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ENHANCING ONSET OF ACTION OF CANDESARTAN CILEXETIL BY THE PREPARATION OF FAST DISSOLVING FILM CONTAINING LOADED CANDESARTAN CILEXETIL NANOEMULSION

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ABSTRACT

Candesartan cilexetil has limitation both in less bioavailability and onset of action. Therefor formulation to optimized the use of candesartan cilexetil. The purpose of this study was to develop nanoemulsion by using an appropriate oils, surfactant and co surfactant. Nanoemulsion was prepared by the spontaneous mixture of labrafil 2125, kolliphor RH 40 and PEG 400 with the ratio (0.5:9:5). The nanoemulsions were prepared by aqeous titration method. The solubility of drug was checked in different solvents, surfactant, oils and co surfactant in regulate to select the best solubilizing componenets for the preparation of nanoemulsion and then pseudoternary phase diagram was used as a useful device to evaluate the nanoemulsion area. The film was prepared by using a polymers (HPMC 5, HPMC 6, HPMC 15, and HPMC 50) and plasticizers. The method used for the preparation of film was petri plate method. It is an easy and low cost price method. The main purpose behind for the preparation of film was enhanced the onset of action and reduce the side effect. The evaluation of film was Invitro dissolution release, weight variation, folding endurance, film thickness, disintegration time, drug content release determination by used a zero order, first order, higuchi, koshmeyer plot graphs. The serious feature was type and ratio of oils, surfactant, co surfactant, and polymer and plasticizer concenteration influenced the film characters.

KEYWORDS: Ternary plot, Polymer, Invitro drug release.

INTRODUCTION

The various range of routes of drug delivery, oral route is the most ideal route for the patient as well as for doctor or clinicial.[1] On the other hand, It has certain disadvantages such as the drug is undergo first pass metabolism and enzymatic degradation within the gastrointestinal tract, that inhibit oral administration of lipid soluble drugs. as a result, lipophilic drugs undergo poor absorption in GIT mucosal membrane and decrease the onset of action as well as bioavailability of drug. Candesartan cilexetil is belongs to BCS II.[2] It is a lipophillic in nature. Transdermal routes of drug delivery is the best option over the oral route of administration. It has advantage avoid the hepatic 1st pass metabolism and protect the drug from enzymatic degradation in GIT tract. Fast dissolving film was prepared on the principle of transdermal patches. Only difference is that between transdermal patches and fast dissolving films, Trandermal patches applied over the skin it makes contact with skin surface after few minutes patches get melt by body heat and drug is released from matrix and directly undergoes the systemic circulation. If we are talking about fast dissolving film it placed over the tounge or below the tounge or inside the buccal cavity it makes contact with mucosal membrane get wet by saliva and released the drug from drug reservoir and directly enter through sublingual vien into systemic circulation which is directly connected with heart, result is that patient get instant relief.

Candesartan cilexetil is a prodrug. Everyone know the solid dosage form of drug is metabolized by hepatic first pass metabolism by the help of cytochrom p450 enzyme. Basically prodrug form use for enhance the bioavailability.[3] Although absolute bioavailability is relatively poor at 15% to 40% percent.[4,5,6] Fast dissolving film of containing loaded candesartan cilexetil nanoemulsion has benefit over the other dosage form provide the large surface area, enhance the bioavalibilty, absorbtion of drug, enhance onset of action, reduce the dose and reduce the side effect. Fast dissolving film is convenient for paediatric and geriatric patients.[7]

Fast dissolving film was used as carrier for the delivery of oral dosage form of candesartan cilexetil loaded nanoemulsion was convereted into hydrophilic film for oral route. Nanoemulsion was prepared by using a Kollphor RH 40, Labrafil 2125 and PEG 400. The ratio was selected by pseudoternary plot 0.5:9.5. The film was prepared by using a polymers and plasticizers. The method used for the preparation of film was petri plate method. It was foumd to be HPMC 50 formed better film for oral dosage form.

MATERIAL AND METHODS

Materials

The following materials was from Candesartan cilexetil (Aurobindo Pharmaceuticals, Hyderabad), Potassium di-hydrogen-*o* phosphate (Signet Chemical Corp. Pvt. Ltd., Mumbai), Sodium hydroxide palates, Ethanol (Changshu Yanguan Chemical, China), Methanol (Fisher Chemical Ltd., Ahmedabad), Acetone (Fisher Chemical Ltd., Mumbai), Di-chloro methane (Fisher Chemical Ltd., Mumbai), Chloroform (Fisher Chemical Ltd., Mumbai), Di-methyl sulphur oxide (Fisher Chemical Ltd., Mumbai), Di- methyl foramide (Fisher Chemical Ltd., Mumbai), n-octanol (Fisher Chemical Ltd., Mumbai), Span 20 (Gattefosse, Ltd., Mumbai), Span 80 (Gattefosse, Ltd., Mumbai), Tween 20 (Gattefosse, Ltd., Mumbai), Tween 80 (Gattefosse, Ltd., Mumbai), Kolliphor RH 40 (Gattefosse, Ltd., Mumbai), Kolliphor EL (Gattefosse, Ltd., Mumbai), Kolliphor HS 15 (Gattefosse, Ltd., Mumbai), PEG 200 (Gattefosse, Ltd., Mumbai), PEG 400 (Gattefosse, Ltd., Mumbai), Labrafil 2125 (Gattefosse, Ltd., Mumbai), Capmul MCM (C8) (Gattefosse, Ltd., Mumbai), Labrafil PG (Gattefosse, Ltd., Mumbai), Capmul MCM EP/NE (Gattefosse, Ltd., Mumbai), Oliec acid (Fisher Chemical Ltd., Mumbai).

Equipments

The following equipments were used UV Spectrophotometer (Shimadzu, Japan), Hot air oven (NSW India 143, New Delhi), Hot metal plate (REMI), pH meter (Ohaus, USA), Digital Balance (Shimadzu, Japan), Orbital shaker (REMI Equipment, Vasai India), Melting Point Apparatus (Remi Equipment, Mumbai), Vortex mixer (REMI Equipment, Mumbai), Cooling centrifuge (REMI Equipment), Sonicator (PCI Analytic), Eppendorf tubes (Tarsons Products Pvt.Ltd), FTIR spectroscopy (Brucker), Dissolution (Orchid Scientific).

Methods

Pre-formulation studies

Organoleptic Properties

The organoleptic studies like general appearance like nature, color, odor etc. were performed by visual observations.

Color: Small quantity of drug was taken in butter paper and viewed in well illuminated place.

Odor: Very less quantity of drug was smelled to get the odor.

Melting Point

For determination of melting point USP method was followed. Melting point of drug was determined by capillary fusion method. A small amount of drug was filled in capillary and it was placed in melting point apparatus. Then the temperature at which drug crystals started melting and turned into liquid was noted down.

Determination of absorption maxima in Methanol

Process: 10 mg of candesartan cilexetil was accurately weighed separately and dissolved in 100 ml of methanol to prepare stock solution of 100µg/ml respectively. From stock solutions of each drug of the respective media, 1 ml aliquots were withdrawn and diluted to 10 ml with their respective media to obtain solutions of concentrations of 10 µg/ml accordingly. These solutions were scanned between 200 nm to 400 nm against their respective media as their blanks using a double beam UV Visible spectrophotometer and the wavelength was showing maximum absorbance, selected as absorption maxima of drug.

Preparation of Calibration Curve of Candisartan cilexetil in methanol

Stock solution of 100 µg/ml was prepared by dissolving 10 mg drug in 100 ml of methanol. Dilutions in the range of 4 µg/ml to 24 µg/ml were prepared from stock solution. Dilutions were scanned for determining λ max from 200-400 nm through UV spectrophotometer.

Solubility Studies

For quantitative solubility study, excess amount of drug was taken in thoroughly cleaned culture tubes containing 5 ml of different solvents, oils, surfactants and cosurfactants (Methanol, Ethanol, pH 6.8, pH 7.4, Acetone, DMF, DMSO, DCM, Chloroform, Span 40, Span 20, Tween 20, Tween 80, Kolliphor RH 40, Kolliphor EL, PEG 200, PEG 400, Propylene glycol, Labrafil 2125, Labrafil MCM, Labrafil PG, Oliec acid, Capmul MCM, Kollisolv MCT) and culture tubes were tightly closed. These culture tubes were shaked on water bath shaker for 24 hrs at room temperature. After 24 hrs each sample was centrifuged and filtrate was suitably diluted and determined spectrophotometrically.

Determination of solubility of drug in various solvents

The solubility of candesartan cilexetil in various solvents was determined by dissolving excess amount of candesartan cilexetil in 1ml of each of the selected solvents in 5ml capacity Stoppard vials separately. Each glass vial was then `mixed for 10 min using a vortex mixer.

The mixture vials were then kept at 37±1.0 °C in a shaker bath for 24 hrs to get equilibrium. The equilibrated samples were removed from shaker and centrifuged at 15000 rpm for 30 min. The supernatant was taken and filtered through a 0.45µm membrane filter. The concentration of API was determined in each solvent by UV spectrophotometer by scanning from 200-400nm.

Partition Coefficient of Drug

Partition coefficient (oil/water) is a measure of a drug's lipophilicity and an indication of drug's ability to cross cell membranes. It is defined as the ratio of unionized drug distributed between the organic and aqueous phases at equilibrium. Partition coefficient provides a means of characterizing the lipophilic/hydrophilic nature of the drug. Drugs having values of P much greater than 1 are classified as lipophilic, where as those with values much less than 1 are indicative of a hydrophilic drug. The partition coefficient is commonly determined using an oil phase of n-octanol and water. In the case n-octanol and water:

- Shake flask method

The partition coefficient determination study was performed by using shake flask method. Excess amounts of the drug (*Candesaratan cilexetil*) dissolved in 5 ml of two solvents (n-octanol: Water) together (1:1) and placed for 24 hrs. After 24 hrs, the two layers were separated and centrifuge for 15 minutes at 15,000 rpm. The absorbance was taken in UV spectrophotometer at the respective λ max after appropriate dilution.

Drug and excipient compatibility study by FTIR spectroscopy

Fourier transform infrared Spectroscopies of different compounds were performed for identification of that particular compound. FT-IR Spectroscopy of pure drug (Candesaratan cilexetil*)* and PVA was done using KBr pellets. Various peaks in FT-IR Spectrum were interpreted for identification of different group in the structure of pure drug (Candesaratan cilexetil), its mixture and optimized suspension. FT-IR Spectroscopy can also be used to investigate and predict any physicochemical interactions between different components.

