5. MATERIALS AND METHODS

1. Reagents and Chemicals used

Potassium dihydrogen ortho phosphate, sodium hydroxide and triethylamine were obtained from Qualigen Fine Chemicals, Mumbai. Tween 80, methanol HPLC grade and acetone HPLC grade were supplied by Sisco Research Laboratories Pvt. Ltd., Mumbai. Acetonitrile HPLC grade and ortho-phosphoric acid were supplied by Rankem, New Delhi. Ethanol and potassium bromide IR grade were obtained from S.D Fine Chem Limited, Mumbai. Hydrochloric acid was supplied by Fisher Chemicals Ltd., Mumbai. All the reagents and chemicals used were of HPLC or Analytical grade.

Olanzapine was obtained as a gift sample from Ind Swift Pvt. Ltd., Chandigarh. Poly (lactic-co glycolic acid), Tripalmitate and Pluronic F-68 (Poloxamer 188) were supplied by Sigma Aldrich, Mumbai. Soy lecithin (Lipoid S 75) was obtained from Lipoid GmBH, Germany. Stearyl amine was obtained from Hengsen (Shanghai) Co Ltd., China. Dialysis bag was obtained from Himedia Labs, Mumbai.

1.1. Instruments and equipments used

i. Sartorius electronic balance
ii. Systronics pH meter
iii. Shimadzu LC 2010A HT HPLC system
iv. Shimadzu UV 1700 spectrophotometer
v. Shimadzu FT IR 8400S spectrophotometer
vi. Sonicator, Bandelin RK 100 H, Germany
vii. Triple blade stirrer, Remi Motors
viii. Freeze drier, Christ Alpha 1-2
ix. Research Centrifuge and Tissue homogenizer, Remi Instruments
x. Deep freezer, Lab-Line Instruments
xi. Magnetic stirrer, REMI Equipments
xii. Isothermal shaker, IKA® KS 4000i, Germany
xiii. Zeta sizer, Malvern Instruments
2. Experimental

This chapter describes the experimental details of the preformulation study, preparation of nano particles, bio availability study design and data handling, optimization of the bio analytical methods for the estimation of olanzapine in rat plasma samples, preparation of standard and sample solutions, development of in vitro dissolution methods, in vitro data analysis, in vivo data analysis and statistical analysis of pharmacokinetic data.

2.1. Preformulation Study

Preformulation in the broadest sense encompasses all the activities and studies that are required to convert an active pharmacological substance into a suitable dosage form. It can be defined as an investigation of the physical and chemical properties of a drug substance alone and also when combined with the excipients. In the present study, therefore, evaluation of nano particles, development of in vitro dissolution method and the compatibility between the drug and the excipients were determined.

2.1.1. Solubility studies

The solubility of the olanzapine was determined in various solvents by adding an excess amount of drug to 10 ml of solvents in conical flasks. The flasks were kept at 25± 0.5 °C in isothermal shaker for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 4000 rpm for 15 min. The supernatant liquid was taken and filtered through 0.45 µm membrane filter. The concentration of olanzapine was determined in the supernatant liquid after suitable dilution at the wave length of maximum absorption ($\lambda_{\text{max}}$) of 258 nm.
2.2. Differential Scanning Calorimetry

The possibility of drug-excipient interaction was investigated by differential scanning calorimetry. The DSC thermograms of pure drug olanzapine, individual excipients and drug-excipient mixtures were recorded.

2.3. Compatibility Studies

Infrared spectral matching approach was employed to detect any possible chemical interaction between olanzapine, lipid and surfactants. Physical mixtures of the drug and the excipient (1:1) were mixed with 400 mg of potassium bromide (IR grade). About 100 mg of the mixture was taken and compressed to form a transparent pellet in a hydraulic press at 15 tonnes pressure. The samples were scanned from 4000 to 400 cm\(^{-1}\) in a Shimadzu FT IR spectrophotometer. Physical appearance of the samples and appearance/disappearance of peaks in the spectra were observed to assess any possible physical and chemical interactions.

2.4. Development of calibration curve

A stock solution of olanzapine was prepared by dissolving 100 mg of drug in 10 ml of 0.1N HCl and made up to 100 ml (with different buffers viz., 0.1 N HCl, pH 6.8 and pH 7.4) with a concentration of 1 mg/ml. From this stock solution, 5-25 µg/ml dilutions were prepared and absorbances were measured at the wave length of maximum absorption (\(\lambda_{\text{max}}\)) of 258 nm. The \(\lambda_{\text{max}}\) of the drug was determined by scanning between 200 and 400 nm using a UV-Visible spectrophotometer. At the maximum wave length of maximum absorption (\(\lambda_{\text{max}}\)) of 258 nm, the absorbance of all the solutions was measured against a blank and the calibration curve between concentration and absorbance was plotted.

