioavailability studies were carried out in Wistar rats. The study indicated that PTN12 comprising of 40% capmul PG8, 40% acrysol K140, and 30% transcutol P displayed minimum droplet size (24.8nm), optimal zeta potential (-10.4 mV), and maximum drug release (98.75%). The SEM data revealed that droplet size is in the nanometer range. The pharmacokinetic studies conducted in rats indicated that C_{max} of optimized PTN SNEDDS (2.25±0.02ng/mL) was higher than pure PTN suspension (0.75±0.03ng/mL) and optimized SNEDDS exhibited superior oral bioavailability about 4 times of AUC, along with higher plasma concentration than pure drug. The above results indicated that the application of SNEDDS formulation technique for PTN increases solubility and dissolution.

Introduction

Although the oral route is more preferred over the other administration routes, it is limited for drug molecules with sufficient solubility in water and permeability across gastric mucosa. Only 9% of the new drug entities belonged to Biopharmaceutics Classification System (BCS) Class-I category (high solubility-high permeability), most of the new drug molecules have poor solubility in water,[1] which cause poor oral bioavailability and therapeutic failure. The most challenging problem for poorly water-soluble compounds is their formulation as oral dosage forms.[2] The most effective technique used to develop the oral bioavailability of lipophilic compounds and hence increase the clinical efficacy is the inclusion of the lipophilic drugs into a lipidic vehicle such as self-emulsifying formulations.[3] Self-emulsifying drug delivery systems are isotropic mixtures of oils (natural or synthetic), surfactants (solid or liquid), hydrophilic solvents, and co-solvents/surfactants.[4] Self-emulsifying systems possess many unique properties compared to other formulation strategies such as nanoparticles, solid dispersions, and lipid-based formulations.[5,6] These systems can emulsify rapidly and spontaneously in the gastrointestinal tract (GIT) when diluted with GI fluids and create delicate oil/water micro and nano-emulsions upon mild agitation.[7]

Pitavastatin (PTN) is an antilipidemic agent used for treating hypercholesterolemia and cardiovascular diseases; it functions by catalyzing the rate-determining step in cholesterol biosynthesis. It belongs to the class II drug in BCS classification, with low water solubility and bioavailability (50%). Examination of potential formulation techniques for enhancing the bioavailability of PTN is subject of importance.[8-10]

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The present research was focused on formulation and *in-vivo* evaluation of PTN SNEDDS using BBD for enhanced oral solubility and bioavailability.

Material And Methods

Chemicals and Reagents

Aurobindo pharma Ltd, Hyderabad gifted pitavastatin. Captex 355, capmul PG8, capryol 90, imwitor 742, IPM, labrafil M2, labrafac CC, maisine 35-1, miglyol 812, paceol, sefsol 218,olive oil, castor oil, acrysol K140, acconon CC400, acconon sorb20, capmul GMO 50,caprol PGE 860, solutol HS15, labrasol, tween 8, gelucire 44/14, tween 20, tween 80, triton-9100, capmul MCMC8, lauroglycol 90, PEG 400, EG, plurololeique CC497, triacetin, and transcutol P procured from Gattefosse, France.

Selection of Oil, Surfactants, and Co-surfactants

The solubility of PTN in various surfactants and co-surfactants was determined. An excess amount of PTN (150 mg) was placed in 2 mL of the excipient in a screw-capped glass tube. The mixture was shaken at 25°C by an isothermal shaker for 48 hours to attain equilibrium. Then, each tube was centrifuged at 5000 rpm for 10-15 minutes. The supernatant was diluted with methanol, and the drug concentration was determined spectrophotometrically at 245nm. This study was conducted in triplicates to record the mean value.[10-13]

Construction of Pseudoternary Phase Diagram

Capmul PG8 (oil), acrysol K140 (surfactant), and transcutolP (co-surfactant)were chosen for formulating SNEDDS considering solubility data. Surfactant: co-surfactant (S_{mix}) were mixed in varying ratio (1:1, 1:2, 1:3, 2:1, 3:1 and 4:1). About seventeen compositions of ratios from 1:9 to 9:1 prepared. 0.1 mL of samples drawn from each of these compositions were put into a beaker containing 100 mL water, and were gently mixed with a magnetic stirrer, checked for % transmittance at 245 nm spectrophotometrically. The resultant emulsion was observed for clarity and phase separation. The emulsions that exhibit phase separation and coalescence were considered unstable.[14,15]

Box–Behnken Experiment Design (BBD)

A 3^3 BBD, employed for optimizing the main, interaction, and quadratic effects of formulation components on characteristics of SNEDDS. Seventeen experiments were conducted randomly for chosen independent variables that include 5 repetitions at the center (asterisk-marked) obtained from 3 factors, 3-level BBD, and their subsequent responses were noted[16] (Tables 1 and 2).