Screening of oil and surfactant

Process: For this study, 100 mg of surfactant were added to 100 mg of oily phase and then this mixture was heated at 50ºC for homogenization of the components. Then from each prepared mixture, 100 mg was withdrawn and diluted to 100ml in a volumetric flask. The ease of emulsification was judged by the number of flask inversions required to yield

homogeneous emulsion. The emulsions were allowed to stand for 24 hrs and then % transmittance was evaluated at 638 nm by using UV spectrophotometer. They were also observed for turbidity or phase separation visually.

Different trials for Ternary Phase Diagram

Process: Phase diagrams involve the plotting the three components surfactant: co-surfactant (Smix), oil and water each of them representing an apex of triangle. Ternary mixtures with varying compositions of the components were formed. For any ternary mixture formed the total of surfactants, co-surfactants and oil concentrations always added to 100%. The required amount of the three components was weighed accurately. The mixture was then gently heated at 45–50ºC and vortex to form homogenous mixture. To this mixture distilled water was added drop by drop until a transparent solution was formed. The surfactant and co-surfactant was varied in mass ratios 1:1, 1:2, 2:1, The different concentration ratios of oil and mixture of surfactant and co surfactant were taken as 0.5:9.5, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8, 3:7, 4:6 and 5:5 Ternary mixtures were formed in these ratios and then quantity of water forming transparent solution was plotted with other components in the pseudo-ternary phase diagram.

Preparation of nanoemulsion

Process: Nanoemulsion was prepared by spontaneous emulsification method, Briefly 10mg candesartan cilexetil dissolved was dissolved in oil followed by additional surfactant and co surfactant. The mixture was then subjected to vortexing for 1min and gentely heated at 40 to 50˚C for 5 minutes and diluted to 5 ml water.

Preparation of oral fast dissolving film by petri plate method

Optimization of polymer type

In fundamental investigations, a few polymers were assessed for their film forming limit by making different arrangements of polymers with plasticizers and throwing them on various surface regions.

The effect of different polymers on the milling efficiency was evaluated. Formulation F11 and F16 with HPMC E5, HPMC E15, and HPMC E50, showed good physical compatibility with nanoemulsion. Formulation F12, F-14, F-15 with HPMC E5, HPMC E15, and HPMC E50 showed good milling efficiency but E50 producing a high viscous emulsion. The polymer took with a selected ratio, oil (49.6 mg), surfactant (471.2mg), cosurfactant (471.2mg) and volume make up upto 4ml of final nanoemulsion formulation.

Characterization of film

Scanning electron microscopy

The diameter of nanoemulsion particals was examined by scanning electron microscopy. A sample of nanoemulsion was placed on glass stub and vacuum dried. After the stub containing the sample was placed in the scanning electron microscope chamber and coated with gold palladium and observed microscopically at accelerating voltage of 10 Kv.

Mechanical folding endurance

Number of times a film can be folded without breaking or visibly cracking it is defined as the folding endurance. The method was used to determine the folding endurance and flexibility of the films (1×1 cm^2) were held with forceps and folded to 180° for a limit of multiple time or until it breaks.

Drug loading capacity (%)

The Nanoemulsion loaded film drug loading capacity was determined by dissolving the film (2×2 cm^2) 10 mg in 10ml solvent mixture comprising of methanol an aliquot of sample was taken in micro centrifuge tubes and followed by centrifugation at 15,000 rpm for 20 min. The supernatant was separated, suitably diluted with methanol, and analyzed by UV-Visible spectrophotometer at 255nm. The % drug loading capacity of the nanoemulsion loaded formulation was determined by measuring the concentration of drug in the dispersion medium. The experiment was performed in triplicate, and % drug loading capacity of Candisartan cexetil in film was calculated from the following equation: Each sample solution was passed through the 0.45 µm syringe filter and then 1 ml of filtrate was analyzed for drug content by UV method.

Drug loading capacity (%) = Amount of drug content in film / Total drug fed in film× 100

Disintegration time

10 ml of distilled water was poured in a Petri dish and one film was included on the surface of the water and the time estimate until the oral film was broken down totally. The estimation were carried out for three distinct films and the range was accounted.

In-vitro drug release studies

The dissolution investigation of prompt discharge films of candesartan cilexetil was carried out in a beaker containing 30ml of the phosphate buffer pH 6.8 as a dissolution medium kept up at 37±0.5˚C. The medium was stirred at 50 rpm. Aliquots 1ml of the dissolution medium

was withdraw at 1,2,3,4,5,6,7,8,9,10,15,20,25 and 30 minutes time interval and the same amount was added with the fresh medium in regulation to maintain the sink conditions. Samples were measured spectrophotometerically at 255nm.

The release profile of fast dissolving films (F-15) was compared with that of pure drug of fast dissolving film and it was observed that the drug release from nanoemulsion loaded in fast dissolving films was much faster than that from raw drug of fast dissolving film.

Drug release kinetic studies

In the present study, raw data obtained from in vitro release studies was analysed, wherein data was fitted to different equations and kinetics model to calculate the percent drug release and release kinetics of *Candesartan cilexetil* from fast dissolving film. The kinetic models used were a Zero-order equation, First-order, Higuchi's model and Korsmeyer-Peppas equation.

Zero-order kinetics

A zero-order release would be predicted by the following equation.

$$A_t = A_0 - K_0 t \quad (1)$$

Where:, A_t is the drug release at time't'

A_0 is the initial drug concentration

K_0 is the zero order rate constant (hr^{-1})

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K_0.

First order kinetics

A first-order release would be predicted by the following equation.

$$\text{Log } C = \text{Log } C_0 - K_t/2.303 \quad (2)$$

Where C is the amount of drug remained at time's'

C_0 is the initial amount of drug

K is the first-order rate constant (hr^{-1})

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follows first-order kinetics. The constant 'K' can be obtained by multiplying 2.303 with slope values.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation:

$$Q = [D\varepsilon/\tau(2A - \varepsilon C_s)] C_s t^{1/2} \quad (3)$$

Where: Q is the amount of drug released at time't'

D is the diffusion coefficient of the drug in the matrix

A is the total amount of drug in unit volume of matrix

C_s is the the solubility of drug in the diffusion medium

E is the porosity of the matrix

τ is the tortuosity

t is the time (hrs) at which 'Q' amount of drug is released.

Equation may be easy if one may assume that Dε, Cs and A are constant. Then equation becomes:

$$Q=K.t^2 \quad (4)$$

When the data is plotted according to equation i.e., cumulative drug released versus square root of time, yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsmeyer-Peppas Model

The release rates from controlled release polymeric matrices can be described by the equation proposed by Korsmeyer et.al.

$$Q = K_1 t^n \quad (5)$$

Where: Q is the percentage of drug released at time't'

K is the Kinetic constant incorporating structural and geometric characteristics and 'n' is the diffusion exponent indicative of the release mechanism.

For Fickian release, n = 0.45 while anomalous (Non-Fickian) transport, n ranges between 0.45 and 0.89 and for zero-order release, n = 0.89.

RESULT AND DISCUSSION

Organoleptic properties of drug: The Organoleptic properties of drug as shown in bullet points,

- **Taste:** Slightly bitter
- **Odor :** pungent smelling
- **Colour Crystal:** White to off-white, crystalline powder
- **Melting point determination**

The melting point of a substance is the temperature at which the solid phase gets converted into liquid phase under the one atmosphere of pressure. The melting point determination implies the purity of drug. Melting point of candesartan cilexetil was determined by capillary tube method and was observed to be quite similar to the reported melting point as shown in Table No.1.

Table No. 1: Melting point.

S. No.	Start point	Mean	Std.dev	End point	Mean	Std.dev
1	180	180.33	0.577	182	182.33	0.577
2	181			183		
3	180			182		

Discussion: The melting point of candesartan cilexetil was found to be in range 182-183°C ± 0.57 which is of the pure drug. Hence drug sample was free from any type of impurities. Shown Table No.1.

Partition coefficient

Partition coefficient determine by using a separating funnel.

Table No. 2

S.No.	Reported Partition coefficient	Observed Partition coefficient
01	3	2.95±0.009

Discussion: The partition coefficient of candesartan cilexetil in n- Octanol: Water was found to be 2.95±0.009; this indicates that the drug is lipophillic in nature. Shown in Table No.2.

UV Spectroscopy[8]

UV-VIS spectroscopy is fundamentally used for quantitative examination and fills in a helful auxiliary device for structural clarification of various drugs to obtain specific information on the chromophoric part of the molecules in solution. When the light passes throught the solution in the visible/UV region of the spectrum light is absorbing at particular wavelength that depend on the electronic transition. The UV spectrum is commonly recorded as a plot of absorbance versus wavelength.

Determination of absorption maxima in Methanol

Absorption maxima of candesartan cilexetil were found to be at 255 nm similar to literature as shown in Figure No.1 and Figure No.2.

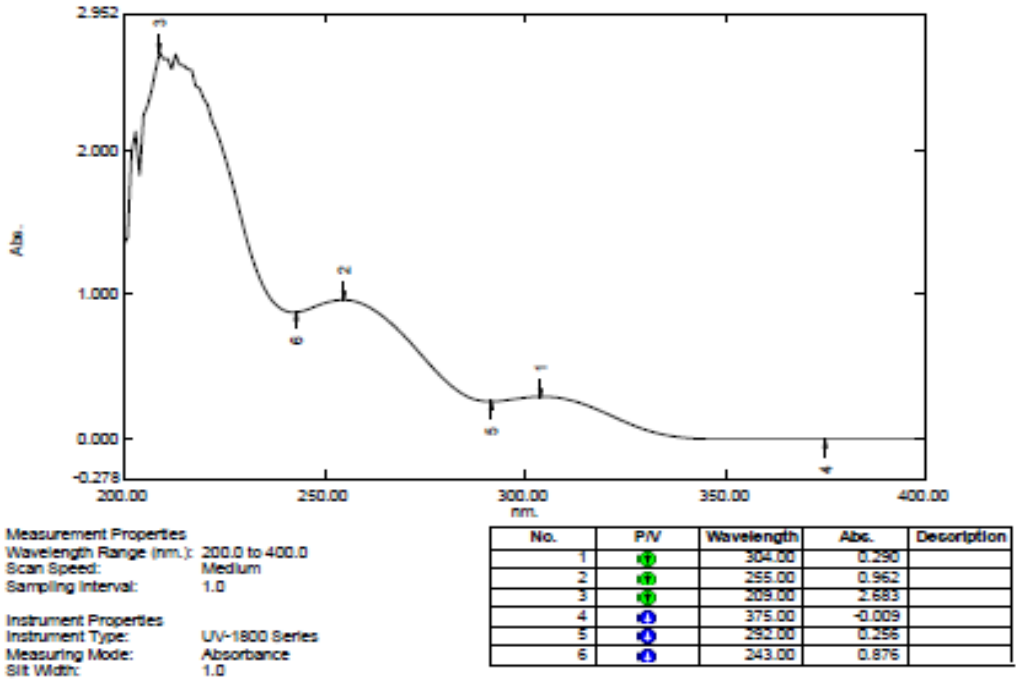

Figure No. 1: UV spectrum graph of candesartan cilexetil in methanol (4-24 µg/ml).