2.5. Partition Coefficient studies

Partitioning behavior of olanzapine was determined with a lipid of glyceryl tripalmitate\(^1\). 10 mg of Olanzapine was dispersed in a mixture of melted lipid (1g), 1 ml of hot phosphate buffer pH 7.4 and shaken for 30 min in a water bath shaker maintained at 10°C above the melting point of lipid. The aqueous phase of the above mixture was separated from the lipid by
Materials and methods

centrifugation at 10000 rpm for 20 min. The clear supernatant liquid was suitably diluted with 0.1 N HCl and olanzapine content was determined in UV-Visible spectrophotometer at 258 nm against solvent blank. The partition coefficient was calculated as:

\[ PC = \frac{(C_{OLI} - C_{OLA})}{C_{OLA}} \]

Where, \( C_{OLI} \) = the initial amount of olanzapine added (10mg)
\( C_{OLA} \) = the concentration of olanzapine in pH 7.4 phosphate buffer

3. Preparation of nano particles

3.1. Preparation of olanzapine solid lipid nanoparticles

Solid lipid nanoparticles were prepared by using microemulsion technique\(^{52,53}\) and \( o/w \) microemulsions were initially prepared. The oil phase, lipophilic surfactant and continuous phase used are glyceryl tripalmitate, soy lecithin and pluronic F-68 (hydrophilic surfactant) respectively. The lipid and soy lecithin were melted at 70\(^{\circ}\)C and the drug was added with constant stirring. 10 ml of aqueous surfactant solution containing pluronic F-68 heated at the same temperature was added to the melted lipid with mechanical stirring for 15 min. A clear microemulsion was obtained at a temperature close to the melting point of the lipid used. Stearyl amine was used as a positive charge inducer and added to melted lipid. Solid lipid nanoparticles were obtained by dispersing the warm \( o/w \) microemulsion which is added drop wise into ice cold water in a beaker under continuous stirring. After completion of stirring, the solid lipid nanoparticles dispersion was subjected to ultrasonication for 15 min.

3.1.1. Study on the effect of lipid quantity

The effect of lipid quantity on the particle size was studied by varying one parameter, keeping the others constant. Three different batches of solid lipid nanoparticles were prepared corresponding to varying concentrations of lipid such as 50, 100 and 200 mg keeping the amount of soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v), stirring time (3 hours) and stirring speed (1500 rpm) constant.
3.1.2. Study on the effect of formulation process variables

The effect of formulation process variables such as stirring time, stirring speed, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

3.1.2.1. Effect of stirring time

Five different batches of solid lipid nanoparticles were prepared corresponding to 1, 2, 3, 4, 5 hours of stirring time keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v) and stirring speed (2000 rpm) constant.

3.1.2.2. Effect of stirring speed

Four different batches of solid lipid nanoparticles were prepared corresponding to 1000, 1500, 2000 and 2500 rpm of stirring speed keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), pluronic F-68 (1% w/v) and stirring time (4 hours) constant.

3.1.2.3. Effect of surfactant concentration

Four different batches of solid lipid nanoparticles were prepared corresponding to 0.5%, 1%, 1.5% and 2% w/v of pluronic F-68 keeping the lipid concentration (50 mg), soy lecithin (1% w/w), stearyl amine (1% w/w), stirring time (4 hours) and stirring speed (2000 rpm) constant.

3.1.3. Preparation of drug loaded solid lipid nanoparticles batches

Eight different batches of drug loaded solid lipid nanoparticles were prepared by microemulsion method and the composition of drug loaded batches are given in Table 2.

3.2. Preparation of olanzapine polymeric (PLGA) nano particles

Polymeric (PLGA) nano particles were prepared by using nanoprecipitation technique. PLGA was dissolved in 10 ml of acetone and the drug was dissolved in polymer/acetone solution. This organic phase was injected at the rate of 10 ml/min in 20 ml of water containing Pluronic F-68 under stirring at room temperature. Acetone was eliminated by evaporation under reduced pressure and the final volume of suspension was adjusted to
10 ml. The final nanosuspension was centrifuged, freeze dried and used for further characterization.

3.2.1. Study on the effect of polymer quantity

The effect of polymer quantity on the particle size was studied by varying one parameter, keeping the others constant. Three different batches of PLGA nanoparticles were prepared corresponding to varying concentrations of PLGA such as 125, 150 and 175 mg keeping the amount of pluronic F-68 (250 mg), acetone (20 ml), water (10 ml), stirring time (1 hour) and stirring speed (3000 rpm) constant.

3.2.2. Study on the effect of formulation process variables

The effect of formulation process variables such as stirring time, stirring speed, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

3.2.2.1. Effect of stirring time

Five different batches of PLGA nanoparticles were prepared corresponding to 1, 2, 3, 4, 5 hours of stirring time keeping the polymer concentration (150 mg), pluronic F-68 (250 mg) and stirring speed (3000 rpm) constant.

3.2.2.2. Effect of stirring speed

Four different batches of PLGA nanoparticles were prepared corresponding to 1000, 2000, 3000 and 4000 rpm of stirring speed keeping the polymer concentration (150 mg), pluronic F-68 (250 mg) and stirring time (3 hours) constant.

