Self Nanoemulsifying Drug Delivery System of Candesartan Cilexetil with Improved Bioavailability

J. Venkateswara Rao*, T. Rama Mohan Reddy

Mewar University, NH-79, Gangrar, Chhitorgarh-312901, Rajasthan, India

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ABSTRACT
Candesartan is an angiotensin II receptor blocker with inherent low bioavailability. The present studies entail the optimization and evaluation of self-nanoemulsifying drug delivery system (SNEDDS) of Candesartan in order to enhance its oral bioavailability. For this Capryol 90, Captex 500, Labrasol were used as oil, surfactant and co-surfactant respectively. FTIR studies revealed no interaction among the drug and polymers used in the formulation. Based on the physicochemical parameters and in-vitro dissolution studies, F17 prepared with Smix (Surfactant: Co-surfactant) of 3:1 and Oil:Smix of 6:4, was found to be an optimum one. The formulation F17 was found to release 99.12 ± 5.10% drug at the end of one hour and scanning electron microscopic analysis showed nanosized particles. The droplet size of the optimized formulation was found to be 51.7 nm & Z-Average of 59.2 nm. The zeta potential of the optimized formulation (F17) was found to be -15.5 mV.

INTRODUCTION
Lipid based drug delivery has become one of the promising technology over the past decade due to the multiple roles of lipids in enhancing oral bioavailability. The success of these lipid based systems may also be attributed to the development of novel...
excipients with acceptable regulatory and safety profiles. [1] Depending upon the excipients and formulation techniques, a variety of systems such as physical mixture, liquid/solid solutions, solid dispersions, self-micro and self-nanoemulsifying drug delivery systems (SMEDDS/SNEDDS) may be developed. [2-3] Candesartan cilexetil is a prodrug which has poor bioavailability because of poor solubility and belongs to BCS Class II. It is practically insoluble in water and has a partition coefficient of 9.8 at pH 7.4 (According to the BCS classification developed by Amidon in conjugation with FDA guidance). [4] Candesartan cilexetil is an antihypertensive drug used in the treatment of hypertension. Due to its low solubility, it is required to be administered in high doses for therapeutic effectiveness. [5] Candesartan has a low bioavailability of only 15% and is weakly acidic in nature. [6] Lipids are known to promote the transport of drugs through lymphatic route. [7] The possible mechanisms for this are increased transcellular absorption by increasing membrane fluidity, enhancing the paracellular transport by opening the tight junction, inhibiting the P-glycoprotein and cytochrome P450 to increase the intracellular concentration and formation of lipoprotein or chylomicron by phospholipid. [8] Hence, theoretically one can assume an increase in oral bioavailability of Candesartan cilexetil.

In the present study SNEDDS of candesartan cilexetil were formulated and evaluated for in vitro drug release, FTIR, zeta potential, Scanning electron microscopy and particle size.

MATERIALS AND METHODS
Candesartan cilexetil was obtained as a gift sample from Aurobindo Pharma Limited, Hyderabad. Capryol 90, Labrafil 1944 Cs and Labrafil 2125 were procured from Gattefosse France. Sesame oil was obtained from Dr Reddy’s Laboratories, India. Peceryl and Castor oil were obtained from Croda Chemicals. Capmul MCM C8 was obtained from Strides Arcolab, Bangalore, India. Brij 35 was procured from Ind Chem International c/o Abitec Corporation, USA. Glycerol and Captex 500 were obtained from Loba Chemie Pvt Ltd, Mumbai. Propylene Glycol was obtained from Suvidinath Laboratories, Baroda, India. Cremophor EL was obtained from Sigma Chemicals Corporation Pvt Ltd. Mumbai. Tween 80 was obtained from Sigma Aldrich, USA. Transcutol P and Labrasol were procured from Gattefosse India. PEG 400 was obtained from Otto Chemie Pvt Ltd. Mumbai, India. Lauroglycol 90 was obtained from Ranbaxy Laboratories India. Captex 200 and Ethanol were procured from Abitec Ltd, Janesville.