The analysis was carried out by Design Expert® software (Version 7.0, Stat-Ease Inc., USA).

The 2nd order quadratic equation generated as:

$$Y = \beta_1 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_2 X_3 + \beta_6 X_1 X_3 + \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2$$

Y - Level of response

β_0 – intercept

β_1 - β_9 - regression coefficient

X_1, X_2, and X_3 main effects

X_1X_2, X_2X_3, and X_1X_3 - interaction between main effects

X_1^2, X_2^2, and X_3^2 - quadratic terms of independent variables.

Preparation of PTN Loaded SNEDDS

Based on drug solubility in the oil (capmul PG8), surfactant (acrysol K140), and co-surfactant (transcutol P) were used for the formulation of PTN SNEDDS. All the components were premixed in a glass vial, heated to 37°C for 5 min to attain a standardized blend. A total of 2 mg accurately weighed drug was subsequently added to respective SNEDDS by refluxing at 50–60°C. Each mixtures was vortexed for a further 5–10 minutes on an Orbital shaker (BT Lab systems 926). After complete drug dissolution, a clear and transparent solution was obtained, which was allowed to equilibrate at 25°C for 48 h before analysis. Seventeen of such formulations are prepared and packed into zero-size capsules.[17]

Evaluation of PTN SNEDDS

All 17 PTN SNEDDS were evaluated for different parameters in terms of droplet size, zeta potential(ZP),[18] entrapment efficiency (EE),[19] drug content,[20] % cumulative drug release (CDR),[21] as referred.

Table 1: List of dependent and independent variables in Box-Behnken design

Independent variables			*Levels*		
Variable	*Name*	*Units*	*Low(-1)*	*Middle (0)*	*High(+1)*
A	Amount of capmul PG8	mg	30	40	50
B	Amount of acrysol K140	mg	20	30	40
C	Amount of transcutol P	mg	10	20	30
Dependent variable			*Goal*		
Y1	Droplet size	nm	Minimize		
Y2	Zeta Potential	mV	Minimize		
Y3	Cumulative % drug release in 60 min(% CDR)	%	Maximize		

Table 2: Box-Behnken design with observed responses

Run	*Amount of capmul PG8 (mg)*	*Amount of acrysol K140*	*Amount of transcutol P*	*Droplet size (nm)*	*Zeta potential (-mV)*	*% CDR after 60 min (%)*
1	30	20	20	37.5	19.5	79.45
2	50	20	20	48.6	29.6	87.38
3	30	40	20	41.6	12.3	84.58
4	50	40	20	49.8	26.8	90.19
5	30	30	10	35.1	15.6	91.77
6	50	30	10	50.4	18.3	88.46
7	30	30	30	49.5	21.9	77.54
8	50	30	30	36.8	16.7	89.43
9	40	20	10	51.2	13.5	84.39
10	40	40	10	36.1	25.2	89.25
11	40	20	30	44.5	17.5	86.37
12	40	40	30	24.8	10.4	98.75
13	40	30	20	50.3	20.1	85.11
14	40	20	20	47.5	20.2	90.15
15	40	40	20	35.3	14.1	92.34
16	40	30	10	51.8	14.7	88.45
17	40	30	20	31.6	21.13	84.58

Characterization of PTN SNEDDS

The optimized formulation analyzed for droplet size, zeta potential,[18] FTIR, and Scanning electron microscopy (SEM)[24]

Stability Study

Stability testing was conducted at 40°C ± 2°C/75% RH ± 5% RH for 3 months using a stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 60, and 90 days and were estimated using a UV-visible spectrophotometer.[25]

Pharmacokinetic Studies of PTN

Animal Preparation

Male Wistar rats weighing between 150–180 g were used for this study and ensured that all animals remained healthy throughout the study. These animals were maintained at a temperature of 25°C, 45% RH with alternating night and daylight cycles, and 100% ventilated room maintained with fresh air exchange and continuous water supply. A standard diet was provided to all the animals and *water ad libitum.* The institutional animal ethics committee approved the animal study with No: IAEC NO: 1292/ac/09/CPCSEA/52.