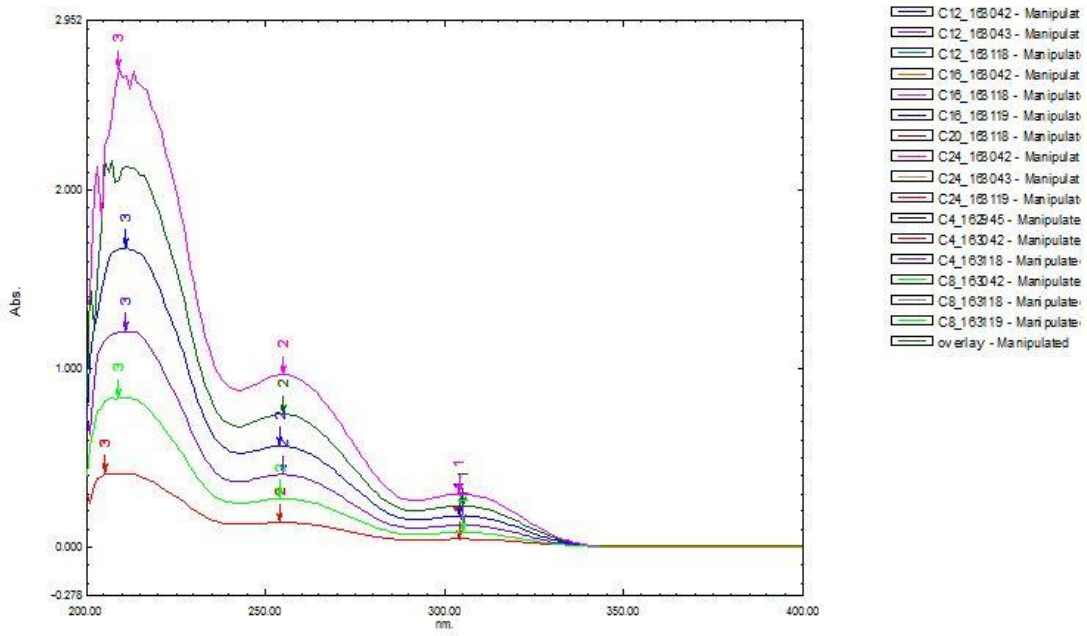

Figure No. 2: Overlay of UV spectrum graph of candesartan cilexetil in methanol (4-24 µg/ml).

Discussion: Absorption maxima of candesartan cilexetil were found to be at 255 nm.

Standard Calibration curve in methanol at 255 nm

Table No. 3: Calibration curve of candesaratn cilexetil in methanol (λ_{max}= 255nm).

S. no	Conc.(µg/ml)	Absorbance
1	4	0.122
2	8	0.270
3	12	0.433
4	16	0.592
5	20	0.754
6	24	0.937

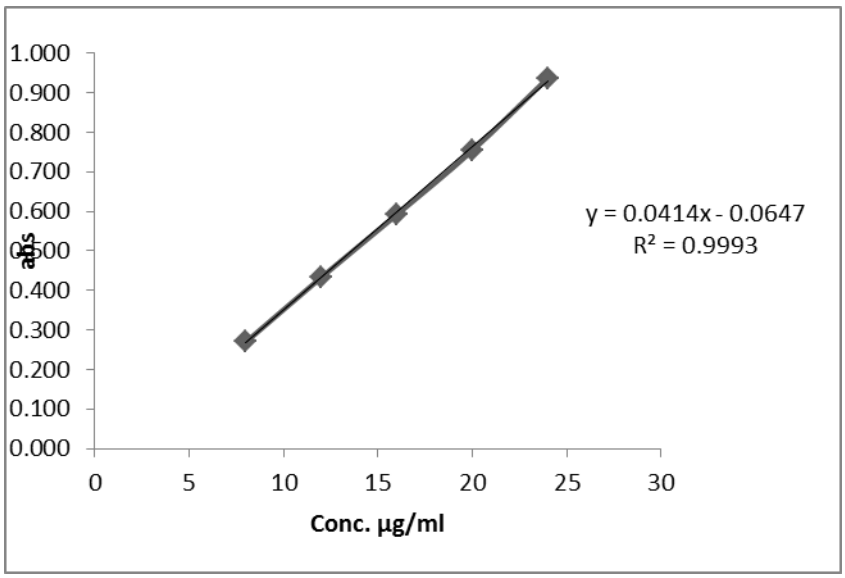

Figure No. 3: Graph of standard calibration curve of Candesartan cilexetil in methanol.

Table No. 4: Result of regression analysis of UV method for estimation of Candesartan cilexetil.

Statistical parameters	Results
λ max	255 nm
Regression equation ** Y=mx+C	Y=0.041x+0.064
Slope (b)	0.041
Intercept (C)	0.064
Correlation coefficient (r^2)	0.999

Discussion: - The calibration curve for candesartan cilexetil was obtained by using the 4 to 24 µg/ml concentration of candesartan cilexetil in methanol. Shown in Table No.3. The absorbance was measured at 255 nm. Calibration curve of candesartan cilexetil shows in graph indicated the regression equation Y=0.041x +0.064 and R^2 value 0.999, which shows good linearity as shown Table No.4 and Figur No.3.

FT-IR spectral analysis

FT-IR analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample. The FT-IR spectrum of candesartan cilexetil is shown in Figure No.4 and interpretation of data is given in Table No.5.

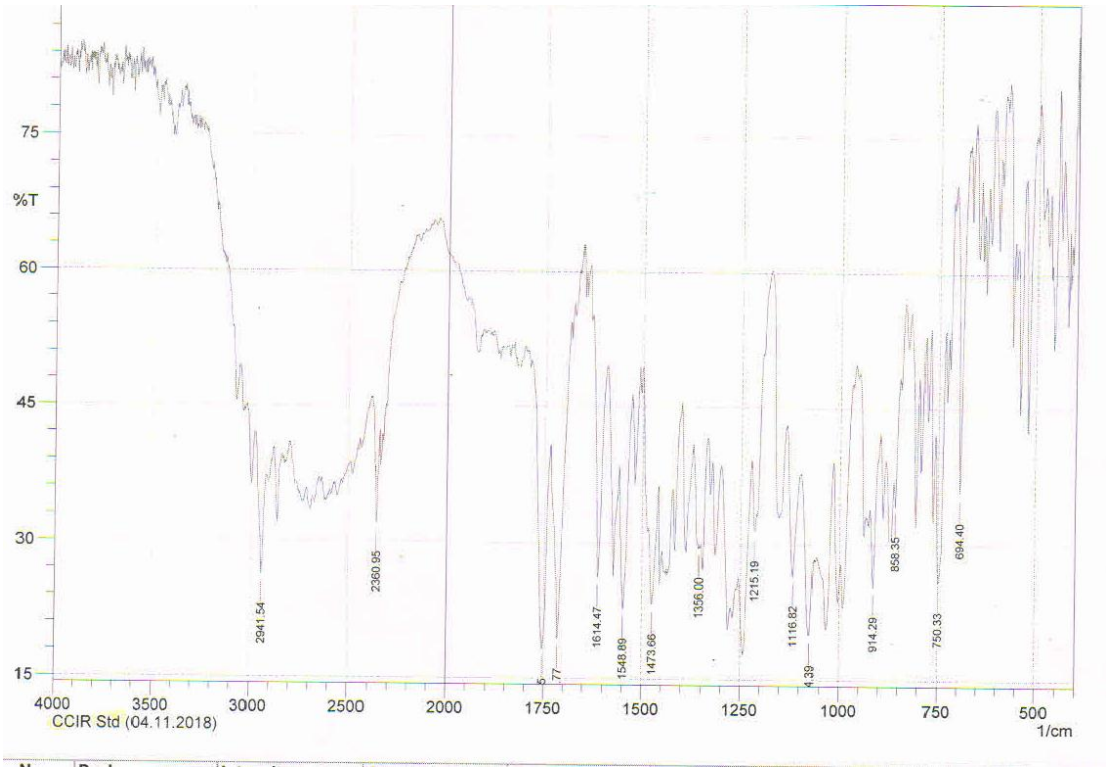

Figure No. 4: FTIR spectrum of pure drug (candesartan cilexetil).

Table No. 5: Interpretation of Pure drug (candesartan cilexetil).

Functional Peak (Vibration)	Reference Peaks (cm^{-1})	Observed Peaks(cm^{-1})
=C-H Bend, Alkenes	1100 - 650	694.4
C-Cl Stretch, Alkyl halides	851 - 550	750.33
C-H, Aromatics	901 - 675	858.35
O-H Bends, Carboxylic acid	951 - 910	914.29
C-N Stretch, Aliphatic amine	1251 - 1020	1074.39
C-O Stretch, Alcohols, Carboxylic acid, Ester, Ether	1321 - 1000	1116.82
C-H, Alkyl halide	1301 - 1150	1215.19
N-O Symmetric stretch, Nitro compounds	1361 - 1290	1356
C-C, Stretch, Aromatics	1501 - 1400	1473.66
N-O, Asymmetric stretch, Nitro compounds	1551 - 1475	1548.89
N-H Bond, 1˚Amine	1651 - 1580	1614.47
C=O Stretch, α,β-Unsaturated esters	1731 - 1715	1714.77
C=O Stretch, Esters satrurated aliphatic	1750 - 1735	1735.35
-C=C Stretch-, Alkynes	2261 - 2100	2360.95(shifting peak)
C-H Strecth, Alkanes	3001 - 2850	2941.54

Table No.5 showed characteristic peaks of candesartan cilexetil. The infrared spectrum of candesartan cilexetil is consistent with their structure.