Methods
Solubility studies
The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for Candesartan. An excess amount (2 mg) of Candesartan was added into 2 ml of each excipients (Oils - Capmul MCM C8, Castor oil, Capryol 90, Peceryl, and Sesame oil). Surfactants – (Captex 500, Cremophor EL, Propylene glycol, Glycerol, Brij 35, Labrafil 2125 and Labrafil 1944Cs). Co-surfactants (Ethanol, Labrasol, Captex 200, Transcutol P, Tween 80, Lauroglycol 90 and PEG 400) and kept in mechanical shaker for 24 hours and centrifuged at 10,000 rpm for 20 min using a centrifuge. Supernatant was filtered through membrane filter using 0.45µm filter disk. Filtered solution was appropriately diluted with methanol, and UV absorbance was measured at 257 nm. Concentration of dissolved drug was determined spectrophotometrically. [9]

Pseudo ternary phase diagram
To determine the concentration of components for the existing range of SNEDDS, pseudo ternary phase diagram was constructed using water titration method at ambient temperature (25°C). Surfactant and co-surfactant (Smix) in each group were mixed in different volume ratio (1:1, 2:1, 3:1). Oil and surfactant/co-surfactant mixture (Smix) were mixed thoroughly in different volume ratios 1.9 to 9.1 (1.9, 2.8, 3.7, 4.6, 5.5, 6.4, 7.3, 8.2 and 9:1) w/w for all the three Smix ratios 1:1, 2:1, 3:1. The mixture of oil, surfactant and co-surfactant at certain ratios were titrated with water by drop wise addition under gentle agitation. Pseudo ternary plots were constructed using Chemix software. Deionized water was used as diluting medium and added into the formulation. The proper ratio of one excipient to another in the SNEDDS formulation was analyzed. [10]

Visual observation
A predetermined volume of mixture (0.2 ml) was added to 300 ml of water in a glass beaker under stirring and temperature was maintained at 37°C using a magnetic stirrer. The tendency of formation of emulsion was observed. If the droplet spreads easily in water, it was judged as ‘good’ and judged as ‘bad’ when there was milky or no emulsion or presence of oil droplets. By performing this test one can assess the self-emulsification property of the final formulation. [11]

Development of SNEDDS formulation
Based on solubility studies, pseudo ternary phase diagram and visual observation, various formulations of Candesartan SNEDDS were prepared. Here, Capryol 90 was used as oil phase, Captex 500 and Labrasol were used as surfactant and co-surfactant respectively. The composition was shown in Table 1. Candesartan was added in accurately weighed amount of oil into screw-capped glass vial and heated in a water bath at 40°C. The surfactant and co-surfactant were added to the oily mixture using positive displacement pipette and stirred with magnetic bar. The formulation was further sonicated for 15 min and stored at room temperature.

Freeze Thawing (Thermodynamic Stability Studies)
Formulations were subjected to freeze cycle (-20°C for 2 days followed by 40°C for 2 days). Only stable
formulations were selected for further studies. The main objective of this study was to evaluate the phase separation and effect of temperature variations on SNEDDS formulations.

Table 1: Formulation trials of Candesartan SNEDDS

| Smix (Surfactant: Co-surfactant) | Oil : Smix | Formula code | Drug Candesartan (mg) | Oil (Capryol 90) (ml) | Surfactant (Capte x 300) (ml) | Co-surfactant (Labrasol) (ml) |
|----------------------------------|-----------|--------------|----------------------|----------------------|-----------------------------|-----------------------------|
| 1:9                             | F1        | 8            | 0.15                 | 0.675                | 0.675                       |
| 2:8                             | F2        | 8            | 0.3                  | 0.6                  | 0.6                         |
| 3:7                             | F3        | 8            | 0.45                 | 0.525                | 0.525                       |
| 4:6                             | F4        | 8            | 0.6                  | 0.45                 | 0.45                        |
| 5:5                             | F5        | 8            | 0.75                 | 0.375                | 0.375                       |
| 6:4                             | F6        | 8            | 0.9                  | 0.3                  | 0.3                         |
| 4:6                             | F7        | 8            | 0.6                  | 0.6                  | 0.6                         |
| 5:5                             | F8        | 8            | 0.75                 | 0.5                  | 0.5                         |
| 6:4                             | F9        | 8            | 0.9                  | 0.4                  | 0.4                         |
| 7:3                             | F10       | 8            | 1.05                 | 0.3                  | 0.15                        |
| 8:2                             | F11       | 8            | 1.2                  | 0.2                  | 0.1                         |
| 9:1                             | F12       | 8            | 1.35                 | 0.1                  | 0.05                        |
| 2:8                             | F13       | 8            | 0.3                  | 0.9                  | 0.3                         |
| 3:7                             | F14       | 8            | 0.45                 | 0.787                | 0.262                       |
| 4:6                             | F15       | 8            | 0.6                  | 0.675                | 0.225                       |
| 5:5                             | F16       | 8            | 0.75                 | 0.5625               | 0.187                       |
| 6:4                             | F17       | 8            | 0.9                  | 0.45                 | 0.15                        |
| 7:3                             | F18       | 8            | 1.05                 | 0.375                | 0.1125                      |

Centrifugation
Centrifugation was performed at 3000 rpm for 5 minutes and observed for phase separation. Stable formulations without any phase separation were selected for further studies.