Study design

All the animals were categorized into two groups (each group of 6 animals), fasted 24 hours before the experiment, and were offered a diet 4hours before drug administration. Group A: Administrated orally with 0.0312 mg pure PTN suspension in 0.5% methocel.

Group B: Administrated orally with 0.0312 mg of PTN SNEDDS suspension in 0.5% methocel.

A sampling of 200 µL of blood collected from femoral artery at predefined intervals, stored in an Eppendorf tube filled with heparin, and samples centrifuged at 5000 rpm for 5-10 min and preserved at −20°C.[26]

The determination of PTN in rat plasma was done according to the informed method. The pharmacokinetics analyzed with the reported method. Using the chromatographic separation method, the determination of pitavastatin and internal standard paracetamol was carried out. Chromatographic studies on HPLC systems (waters 1525) are composed of binary HPLC pumps connected to water 2487 dual-wavelength absorbance detectors. The Phenomenex C18 (250 x 4.60) is a 5 µ particle size column used in the isocratic mode at room temperature. The sample was introduced through an injector valve with a 20 µL sample loop. 0.5% acetic acid: acetonitrile 35:65 (%, v/v), is used as mobile phase with 1 mL/min flow rate and UV detection performed at 245 nm.[27]

The pharmacokinetic parameters evaluated include maximum plasma concentration (C_{max}), time to attain C_{max}i.e., T_{max} and t ½ values, the area under the plasma concentration-time curve from zero to the last sampling time ($AUC_{0\text{-}t}$), area under plasma concentration-time curve from zero to infinity($AUC_{0\text{-}\infty}$). $AUC_{0\text{-}t}$ is calculated by the linear trapezoidal rule and $AUC_{0\text{-}\infty}$ from the following formula:

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

Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software,

San Diego, CA, USA) using ANOVA followed by Tukey–Kramermultiple comparison test. The difference with $P < 0.05$ was considered statistically significant.

Results

Based on solubility study and pseudo-ternary phase diagram results, the capmul PG8 was chosen as oil, acrysol K140 as surfactant, and transcutol P, as co-surfactant. The range selected on is as follows: 30% ≤Capryol90 ≤ 50%, 20% ≤ acrysol K140 ≤ 40%, 10% ≤ transcutol P ≤ 30%. [28]

Evaluation Parameters of *PTN SNEDDS*

The %drug content of all PTN SNEDDS ranged from 95.38±1.45 to 99.15±1.62%, and the entrapment efficiency varied between 94.38±1.67 to 98.57±1.95%, with maximum value recorded for PTN12. The dissolution study shows that PNT SNEDDS enhanced the dissolution of PTN with the highest release rate for PTN12 (98.75±1.73%) while that of the pure drug is onle12.57 within 60 minutes.[29] (Figs. 1 and 2)

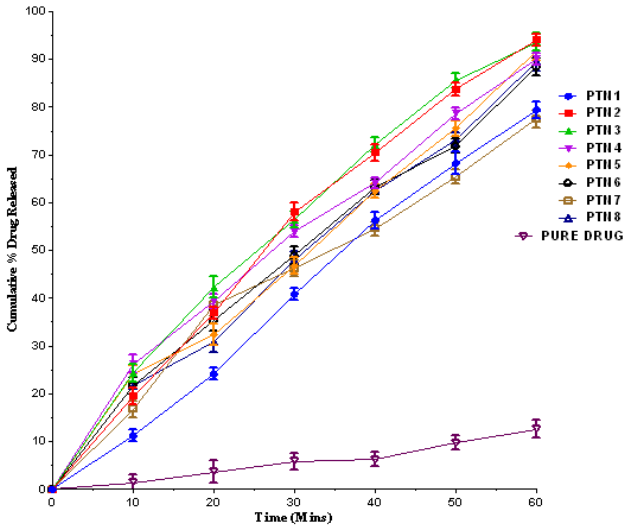


Fig. 1: *In vitro* drug release profile of Pitavastatin SNEDDS (PTN1-PTN8)