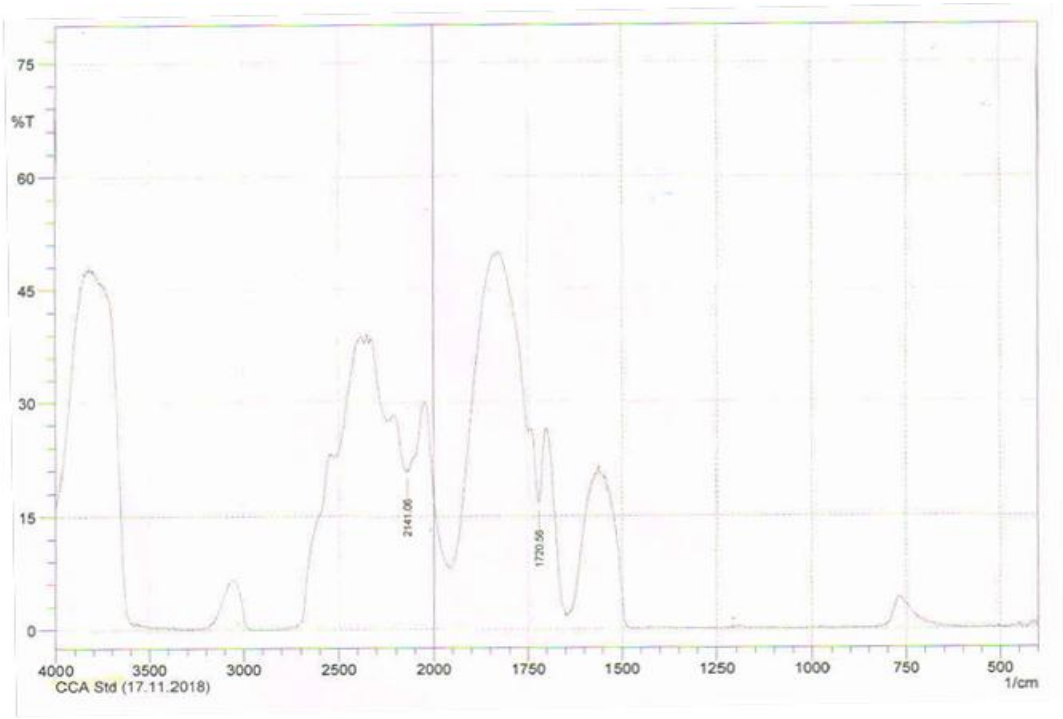

Figure No. 5: FTIR spectrum PEG 400.

Table No. 6: Interpretation of PEG 400.

Functional Peak (Vibration)	Reference Peaks (cm^{-1})	Observed Peaks(cm^{-1})
C=O Stretch, aldehyde, saturated aliphatic.	1740 - 1720	1720.56
-C=C- Stretch, Alkynes	2260 - 2100	2141.06

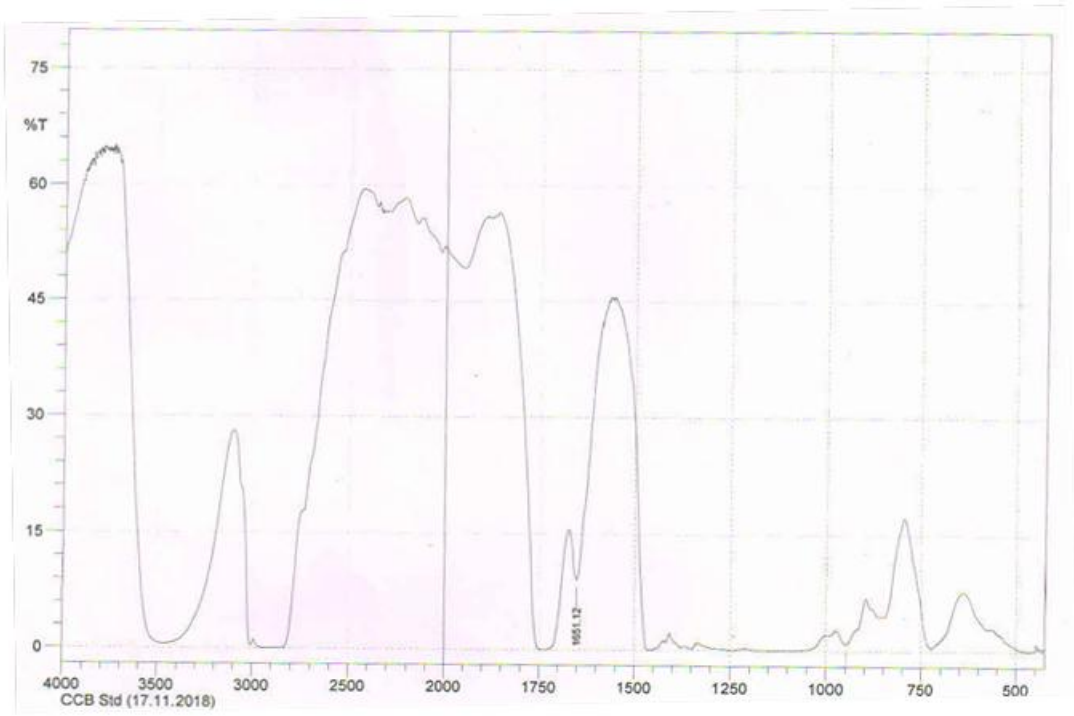

Figure No. 6: FTIR spectrum of labrafil 2125.

Table No. 7: Interpretation of labragil 2125.

Functional Peak (Vibration)	Reference Peaks (cm^{-1})	Observed Peaks(cm^{-1})
O-H Bend, Carboxylic group	950 - 910	949.01
N-H Bend, Primary amine	1650 - 1580	1651.12

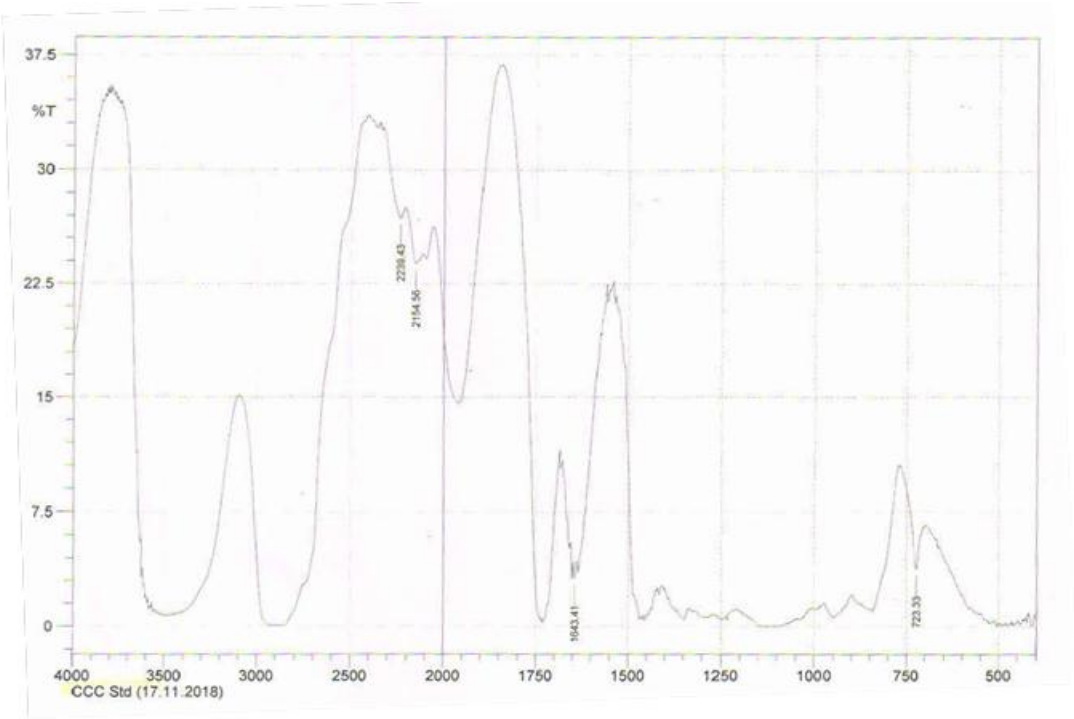

Figure No. 7: FTIR of Kolliphore RH 40.

Table No. 8: Interpretation of Kolliphor RH 40.

Functional Peak (Vibration)	Reference Peaks (cm^{-1})	Observed Peaks(cm^{-1})
C-H rock, Alkenes	725 – 720	723.33
-C=C- Stretch, Alkenes	1680 - 1640	1643.41
-C=C- Stretch, Alkynes	2260 - 2100	2154.56
-C=C- Stretch, Alkynes	2260 - 2100	2239.43

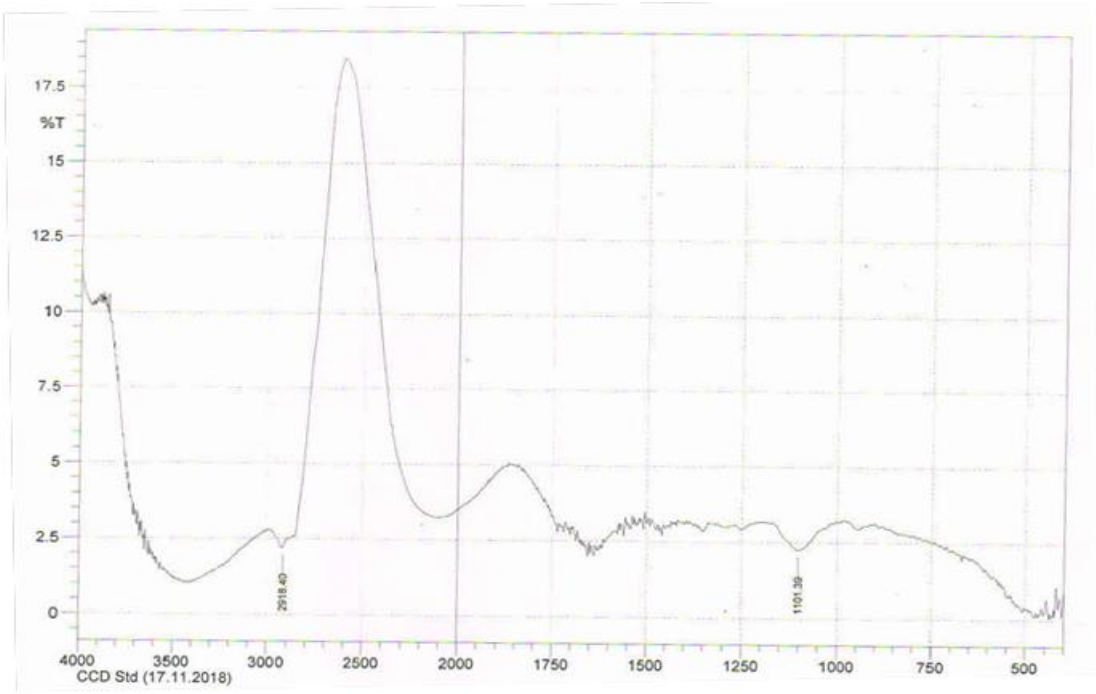

Figure No. 8: FTIR of Physical mixture.