% Transmittance measurement
Various SNEDDS formulations were reconstituted with distilled water and the percent transmittance was measured at 243 nm using UV spectrophotometer against water as a blank.

Determination of drug content
SNEDDS equivalent to 2 mg of Candesartan were weighed accurately and dissolved in 100 ml of 0.1N HCl the solution was filtered, diluted suitable and drug content was analyzed at λmax 257 nm against blank by UV spectrophotometer. The actual drug content was calculated using the following equation as follows:

\[
\text{Actual amount of drug in SNEDDS} = \frac{\text{Drug content}}{\text{Theoretical amount of drug in SNEDDS}} \times 100
\]

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In-vitro dissolution studies
The release of drug from liquid SNEDDS formulations and pure drug was determined using a US Pharmacopoeia Type II dissolution apparatus. SNEDDS of Candesartan equivalent to 8 mg was filled in size “0” hard gelatin capsules. The dissolution media 0.1N HCl and temperature of the dissolution medium was maintained at 37°C operated at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined intervals 2, 5, 10, 15, 20, 25, 30, 45, and 60 mins and filtered through 0.45μm pore size membrane filters. The removed volume was replaced each time with 5 ml of fresh media. The concentrations were assayed spectrophotometrically at 257 nm.

Characterization of SNEDDS
FTIR studies
Analysis of Candesartan pure drug and physical mixtures of the drug with the excipients were carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For each the spectrum, 8 scans were obtained at a resolution of 4 cm−1 from a frequency range of 400-4000 cm−1. An FTIR-8400S Spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflection (ATR) accessory was used to obtain the infrared spectra of drug in the isotropic mixtures of excipients.

Determination of droplet size
Photon correlation spectroscopy technique was used to determine the average droplet size of the optimized formulation. (Malvern Instrument UK). It is used to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement.

Determination of Zeta Potential
The emulsion stability is directly related to the magnitude of the surface charge. In conventional SNEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SNEDDS formulation was measured using a zeta meter system. The SNEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta-potential of the resulting micro emulsion was determined using a Zetasizer.

Scanning Electron Microscopy
Scanning electron microscopy (SEM) was applied to assess the shape and surface morphology of microspheres. The SNEDDS after converting to nanoemulsion were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument (HITACHI, S-3700N).

Percent entrapment efficiency
The contents of free drug were separated from nanoemulsion by ultrafiltration at 3500 Da with centrifugation at 3000g for 5 to 10 minutes, followed by qualification using HPLC method. The Entrapment Efficiency was calculated as follows.

\[
\text{Entrapment Efficiency} = \frac{\text{Total amount of drug in SNEDDS}}{\text{Total weight of ingredients in nanoemulsion}} \times 100
\]

Stability studies
Various in vitro parameters like % yield, entrapment efficiency and in vitro release studies were evaluated during stability testing. It was carried out at 40°C ± 2°C/75% RH ± 5% RH for 6 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 90 and 180 days period according to ICH guidelines.
Pharmacokinetic study

Animals
Healthy Wistar rats were (Weighing 150-180 g) selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25°C, Relative Humidity 45% 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.[20-21] The protocol of animal study was approved by the institutional animal ethics committee (IAEC NO: IAEC/1657/CMRCP/T2/Ph D-16/70).