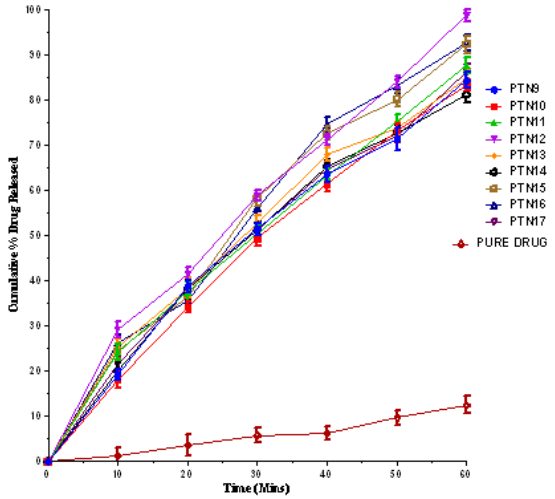


Fig. 2: *In vitro* drug release profile of Pitavastatin SNEDDS (PTN9-PTN17)

Design of experiment

About 17 experiments were performed according to experimental runs generated by 3^3 Box-Behnken design. All responses fitted into 2nd order quadratic equations, and the model's competence was validated by ANOVA tests.

Droplet size (Y1) analysis

The regression equation for Y1 given as

$$Y_1 = 71.25 + 18.52X_1 - 14.36X_2 - 2.13X_3 - 0.549X_1^2 + 0.17X_1X_3 + 11.75X_2^2 - 1.63X_2X_3 + 1.05X_3^2$$

Table 2 specifies droplet size varied between 24.8-51.8nm. The quadratic model generated unveiled that amount of capmul PG8, amount of acrysol K140, and amount of transcutol P have a considerable impact on droplet size. Fig. 3 indicated that B has a major effect on droplet size followed by A&C, which shows a moderate effect. The upsurge in Y1 with associated augment in X1 or decreased X2 and vice versa was detailed in literature earlier. The mathematical model generated for droplet size was significant (F-value = 4632.27), implying that the model is significant.[30-32]

Zeta Potential Analysis

The regression equation for Y2 given as

$$Y_2 = 20.33 + 06.19X_1 + 13.72X_2 + 4.33X_3 + 0.35X_1^2 - 0.71X_1X_3 - 12.59X_2^2 - 2.15X_2X_3 - 3.89X_3^2$$

The zeta potential ranged between -10.4 to -29.6mV. The quadratic model generated reveals that the amount of acrysol K140 and amount of transcutol P possess a significant effect on zeta potential. The regression

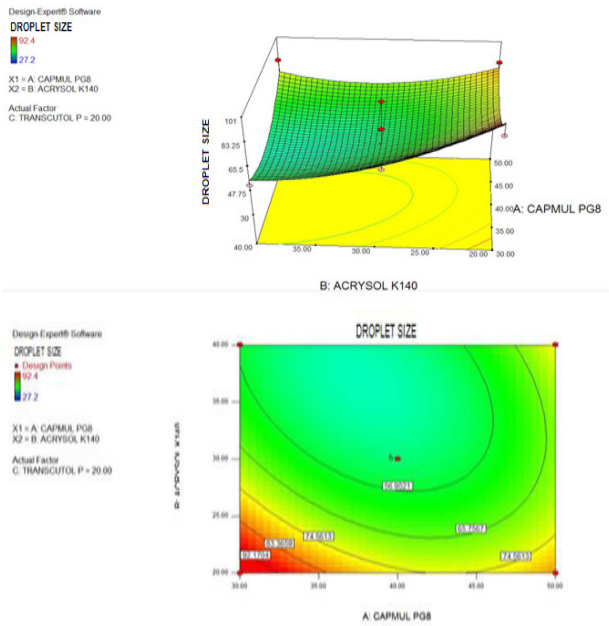


Fig. 3: Response 3D surface and counter plot showing the influence of amount of capmul PG8 and amount of acrysol K140 on droplet size fixed level of C.

equation results indicate that B's effect is noteworthy than A and C. Fig. 4 indicates that B has a significant effect on Y2 followed by C with a moderate effect. The mathematical model generated for zeta potential (Y2) is significant (F-value = 0.0121), implying that the model is significant.