Table No. 9: Interpretation of Physical mixture.

Functional Peak (Vibration)	Reference Peaks (cm^{-1})	Observed Peaks(cm^{-1})
C=O Stretch, Alcohols, Carboxylic acid, Esters, Ethers	1320 - 1000	1101.39
C-H Stretch, Alkanes	3000 - 2850	2918.4
C-N Stretch, Aliphatic amine	1150 - 1020	1101.39

Determination of solubility of drug in various solvents

Table No. 10: Solubility Studies of candesartan cilexetil in various solvents at 255 nm.

S.NO.	NAME OF SOLVENT	solubility (mg/ml)
1	pH 7.4	0.01525±0.00025
2	pH 6.8	0.027±0.00025
3	DMF	16.658±0.0381
4	Dmso	16.733±0.0381
5	Chloroform	22.667±0.0144
6	Acetone	24.0833±0.0288
7	Ethanol	25.625±0.025
8	DCM	53.667±0.3818
9	Methanol	74.75±0.25

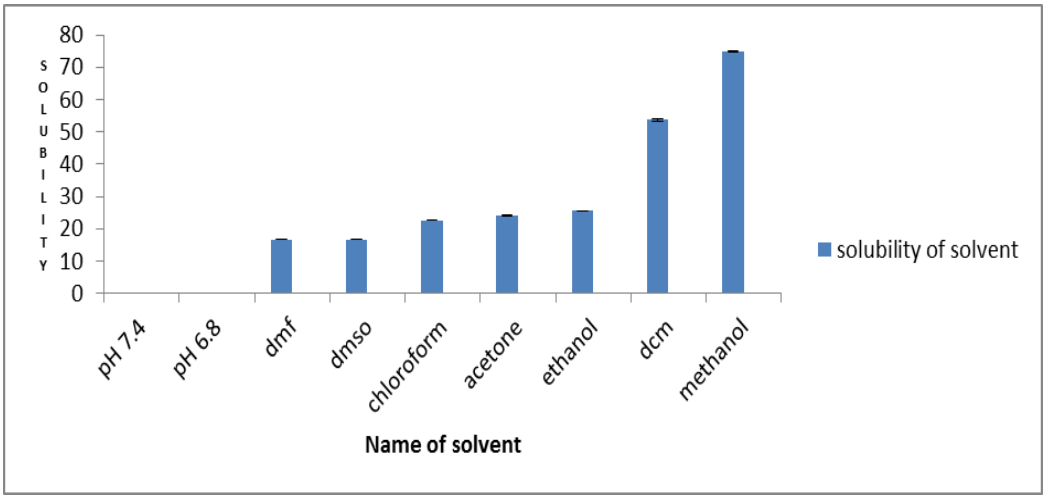

Figure No. 9: Solubility study of drug in different Solvents.

Discussion: Table No.10 and Figure No.9. Demonstrated that among above all mentioned solvents candesartan cilexetil has maximum solubility in methanol.

Table No. 11: Solubility Studies of candesartan cilexetil in various oils at 255 nm.

S.NO.	NAME OF OILS	SOLUBILITY mg/ml
1	capmul mcm	25.983±0.0629
2	labrafac pg	2.466±0.0162
3	labrafil 2125	13.316±0.4679
4	kollisolv mct	1.926±0.0277
5	oleic acid	11.833±0.5928
6	capmul mcm EP/NE	2.464±0.0364
7	labrafil mcm	15.85±0.264

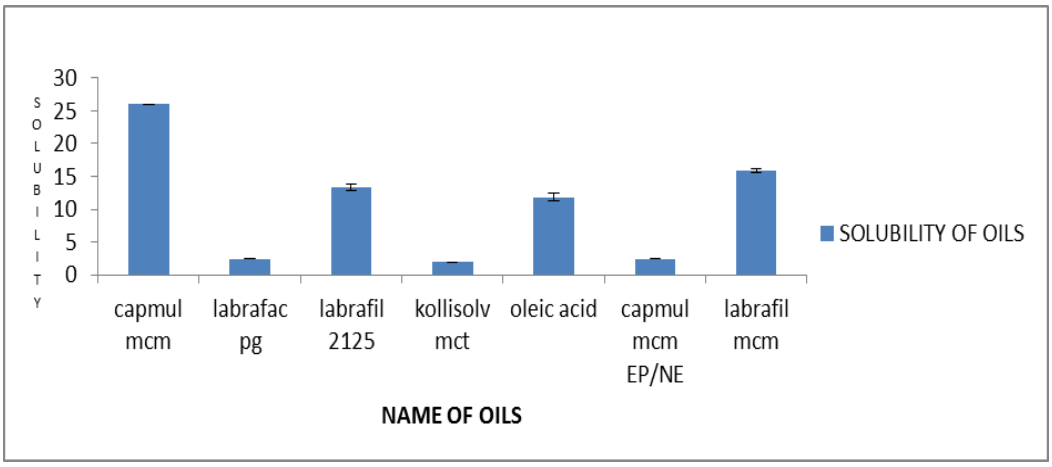

Figure No. 10: Solubility study of drug in different Oils.

Discussion: Table No.11 and Figure No.10 demonstrated that among above all mentioned oil candesartan cilexetil has maximum solubility in Labrafil M 2125 CS, Capmul MCM, oleic acid, Labrafil mcm.

Table No. 12: Solubility Studies of candesartan cilexetil in various Surfactants and co surfactant at 255 nm.

S.No	Name of surfactant and co surfactant	Solubility (mg/ml)
1	kolliphor HS 15	5.125±0.0433
2	kolliphor RH 40	6.35±0.0866
3	tween 80	8.975±0.0901
4	tween 20	8.9±0.173
5	span 20	8.1±0.217
6	span 80	12.441±0.177
7	kolliphor EL	5.541±0.072
8	PEG 400	9.358±0.350
9	Propylene glycol	6.033±0.125

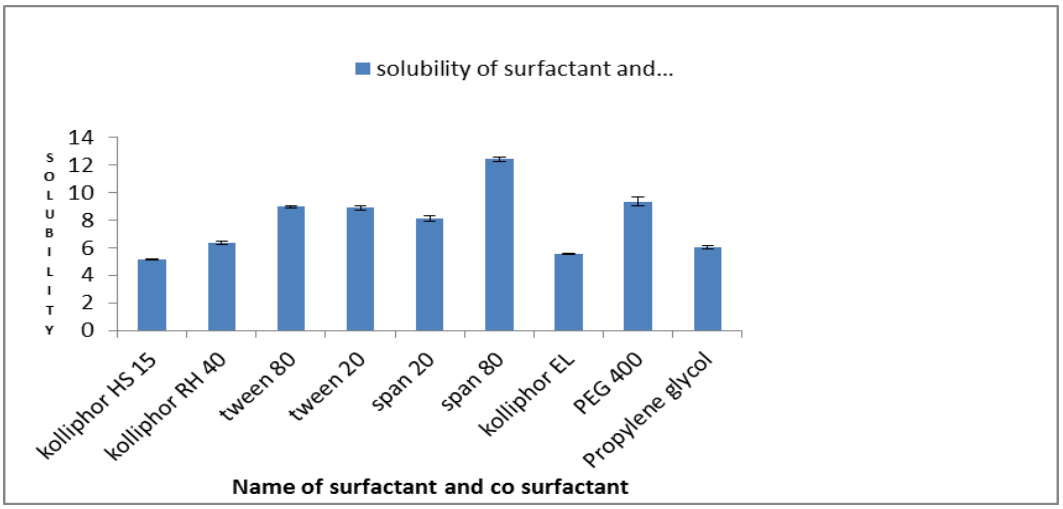

Figure No. 11: Solubility study of drug in different surfactant and co surfactant.

Discussion: Table No.12 and Figure No.11 demonstrated that among above all mentioned surfactant candesartan cilexetil has maximum solubility in PEG400, Span 80, Tween 20 and Tween 80.

5.1.7 Characterization of Screening of Oils, Surfactants and Co-surfactants

Evaluation of Screening of Surfactant and oil

Table No. 13: Selected Formulation for screening of cosurfactant.

Formulation Code	Oil	Surfactant
A2	Labrafil 2125	Kolliphor
A5	Capmul MCM	Span 20
A6	Capmul MCM	Tween 20
A9	Labrafil 2125	Kolliphor RH 40
A8	Labrafil 2125	Kolliphor HS 15
A11	Labrafil 2125	Propylene glycol
A13	Capmul MCM C8	Kolliphor EL
A14	Capmul MCM C8	Kolliphor HS 15
A15	Capmul MCM C8	Kolliphor RH 40

Discussion: Above the data we found that, the maximum % transparency showed in above formulation A9. We selected A9 formulations for further study, Shown in Table No.13.

Different trials for Ternary Phase Diagram

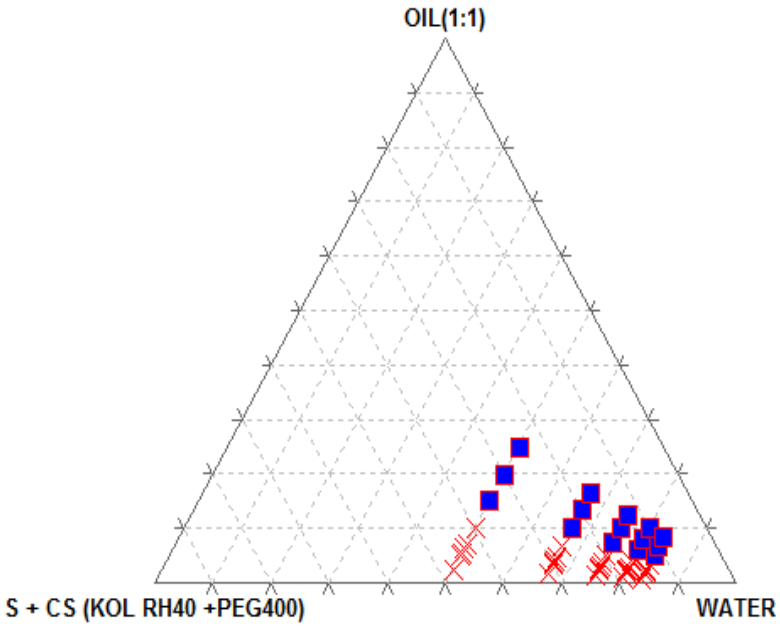

Figure No. 12: Ternary phase diagram of composition of (1:1).