Study Design
Rats were divided in to two groups at random and each group contains 6 rats. The rats were fasted for 24 hours prior to the experiments. After 4 hours of dosing, foods were reoffered. First group was administered with pure Candesartan (as such) made suspension with 0.5% methocel and second group was administered Prepared Candesartan SNEDDS diluted in 0.5% methanol by oral route at a dose of 10 mg/kg. Then, 500µL blood samples were collected from the femoral artery at certain times 0, 0.50, 1, 1.50, 2, 2.50, 3, 4, 5, 6, 8, 12, 16, 20, 24 h post dose and transferred into Eppendorf tubes containing heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5 min to 10 minutes and stored frozen at ~20°C until analysis. [22]

Determination of Candesartan in Rat plasma by HPLC method
Determination of Candesartan cilexetil and internal standard hydrochlorothiazide was carried out by using the chromatographic separation was achieved on Hibar C18 stationary phase (150 mm x 4.6 mm i.d; 5µ) with mobile phase containing phosphate buffer-acetonitrile (55:45) adjusted to pH 4.6 using ortho phosphoric acid was used and injection volume of 20µL, with a flow rate of 1.0 ml/min. and effluents were monitored at 244 nm. The retention times of Candesartan cilexetil and hydrochlorothiazide were 2.6 min and 3.6 min, respectively. [23]

Pharmacokinetic analysis
The pharmacokinetic parameters employed to evaluate were maximum plasma concentration (Cmax), time to attain Cmax i.e., Tmax and t½ values, area under plasma concentration-time curve from zero to the last sampling time (AUC0-t), area under plasma concentration-time curve from zero to infinity (AUC0-∞). AUC0-t was calculated by the linear trapezoidal rule and AUC0-∞ from the following formula

\[ \text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t / K_e \]

Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA) was used to perform the pharmacokinetic parameters. All values are expressed as the mean ± SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. Difference with \( p<0.05 \) was statistically significant.

RESULTS AND DISCUSSIONS

Solubility studies
In the case of SNEDDS initially preliminary solubility analysis was carried out to select the appropriate excipient from various (Oils - Capmul MCM C8, Castor oil, Capryol 90, Pecceol, and Sesame oil). Surfactants – (Captex 500, Cremophor EL, Propylene glycol, Glycerol, Brij 35, Labrafal 2125 and Labrafil 1944Cs); Co-surfactants (Ethanol, Labrasol, Captop 200, Transcutol P, Tween 80, Lauroglycol 90 and PEG 400). The solubility of pure drug was found to be 0.014 mg/ml in water. Based on drug solubility, Capryol 90, Captex 500, Labrasol, were selected as oil, surfactant and co-surfactant respectively. The drug solubility values of these polymers were found to be highest when compared with the pure drug and other polymers (Tables 2, 3, 4 and Figures 1, 2, 3).

| Solubility studies of Candesartan in various oils          |                |                |
|----------------------------------------------------------|----------------|----------------|
| Oils           | Solubility (mg/ml) |
| Capmul MCM C8  | 55.26 ± 1.05     |
| Capryol 90     | 80.25 ± 3.28     |
| Castor oil     | 69.12 ± 3.01     |
| Pecceol        | 45.29 ± 0.99     |
| Sesame oil     | 60.93 ± 2.73     |

| Solubility studies of Candesartan in various surfactants |                |                |
|----------------------------------------------------------|----------------|----------------|
| Surfactants  | Solubility (mg/ml) |
| Glycerol     | 180.23 ± 2.56     |
| Brij 35      | 105.1 ± 1.01      |
| Labrafal 2125| 120.88 ± 1.56     |
| Labrafil 1944Cs| 148.20 ± 1.99  |
| Propylene glycol| 98.26 ± 0.98  |
| Captop 500   | 190.5 ± 2.78      |
| Cremophor EL | 152.9 ± 2.01      |

| Solubility studies of Candesartan in various co-surfactants |                |                |
|-------------------------------------------------------------|----------------|----------------|
| Co-surfactants  | Solubility (mg/ml) |
| Ethanol          | 109.6 ± 1.99     |
| Labrasol         | 156.5 ± 3.53     |
| Captop 200       | 130.2 ± 2.89     |
| Transcutol P     | 95.60 ± 1.58     |
| Tween 80         | 120.1 ± 2.05     |
| Lauroglycol 90   | 89.91 ± 0.85     |
| PEG 400          | 140.25 ± 3.01    |

![Fig. 1: Solubility studies of Candesartan in oils](image-url)
From the solubility studies, Capryol 90, Captex 500 and Labrasol were selected as oil, surfactant and co-surfactant respectively. From the phase diagram indicated as Figure 4, it was observed that self-emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil. Efficiency of self-emulsification was good when the surfactant concentration increased (Figure 4).