%CDR analysis

The regression equation for % CDR (Y_3) is given as

$$Y_3 = 91.52 - 4.12X_1 + 68.18X_2 - 13.45X_3 + 0.22X_1^2 - 23.43X_1X_3 + 04.16X_2^2 - 35.10X_2X_3 + 2.49X_3^2$$

The % CDR of all PNT SNEDDS ranged between 77.54–98.75%. The quadratic model revealed that A, B, and C significantly influence the droplet size. The regression equation indicates that the effect of B is more significant than A and C. The factorial equation for Y3 showed a good correlation coefficient (0.9995). Fig. 5 shown that B has a major effect on Y1 followed by A & C that have a moderate effect. At higher values, A and B have a negative effect on Y3. The mathematical model generated for Y3 was significant with an F-value of 0.0195 implies that the model is significant.

Optimization by desirability function

Based on the optimization process, the responses transformed to a desirability scale where Y1 and Y2 are minimized while Y3 is maximized. The result was analyzed by Design-Expert software. The optimal value was obtained at X1:40, X2:40, and X3:30. To verify the model's competence, three batches of PTN SNEDDS were prepared with optimum composition and analyzed. Results indicate an agreement existed amongst predicted and observed results confirming the adequacy of BBD in the evaluation and optimization of PTN SNEDDS formulations (Table 3).

Characterization of PTN SNEDDS

Fourier Transform Infrared-Spectroscopy Analysis (FTIR) Studies

The FTIR spectra of optimized SNEDDS formulation displayed all major peaks of drugs, indicating the compatibility between drug and excipients used (Fig. 6).

Zeta Potential and Particle Size of PTN SNEDDS (PTN12)

The optimized formulation's PTN12 displayed a droplet size and zeta potential of 24.8 and -10.4mV, respectively. The droplet size of SNEDDSs indicates nanoparticle range that facilitates rapid absorption as drug absorption by oral delivery is enhanced by decreasing the particle size to the nano range. The zeta potential values of SNEDDS formulations indicate good stability.[30-35]

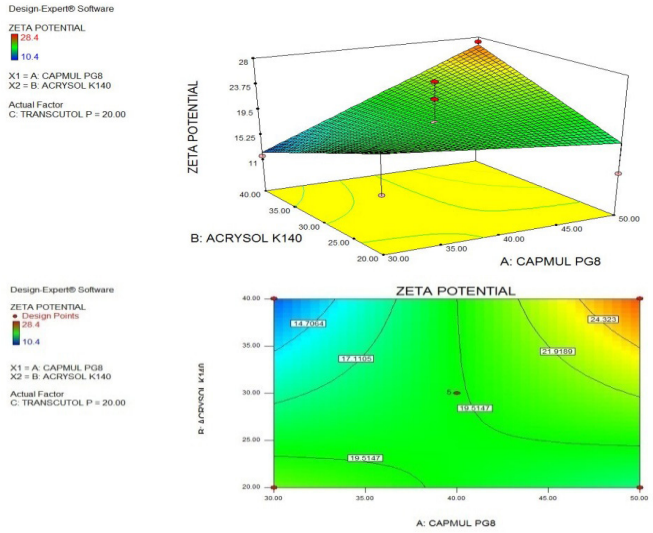


Fig. 4: Response 3D surface plot and counter plots showing the influence of amount of capmul PG8 and amount of acrysol K140 on zeta potential level of C.

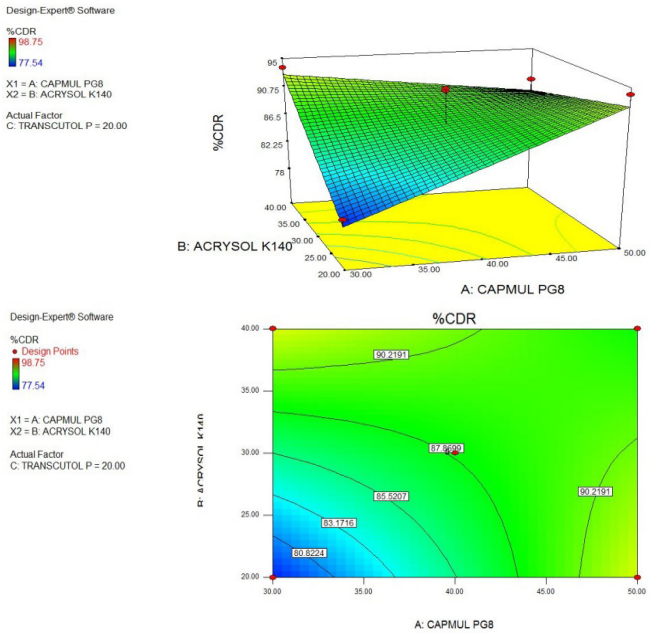


Fig. 5: Response 3D surface plot and counter plot showing the influence of amount of capmul PG8 and amount of acrysol K140 on %CDR level of C.