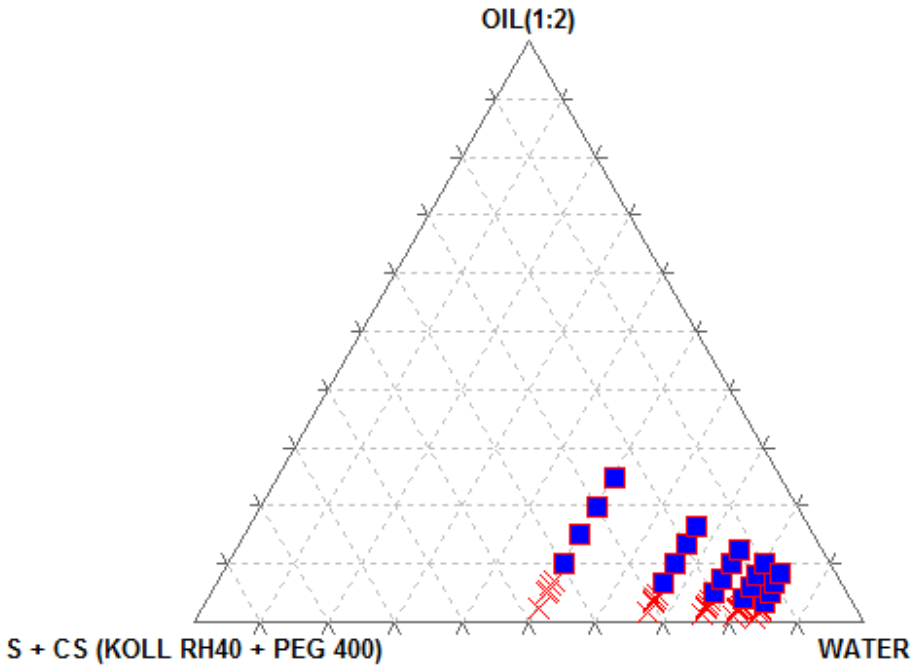

Figure No. 13: Ternary diagram of Combination of ((Surfactant: Cosurfactant (1:2)).

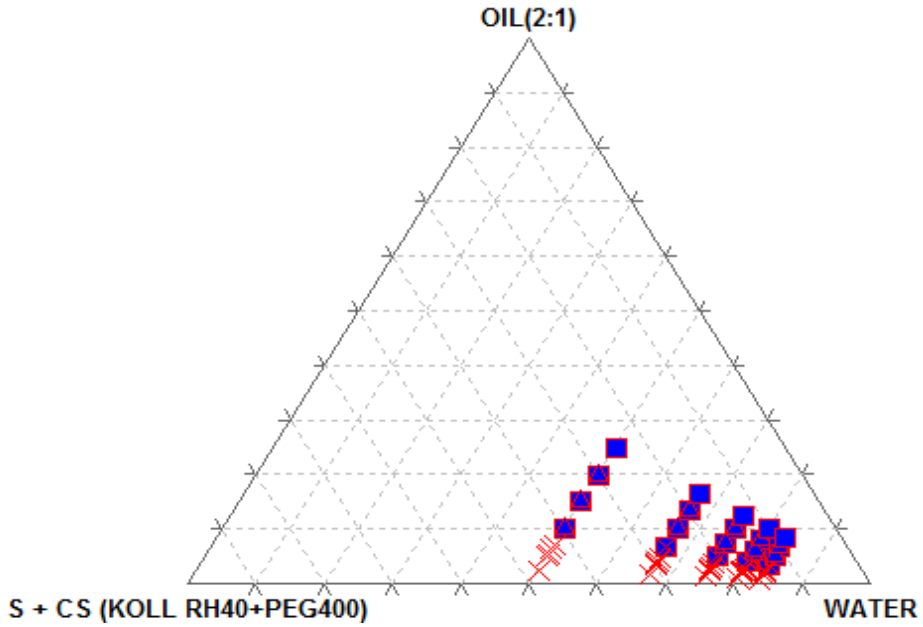

Figure No. 14: Ternary diagram of Combination of ((Surfactant: Cosurfactant (2:1).

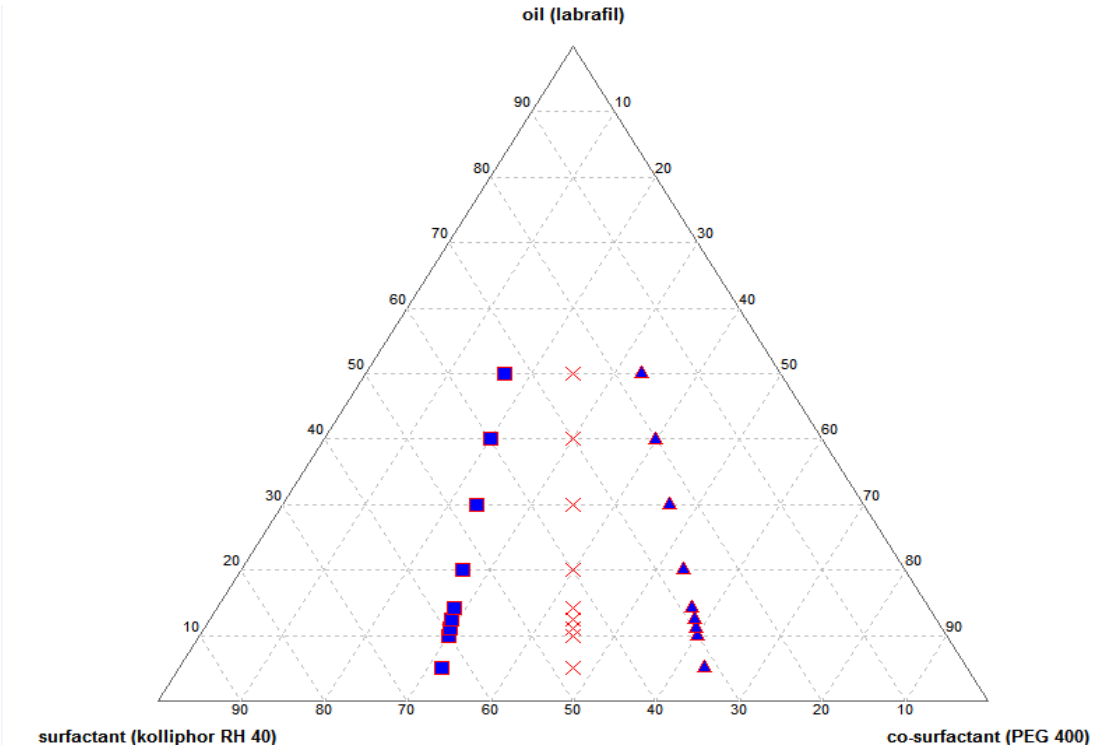

Figure No. 15: Ternary diagram of Combination of physical mixture. The cross sign reperesent the transparent nanoemulsion with partical size (droplet size) 74.0 nm.

DISCUSSION

Ternary plot is use for the determination of solubility of three different components oil, surfactant and co surfactant. On the behalf of ternary plot we plotted different ratio graph (1:1), (1:2), (2:1). This ratio also consist a different ratio like 0:5:9:5, 1:9.1:8, 1:7, 1:6, 2:8,

3:7, 4:6, 5:5 we selected a maximum area plotted graph(1:1) and finalised the ratio (1:1) after selected a ratio (1:1). we checked a percentage transparency or drug content of three different component in different ratio's (0.5:9.5), 1:9, 1:8, 1:7, 1:6, 2:8, 3:7, 4:6, 5:5 and found a maximum drug content present in a (0:5:9:5) ratio. We finalised the ratio (0:5:9:5) for the preparation of nanoemulsion formulation. see Figure No.12,13,14,15.

Characterization of nanoemulsion

Particle Size Distribution

Particle size distribution values are essential characterization parameters of nanoemulsion They have great influence on drug solubility, rate of dissolution, stability, and bioavailability (41). Particle size of the formulated nanoemulsion were 74.0 ± 0.123 nm and PDI 0.201 ± 0.020, respectively. The smaller value, the particle size distribution range becomes, which indicates homogeneity distribution of particles' diameters. The saturation solubility difference between particles is minimized when the particle size distribution is narrow. Hence, drug concentration gradients in the medium decline which helps to prevent incidence of Ostwald ripening (99).

Table No. 14: Final compositions of Nanoemulsion F-10.

Formulation code	Z-average (nm)	PDI
F-10	74.0±0.123	0.201±0.020

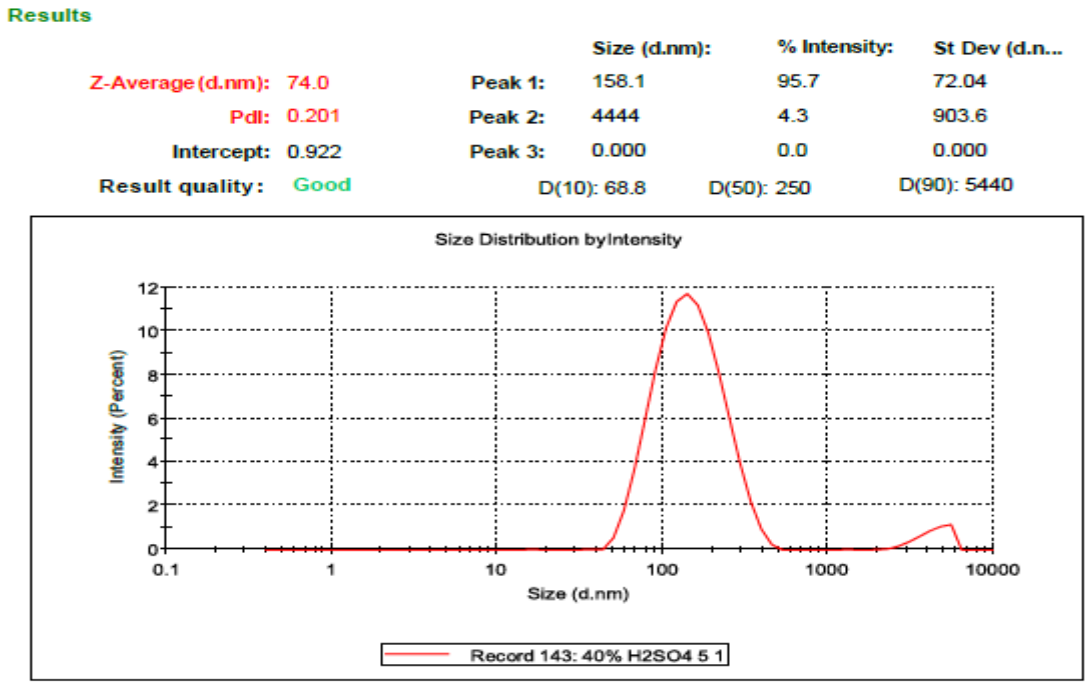

Figure No. 16: Partical size distribution of Nanoemulsion formulation.