Visual observation
With the use of visual observation method, the tendency of formation of emulsion was observed. Visual observation test was performed for different ratios by keeping the surfactant and co-surfactant ratio (Smix) as 1:1, 2:1 and 3:1. Grades were given to the ratios based on the tendency of formation of micro-emulsion. Ratios 2:8, 3:7, 5:5 and 6:4 of Smix 1:1 and 1:9, 4:6, 5:5, 8:2, 9:1 of Smix 2:1 and 2:8, 3:7, 4:6, 5:5 of Smix 3:1 showed rapid formation of micro emulsion within a minute having a clear appearance. Therefore, these ratios were selected for the formulation of SNEDDS (Tables 5, 6 & 7 and Figures 5, 6).

Table 5: Visual observation test for Smix (Surfactant: Co-surfactant) ratio 1:1

| Oil:Smix | Time of self emulsification (min) | Grade |
|----------|----------------------------------|-------|
| 1:9      | <2                               | III   |
| 2:8      | <1                               | I     |
| 3:7      | <1                               | I     |
| 4:6      | <1                               | I     |
| 5:5      | <1                               | I     |
| 6:4      | <1                               | I     |
| 7:3      | <1                               | 1/II  |
| 8:2      | <2                               | III   |
| 9:1      | <2                               | III   |

Table 6: Visual observation test for Smix (surfactant: co-surfactant) ratio 2:1

| Oil:Smix | Time of self emulsification (min) | Grade |
|----------|----------------------------------|-------|
| 1:9      | <1                               | I/II  |
| 2:8      | <1                               | I/II  |
| 3:7      | <2                               | III   |
| 4:6      | <1                               | I     |
| 5:5      | <1                               | I     |
| 6:4      | <2                               | III   |
| 7:3      | <2                               | III   |
| 8:2      | <1                               | I     |
| 9:1      | <2                               | III   |

Table 7: Visual observation test for Smix (surfactant: co-surfactant) ratio 3:1

| Oil:Smix | Time of self emulsification (min) | Grade |
|----------|----------------------------------|-------|
| 1:9      | <1                               | I/II  |
| 2:8      | <1                               | I     |
| 3:7      | <1                               | I     |
| 4:6      | <1                               | I     |
| 5:5      | <1                               | I     |
| 6:4      | <2                               | III   |
| 7:3      | <1                               | I/II  |
| 8:2      | <1                               | I/II  |
| 9:1      | <2                               | III   |
Preparation of Candesartan SNEDDS

SNEDDS of Candesartan were prepared by using Capryol 90 (oil) Captex 500 (surfactant) and Labrasol (co-surfactant). In the present study, fifteen formulations were prepared (Table 8). All the formulations prepared were found to be clear and transparent. Pictorial representations of formulations F1 to F18 were shown in Figure 7.

Thermodynamic stability studies

In thermodynamic stability study, no phase separation and effect of temperature variations on prepared formulations were observed. There was no change in the visual description of samples after centrifugation freeze-thaw cycles. Formulations which are thermodynamically stable only those were selected for further characterization (Table 8).

Transmittance measurement

The visual observation of all the formulations was shown in Table 9. The clarity of nanoemulsions was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w microemulsion since water is external phase. Formulation F17 has % transmittance value greater than 98%. These results indicate the high clarity of microemulsion. In case of other systems %T values were less than 99% suggesting less clarity of microemulsions. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of microemulsion and thereby values of %T are affected (Table 9).

Drug content of SNEDDS

Actual drug content of all 18 formulations are shown in Table 10. The drug content of the prepared SNEDDS was found to be in the range of 88.27–98.77%. Maximum % drug content i.e. 98.77% was found in the formulation F17.

In-vitro dissolution studies of SNEDDS

The faster dissolution from SNEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and upon exposure to dissolution medium results in small droplet that can dissolve rapidly in the dissolution medium. The release from liquid SNEDDS formulation F17 was faster than other SNEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution (Tables 11, 12, 13 and Figures 7, 8, 9).
Table 1: Dissolution profiles of various formulations (F1 to F6)