Table 3: Optimized values obtained by the constraints applies on Y1, Y2 and Y3

		Predicted values				*Observed values*		
Independent variable	*%Nominal values*	*Droplet size (Y1) (nm)*	*Zeta potential (mV) (Y2)*	*%CDR (Y3)*	*Batch*	*Droplet size (Y1) (nm)*	*Zeta potential (Y2) (mV)*	*% CDR (Y3)*
Amount of capmul PG8 (A)	40				1	29.6	-11.6	97.41
Amount of acrysol K140 (B)	40	27.2	-10.47	98.75	2	26.1	-10.4	98.34
Amount of transcutol P (C)	30				3	29.4	-12.4	98.12

SEM Studies

The SEM studies indicated that PTN12 results are in harmony with globule size investigation. The particles are comparatively uniform in shape, exist as spherical particles with smaller size distribution. The SEM images of different scales of 2.00 and 5.00 micrometers and 1mm were shown in Figs. 7 a, b, and c, respectively.

Stability Studies

The formulation PTN12 analyzed for stability study for 3 months at 40 ± 2°C/75% RH ± 5% RH and no significant difference ($p > 0.05$) is found in entrapment efficiency, drug content, and cumulative % drug release.

Pharmacokinetic Studies[37-38]

HPLC analyzes the plasma drug concentrations, and the retention times of HPLC chromatogram of PTN and paracetamol as internal standard was 4.5 and 2.7 minutes, respectively Figs. 8 and Fig. 9 indicates the plasma concentration-time curve post single oral dosage of PTN12 and PTN pure drug suspension. At any given time point, the drug plasma concentration in animals administrated with PTN SNEDDS was considerably superior to those administrated with PTN. From bioavailability studies, the relevant pharmacokinetic parameters of PTN pure drug and SNEDDS formulations PTN12 are tabulated in Table 4. The C_{max} of PTN SNEDDS (2.25 ± 0.02 ng/mL) appreciably increased ($P < 0.05$) in comparison to PTN (0.75 ± 0.003 ng/mL) and $T_{max}$of SNEDDS and pure drug were 1.50 ± 0.03 h and 1.00 ± 0.01h respectively.$AUC_{0-\infty}$ of SNEDDS formulation was four-fold higher (81.21 ± 2.14 ng.h/mL) than the pure drug (20.32 ± 2.12 ng.h/mL). The enhancement in bioavailability of PTN was due to the rapid uptake of nano-emulsion from SNEDDS by enterocytes at the absorption site.[36-38]