Discussion: Particle size distribution values are essential characterization parameters of nanoemulsion They have great influence on drug solubility, rate of dissolution, stability, and bioavailability. Particle size of the formulated nanoemulsion were 74.0 ± 0.123 nm and PDI 0.201 ± 0.020, respectively. The smaller value, the particle size distribution range becomes, which indicates homogeneity distribution of particles' diameters. The saturation solubility difference between particles is minimized when the particle size distribution is narrow. Hence, drug concentration gradients in the medium decline which helps to prevent incidence of Ostwald ripening . See Table No.14 and Figure No.16.

SEM Image

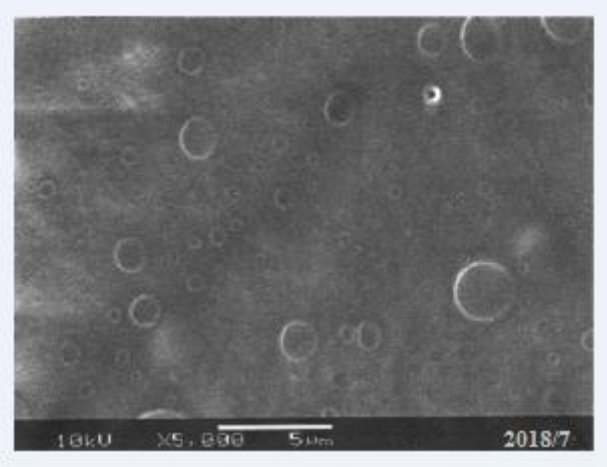

Figure No. 17: SEM Photography of F 15 Film Formulation.

Discussion: SEM images confirmed formation of nanoemulsion and in Figure no.17 SEM analysis of film revealed homogeneous distribution of nanoemulsion of candesartan cilexetil (present as individual and in agglomerated form) within the film matrix. Figure No.17.

Development of formulation

Table No. 15: Optimization of formulation parameters.

Formulation code	HPMC E5	HPMC E15	HPMC E50	FORMULATON (ml)
F-12	200			5
F-14		200		5
F-15			200	5

Calculation of dose of drug to be incorporated in fast dissolving films

The dose incorporated would be one half of the oral conventional dose. Therefore, the amount of cephalexin required for one day is 100mg

Calculation of the amount of drug for circular cast film

Internal diameter of petridish = 9 cm.

Internal surface area of petridish = πr2 = 3.14 x 4.5 x 4.5 = 63.585 cm2

Diameter of fast dissolving film = 4cm

Area of fast dissolving film = πr2

= 3.14 x 2 x 2

= 12.56 cm2 18

Therefore the number of fast dissolving films from one circular cast film. = 63.585 /12.56 = 5.0 films

Since one film contained drug load = 20 mg.

Therefore one circular cast film drug load = 20 x 5

Therefore 100 mg of candesartan cilexetil is needed for one circular cast film.

Evaluation of Oral fast dissolving film

General Appearance: All the films were exhibit in translucent, odorless, round in shape with smooth surface with zero defects.

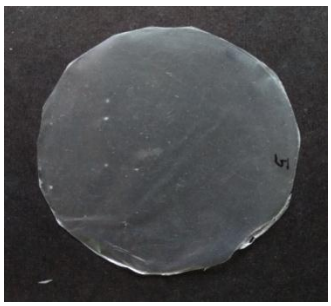

Figure No. 18: Film Formulation using HPMC 50.

Weight variation

The film weight was determined by using digital balance machine, shown in Table No.16.

Film Thickness

Discussion: Thickness of film was determined by using vernier calliper. The thickness of all films was found 57.73± 0.208µm and the film were observed uniformed and maximum thickness was observed in F-15 shown in Table No.16.

Folding endurance

Discussion: The folding endurance of film was determined by folding a film at same place till at breakdown. Film was folded up to 110 times which is represent the good properties of film. The Folding endurance of all films was found to be more in F-15 56 ± 0.23. The folding

endurance was increases with increasing concentration of polymer film matrix, as shown in Table No.16.

Surface pH

Discussion: The Surface pH of all films was found 6.46± 0.416, We find out the surface pH of film observed around 7, it confirmed the surface of films were neutral, as shown in Table No.16.

Disintegration time (DT).

Discussion: The Disintegration time of all films was found to be in range of 15.53 ± 0.585 to 17.06 ± 0.251(n= 3, the data represents the mean of three observations), as shown in Table No.16.

Drug Content

Discussion: The percent drug content of drug in all film batches was found to be within limits. The percent drug content value of Candesrtan cilexetil was found 99.98± 0.900; the values make sure good uniformity in the drug content in Fast Dissolving oral Film of Candesartan cilexetil as shown in Table No.16 and Figure No.19.

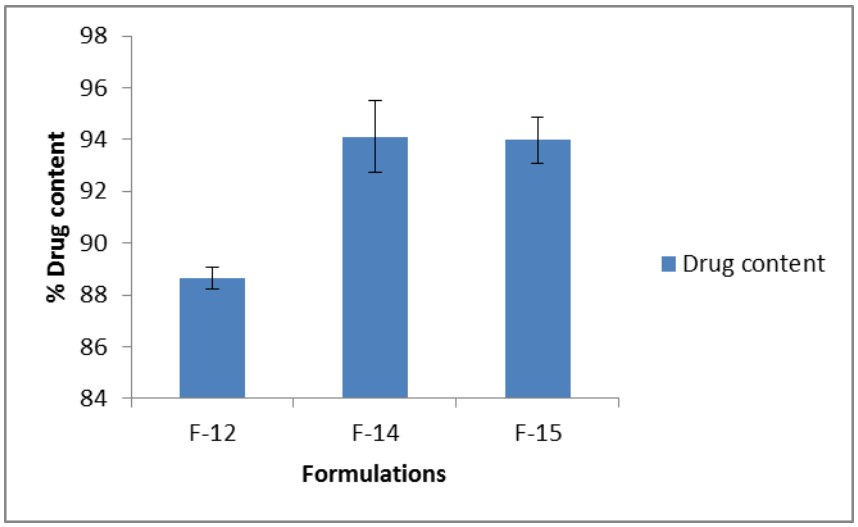

Figure No. 19: % drug content of candesartan cilexetil film.

Table No. 16: Evaluation of fast dissolving film.

FC	Weight variation (mg)	Thickness (µm)	Folding endurance	Surface ph	D T	% Drug content
F-12	16.26 ± 0.152	56.63± 0.378	56 ± 0.23	6.46± 0.416	15.53 ± 0.585	99.98± 0.900
F-14	16.56 ± 0.0577	57.23 ± 0.0577	13.66 ± 0.577	6.47± 0.0608	16.37 ± 0.136	88.64± 0.432
F-15	16.3 ± 0.01	57.73± 0.208	15.33 ± 0.577	6.6± 0.1	17.06 ± 0.251	99.12± 1.390

Tensile strength(TS) and % Elongation

Table No. 17: Tensile strength & % elongation of candesartan cilexetil loaded film.

Formulation code	Tensile strength(MPa)	% Elongation	Young's Modulus
F-12	6.97 ± 0.145	1.23± 0.0173	0.0012
F-14	8.36 ± 0.0577	1.13± 0.0577	0.0009
F-15	13.14 ± 0.0152	1.31± 0.0152	0.0008

Discussion: The tensile strength was found in range between 6.97 ± 0.145 to 13.14 ± 0.0152 MPa and the percentage elongation was found in the range between 1.23 ± 0.0173 to 1.31 ± 0.0152. Table No.17 and Figure No.20.

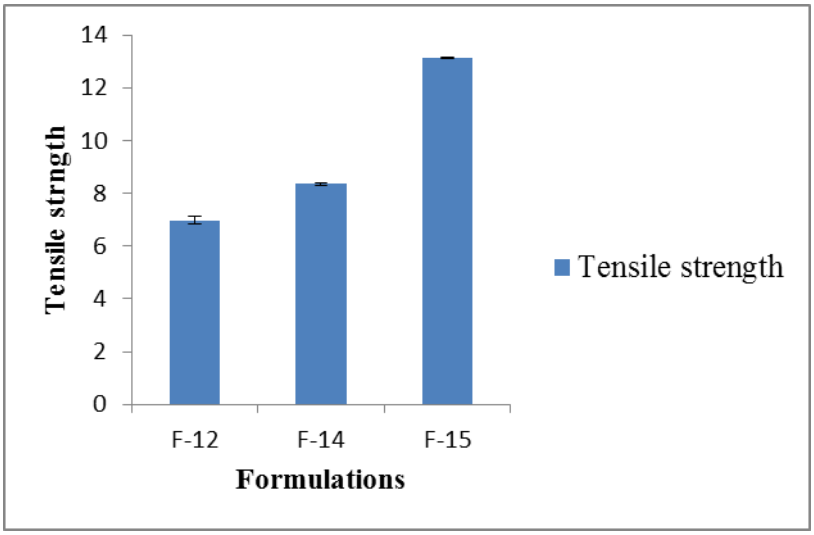

Figure No. 20: Tensile strength and % elongation of film.

In-vitro dissolution studies of pure drug and candesartan cilexetil loaded in fast dissolving film

Table No. 18: In-vitro drug release data of pure drug and candesartan cilexetil loaded in fast dissolving film.

Time(min)	% Drug release F-15	% Drug release of Pure drug
2	38.57±0.129	16.17±0.417
5	63.27±0.129	25.25±0.149
7	75.84±0.074	35.61±0.198
10	87.45±0.129	40.4±0.129
15	98.96±0.149	43.91±0.129

Discussion: The release profile of fast dissolving films (F-15) was compared with that of pure drug of fast dissolving film and it was observed that the drug release from nanoemulsion

loaded in fast dissolving films was much faster than that from raw drug of fast dissolving film. Shown in Table No.18 and Figure No.21.