| Time (min) | F1 | F2 | F3 | F4 | F5 | F6 |
|------------|----|----|----|----|----|----|
| 0          | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 |
| 2          | 4.98±0.02 | 15.12±0.68 | 14.98±0.66 | 14.03±0.66 | 11.26±0.52 | 13.25±0.65 |
| 5          | 7.28±0.09 | 18.25±0.98 | 19.46±0.99 | 17.66±0.97 | 18.01±0.98 | 17.99±0.97 |
| 10         | 11.25±0.52 | 25.89±1.59 | 22.13±1.25 | 22.16±1.25 | 22.16±1.25 | 25.16±1.28 |
| 15         | 14.68±0.61 | 35.69±2.35 | 39.15±2.55 | 39.45±2.65 | 33.25±2.35 | 38.19±2.45 |
| 20         | 22.13±1.25 | 59.56±3.25 | 49.16±2.98 | 48.99±2.96 | 49.45±2.98 | 48.67±2.96 |
| 25         | 29.27±1.89 | 69.25±3.59 | 65.16±3.25 | 69.88±3.45 | 55.12±3.25 | 68.15±3.45 |
| 30         | 33.09±2.20 | 72.19±3.65 | 79.89±3.98 | 79.98±3.99 | 63.12±3.35 | 74.22±3.95 |
| 45         | 39.98±2.56 | 85.99±4.86 | 86.16±4.56 | 89.25±4.99 | 75.16±3.98 | 85.66±4.59 |
| 60         | 46.98±2.98 | 93.12±5.03 | 90.78±5.01 | 92.25±5.02 | 89.99±4.99 | 91.88±5.01 |

Table 2: Dissolution profiles of various formulations (F7 to F12)

| Time (min) | F7 | F8 | F9 | F10 | F11 | F12 |
|------------|----|----|----|-----|-----|-----|
| 0          | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 |
| 2          | 4.98±0.02 | 12.58±0.54 | 8.78±0.09 | 13.25±0.60 | 11.85±0.52 | 10.99±0.45 |
| 5          | 7.28±0.09 | 14.98±0.62 | 15.67±0.68 | 18.36±0.97 | 20.19±0.99 | 18.15±0.98 |
| 10         | 11.25±0.52 | 25.67±1.59 | 22.15±1.25 | 25.67±1.56 | 22.98±1.28 | 28.96±1.99 |
| 15         | 14.68±0.61 | 39.15±2.85 | 38.25±2.84 | 39.16±2.85 | 35.16±2.45 | 34.12±2.33 |
| 20         | 22.13±1.25 | 49.67±2.98 | 50.12±3.22 | 52.16±3.18 | 42.98±2.96 | 43.55±2.97 |
| 25         | 29.27±1.89 | 55.12±3.45 | 59.99±3.45 | 55.89±3.46 | 49.55±2.98 | 54.16±3.44 |
| 30         | 33.09±2.20 | 75.18±3.92 | 65.36±3.55 | 62.13±3.51 | 52.18±3.45 | 69.89±3.38 |
| 45         | 39.98±2.56 | 80.19±4.58 | 78.99±3.95 | 75.99±3.92 | 69.55±3.58 | 79.99±3.96 |
| 60         | 46.98±2.98 | 90.25±5.01 | 85.19±4.86 | 89.99±4.99 | 80.1±4.58 | 91.1±5.02 |

Table 3: Dissolution profiles of various formulations (F13 to F18)

| Time (min) | F13 | F14 | F15 | F16 | F17 | F18 |
|------------|-----|-----|-----|-----|-----|-----|
| 0          | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 | 0.0±0 |
| 2          | 4.98±0.02 | 10.12±0.45 | 13.12±0.56 | 12.11±0.54 | 11.03±0.52 | 17.98±0.81 |
| 5          | 7.28±0.09 | 15.67±0.68 | 18.25±0.91 | 19.20±0.92 | 14.12±0.61 | 25.23±1.89 |
| 10         | 11.25±0.52 | 29.12±1.98 | 29.66±1.98 | 23.52±1.55 | 25.98±1.80 | 38.65±2.51 |
| 15         | 14.68±0.61 | 35.88±2.25 | 48.12±2.98 | 39.16±2.56 | 32.15±2.22 | 55.67±3.45 |
| 20         | 22.13±1.25 | 49.17±2.88 | 52.13±3.46 | 58.12±3.49 | 49.97±2.98 | 69.03±3.88 |
| 25         | 29.27±1.89 | 59.25±3.56 | 65.03±3.62 | 70.25±3.99 | 65.11±3.65 | 78.25±4.01 |
| 30         | 33.09±2.20 | 65.12±3.79 | 72.25±3.91 | 72.19±3.91 | 72.19±3.92 | 80.11±4.45 |
| 45         | 39.98±2.56 | 78.55±4.02 | 81.98±4.56 | 80.15±4.25 | 79.89±4.01 | 88.97±4.72 |
| 60         | 46.98±2.98 | 89.28±4.75 | 90.1±5.01 | 91.25±5.02 | 89.99±4.75 | 99.12±5.10 |

Fig. 7: Dissolution profiles of Candesartan pure drug and formulations (F1 to F6)

Fig. 8: Dissolution profiles of Candesartan pure drug and formulations (F7 to F12)
The FTIR spectra of optimized formulation were having similar fundamental peaks and pattern when compared with the pure drug (Figure 10). Thus there are no significant interactions among the drug and excipients. The optimized formulation of Candesartan spectrum was shown in Figure 11.