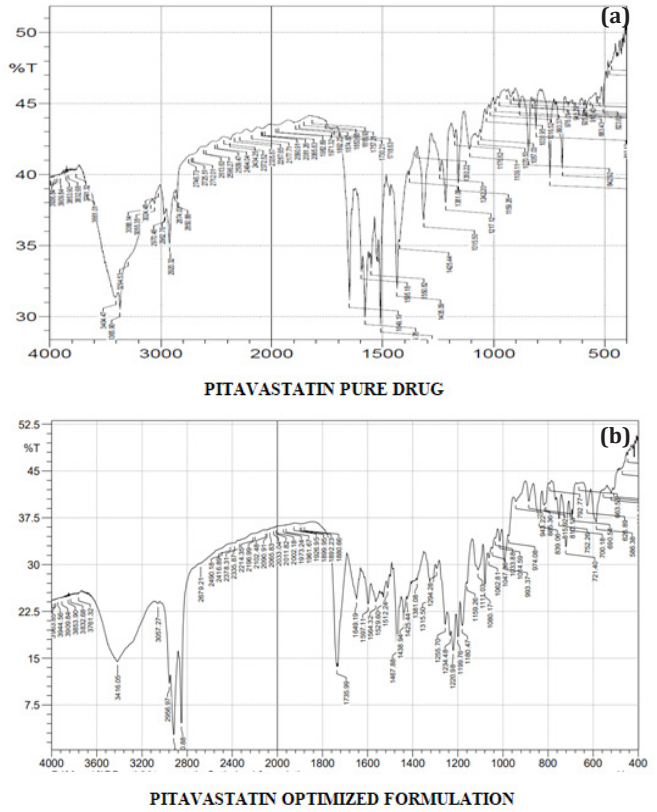


Fig. 6: FTIR of (a) PTN pure drug (b) optimised formulation PTN 12

Table 4: Pharmacokinetic parameters of PTN SNEDDS and pure drug in Wistar rats

Pharmacokinetic parameters	*PTNpure drug*	*PTN12*
C_{max} (ng/mL)	0.75±0.03	2.25±0.02
AUC_{0-t} (ng.h/mL)	12.25±1.02	54.34±2.21
AUC_{0-inf} (ng.h/mL)	20.32±2.12	81.21±2.14
T_{max}(h)	1.00±0.01	1.50±0.03
$t_{1/2}$ (h)	2.32±0.02	3.02±0.02

*Each value represents the mean ± SD (n = 6)

Discussion

The SNEDDS formulation of PTN consisting of capmul PG8, acrysol K140, transcutol was prepared by applying

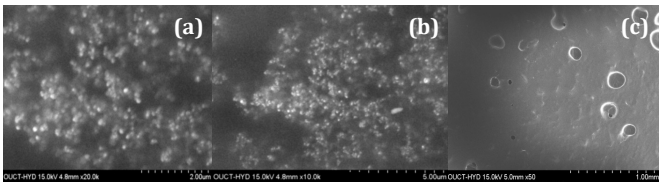


Fig. 7: SEM images of PTN optimized SNEDDS with different scales of (a) at 2µm, (b) at 5µm and (c) at 1mm.

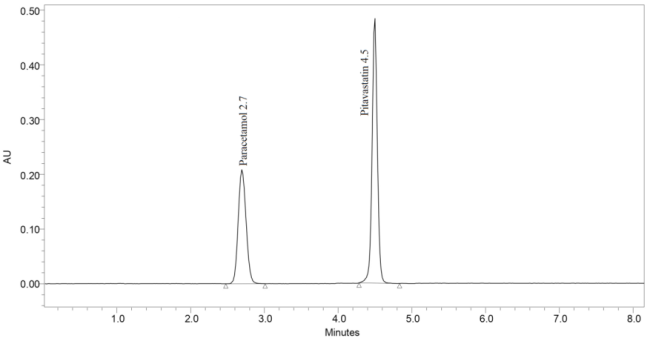
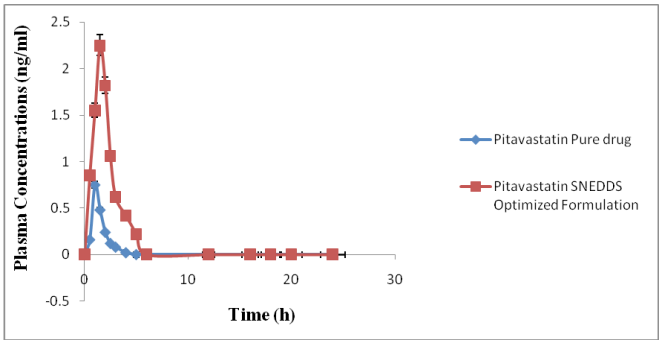


Fig. 8: Standard HPLC chromatogram of Pitavastatin in rat plasma



Each value represents the mean ± SD (n = 6)

Fig. 9: Plasma concentration profiles of PTN SNEDDS and pure drug in Wistar rats.

BBD, which is 3^3 (three-level three factors), considering many factors at a time, where 27 runs were conducted and optimized the best formulation out of all SNEDDS. All the formulations exhibited enhanced drug release compared to the pure drug, with a maximum drug release of 98.75%, superior to that of pure drug release 12.57% in 60 minutes. The C_{max} of optimized SNEDDS (2.25 ± 0.02 ng/mL) was higher than pure PTN suspension (0.75 ± 0.03 ng/mL) and optimized SNEDDS exhibited higher from bioavailability studies oral bioavailability with more than 4 times area under the concentration along with maximum plasma concentration than pure drug. The establishment of safety and efficacy of PTN SNEDDS was done for the first time in this research with *in-vivo* bioavailability studies.

Ethics Approval

The animal studies on Wistar rats were authenticated by the institutional ethical committee no- IAEC NO: 1292/ac/09/CPCSEA/52.

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