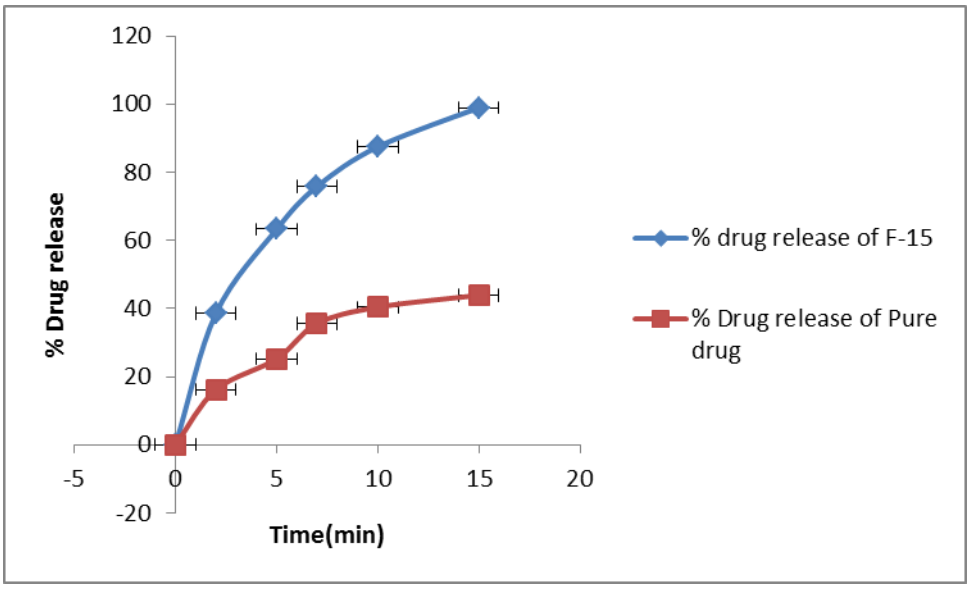

Figure No. 21: Comparison of In-vitro dissolution profile of film formulation F-15 and pure drug (candesartan cilexetil).

In vitro release kinetics[9]

To understand the mechanism by which the drug was released from the polymeric tablet, various release kinetics model including zero order, first order, Higuchi and Korsmeyer-Peppas model were applied as shown in Figure No.22-25.

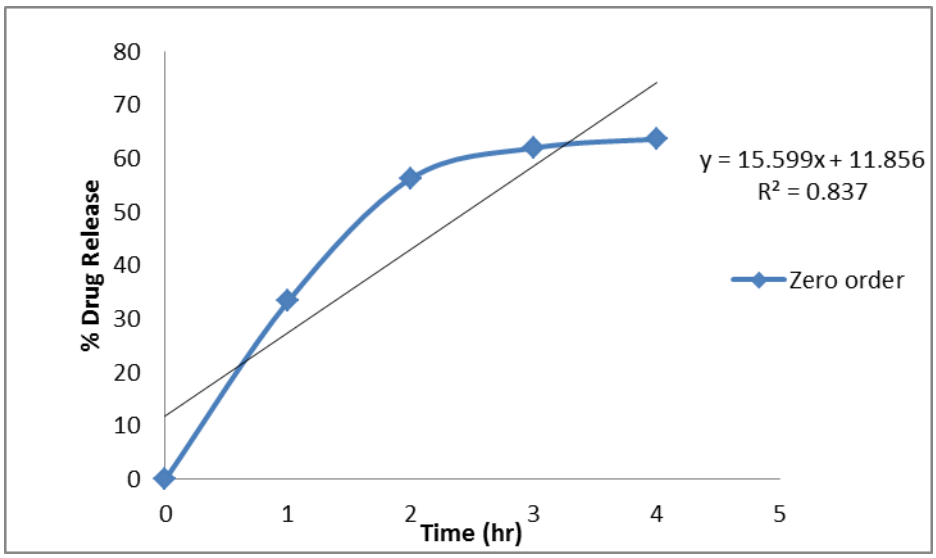

Figure No. 22: Zero order release kinetics of different pulsatile formulations F-15.

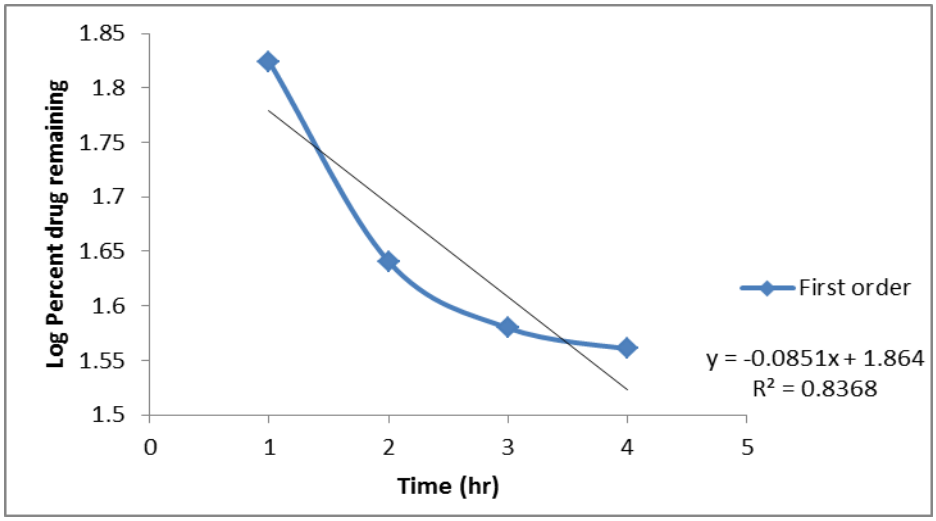

Figure No. 23: First order release kinetics of optimized formulation F-15.

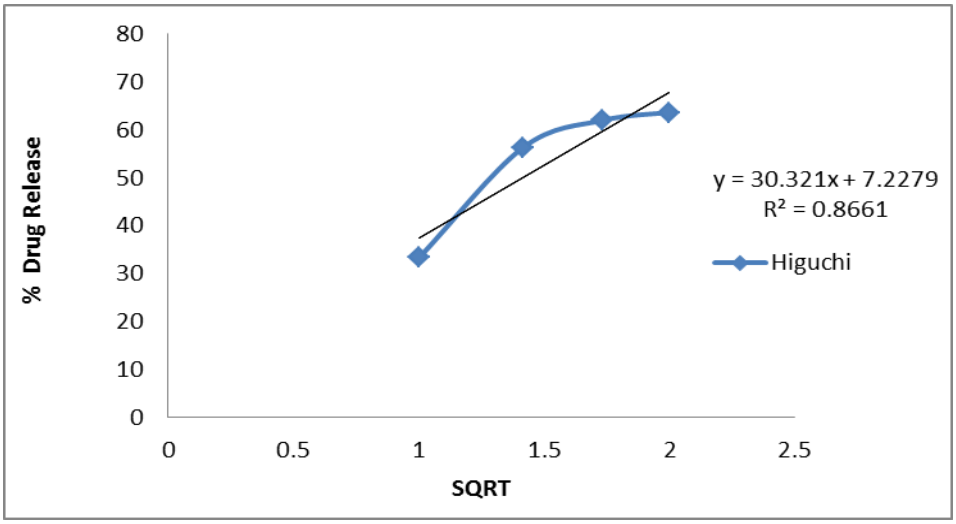

Figure No. 24: Higuchi model release kinetics of optimized formulation F-15.

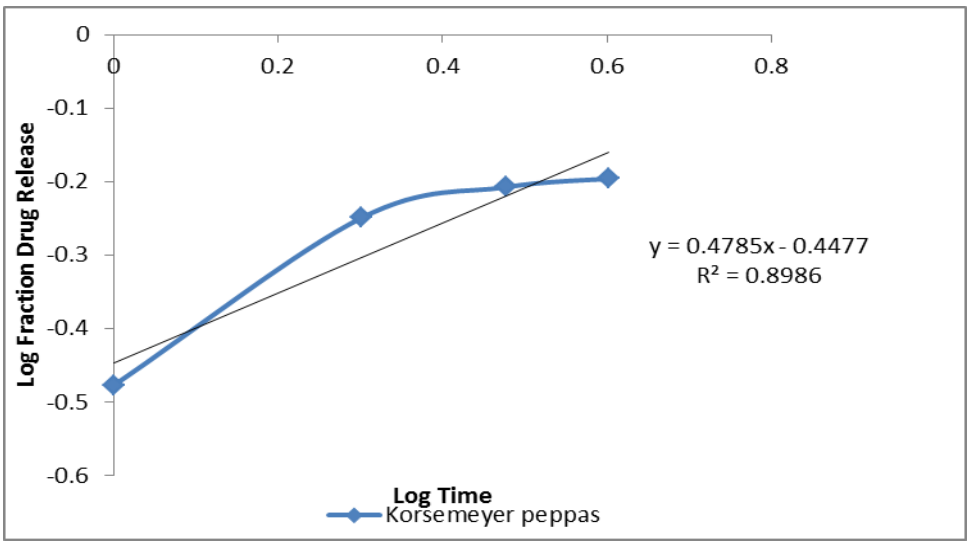

Figure No. 25: Korsemayer-peppas release kinetics of optimized formulation F-15.

Table No.19

Formulation name	Zero order		First order		Higuchi		Peppas	
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0
F15	0.837	15.599	0.8368	0.0851	0.8661	30.321	0.8986	0.4785

Discussion: The *in vitro* drug release of candesartan cilexetil fast dissolving film F-15 Korsmeyer-Peppas was best explained by, as the plots showed the highest linearity (R^2=0.8986), followed by Higuchi kinetics (R^2=0.8661), First order (R^2=0.8368), zero order and (R^2=0.837), suggesting that the diffusion plays an important role in the controlled release. Shown in Figure No.22-25 and Table No.19.

CONCLUSION

Fast dissolving film of candesartan cilexetil enhances the onset of action, reduce the dose, bioavailability, absorption and reduces the side effect. It is convenient for paedriatic and geriatric patient. Polymers are widely used in the pharmaceutical field. They are easily available in market and non toxic in nature. Polymer's is effectively used for the preparation of fast dissolving film. It was found to be nanoemulsion enhance the invitro release and invitro disintegration time of the film. Polymer (HPMC 50) film containing candesratan cilexetil loaded nanoemulsion appeared clear and transparent film with best characteristics, Challenges was faced the types and ratio (0.5:9.5,1:9,1:8,1:7,1:6,2:8,3:7,4:6,5:5) of oil, surfactant, co surfactant, polymer and plasticizer concenteration influence the film character and delayed in drying process of film.

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