**Particle size analysis of SNEDDS**

Droplet size determines the rate and extent of drug release as well as drug absorption. Smaller the particle size, larger the interfacial surface area which may lead to more rapid absorption and improved bioavailability. SNEDDS with a mean droplet size below 200 nm exhibit excellent bioavailability. The particle size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The particle size of the optimized SNEDDS formulation (F17) was found to be 51.7 nm & Z-Average of 59.2 nm indicating all the particles were in the nanometer range (Figure 12).

**Interpretation of FTIR data**
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Fig. 12: Particle size analysis of optimized formulation F17

Fig. 13: Zeta potential of the optimized formulation F17
Zeta potential of SNEDDS
Zeta potential is responsible for the degree of repulsion between adjacent, similarly charged, dispersed droplets. A zeta potential value of ±30 mV is sufficient for the stability of a micro emulsion. The zeta potential of the optimized SNEDDS formulation (F17) was found to be -15.5 mV which comply with the requirement of the zeta potential for stability (Figure 13).

SEM studies
Scanning electron microscope studies of optimized formulation of Candesartan (F17) revealed oval shaped globules. The size is within nanometers. There are clear liquid droplets without any pores (Figure 14).

Stability studies
The Candesartan SNEDDS F17 formulation was filled in hard gelatin capsules as the final dosage form and subjected to stability studies for 6 months. There was no significant change in the drug content and drug release. It was also seen that the formulation were compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There was no significant change in the appearance, or micro emulsifying property.

Pharmacokinetic parameters comparison for pure drug suspension and SNEDDS
Figure 15 shows the plasma concentration–time curve in Wistar rats after a single oral dose of Candesartan SNEDDS formulation as compared to Candesartan pure suspension. At all the indicated time points, the Candesartan plasma concentrations in rats treated with SNEDDS formulation was significantly higher than those treated with pure drug. Pharmacokinetic parameters of Candesartan after oral administration of the two formulations in Wistar rats are shown in Table 9. C\(_{\text{max}}\) of the SNEDDS 35.2 ± 0.02 ng/ml was significant (p<0.05) as compared to the pure drug suspension formulation 25.1 ± 0.03 ng/ml. T\(_{\text{max}}\) of both SNEDDS formulation and pure drug suspension was 1.00 ± 0.03 h and 2.00 ± 0.01 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\(_{\text{0-\infty}}\) infinity for SNEDDS formulation was higher (160.1 ± 1.04 ng. h/ml) than the pure drug suspension formulation 135.3 ± 2.02 ng.h/ml. Statistically, AUC\(_{\text{0-t}}\) of the SNEDDS formulation was significantly higher (p<0.05) as compared to pure drug suspension formulation. Higher amount of drug concentration in blood indicated better systemic absorption of Candesartan from SNEDDS formulation as compared to the pure drug suspension formulation.

Table 9: Pharmacokinetic Parameters of Candesartan SNEDDS formulation and pure drug

| Pharmacokinetic parameters | Candesartan Pure drug | Candesartan - SNEDDS Optimized Formulation |
|---------------------------|-----------------------|------------------------------------------|
| C\(_{\text{max}}\) (ng/ml)  | 25.1 ± 0.03           | 35.2 ± 0.02                              |
| AUC \(_{\text{0-1}}\) (ng. h/ml) | 82.2 ± 1.02          | 104.4 ± 1.01                             |
| AUC \(_{\text{0-\infty}}\) (ng. h/ml) | 135.3 ± 2.02       | 160.1 ± 1.04                             |
| T\(_{\text{max}}\) (h)    | 2.00 ± 0.01           | 1.00 ± 0.03                              |
| 1/2\(_{\text{t}}\) (h)     | 4.52 ± 0.02           | 3.02 ± 0.02                              |

In the present study, SNEDDS comprising of Capryol 90, Captop 500, Labrasol were prepared for enhancing the dissolution and bioavailability of candesartan.
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