3.2.2.3. Effect of surfactant concentration

Four different batches of PLGA nanoparticles were prepared corresponding to 150 mg, 250 mg, 350 mg and 450 mg of pluronic F-68 keeping the polymer concentration (150 mg), stirring time (3 hours) and stirring speed (3000 rpm) constant.
3.2.3. Preparation of drug loaded PLGA nanoparticles batches

Eight different batches of drug loaded PLGA nanoparticles were prepared by nano precipitation method and the composition of drug loaded batches are given in Table 3.

3.3. Preparation of olanzapine nano suspension

The preparation of nano suspension was done by solvent diffusion method. Drug was dissolved in 10 ml of acetone and required quantity of surfactants such as Tween 80 and Pluronic F-68 were dissolved in 20 ml of water by using mechanical stirrer. The drug solution was added dropwise to the above solution and was stirred at 3000 rpm for 30 min and was subjected to sonication at 80 amplitude for 15 min. Cryo-protectant mannitol was added and the prepared nano suspension was subjected to lyophilisation, the dried product was collected and characterized.

3.3.1. Study on the effect of formulation process variables

The effect of formulation process variables such as stirring time, stirring speed, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

3.3.1.1. Effect of Stirring time

Five different batches of nanosuspensions were prepared corresponding 10, 20, 30 and 60 min of stirring time keeping the pluronic F-68 (250 mg), tween 80 (0.5 ml) and stirring speed (3000 rpm) constant.

3.3.1.2. Effect of Stirring speed

Four different batches of nanosuspensions were prepared corresponding to 1000, 2000, 3000 and 4000 rpm of stirring speed keeping the pluronic F-68 (250 mg), tween 80 (0.5 ml) and stirring time (30 min) constant.

3.3.1.3. Effect of Surfactant concentration

Four different batches of nanosuspensions were prepared corresponding to 100 mg, 250 mg, 500 mg and 750 mg of pluronic F-68 keeping the tween 80 (0.5 ml), stirring time (30 min) and stirring speed (3000 rpm) constant.
3.3.2. Preparation of drug loaded PLGA nanoparticles batches

Eight different batches of drug loaded nanosuspension were prepared by solvent diffusion method and the composition of drug loaded batches are given in Table 4.

4. Evaluation of nanoparticles

4.1. Particle size and zeta potential

Particle size and zeta potential of the solid lipid nanoparticles, PLGA-nanoparticles and nanosuspensions were measured by photon correlation spectroscopy using a Malvern Zetasizer.

4.2. Polydispersity Index

Polydispersity was determined according to the equation,

\[
\text{Polydispersity} = \frac{D(0.9) - D(0.1)}{D(0.5)}
\]

Where,

D (0.9) corresponds to particle size immediately above 90% of the sample.
D (0.5) corresponds to particle size immediately above 50% of the sample.
D (0.1) corresponds to particle size immediately above 10% of the sample.

4.3. Entrapment efficiency

Entrapment efficiency was determined by dialysis method. Solid lipid nanoparticles and PLGA nanoparticles entrapped olanzapine were separated from the free drug by dialysis method. The above said formulations were filled into dialysis bags and the free olanzapine dialyzed for 24 hours into 50 ml of phosphate buffer 7.4 saline. The absorbance of the dialysate was measured at 258 nm against blank phosphate buffer 7.4 saline and the absorbance of the corresponding blank phosphate buffer 7.4 saline was measured under the same condition. The concentration of free Olanzapine could be obtained from the absorbance difference based on standard curve. Standard curve was made by measuring the absorbance at 258 nm for known concentrations of Olanzapine solution. The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug.
4.4. External Morphological Study (Scanning electron microscopy):

External morphology of nanoparticles was determined by using Scanning Electron Microscopy (SEM).

4.5. Saturation solubility

Approximately 1.5 ml of prepared nanosuspension was filled in 2 ml centrifugation tube and centrifuged after 24 hours, using sigma centrifuge at 25000 rpm for 30 min. Concentration of olanzapine in the supernatant was measured by using UV-Visible spectrophotometer at 258 nm after suitable dilution with phosphate buffer pH 6.8 using the same solvent as blank. Saturation solubility of pure drug was also measured in the similar manner.

4.6. In vitro release studies

The release of olanzapine from all the three nanoformulations were studied under sink conditions. Formulations which showed higher drug content, entrapment efficiency, saturation solubility were evaluated for in vitro release. 5 ml of formulations equivalent to 1 mg were put in dialysis bags. The dialysis bags were placed in 50 ml of dissolution medium (phosphate buffer saline pH 7.4 and pH 6.8) and stirred under magnetic stirring at 37°C. Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to maintain a constant volume. Samples were withdrawn and analyzed for olanzapine content spectrophotometrically at 258 nm against solvent blank.

4.7. Release kinetics

In-vitro dissolution has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where \( f_t \) is the function of \( t \) (time) related to the amount of drug dissolved from the pharmaceutical dosage system. To compare dissolution profiles between two drug products
Materials and methods

Model dependent (curve fitting), statistic analysis and model independent methods can be used. In order to elucidate mode and mechanism of drug release, the _in vitro_ data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low soluble drugs, coated forms, osmotic systems etc., where the drug release is independent of concentration.

\[ Q_t = Q_0 + K_0 t \]  

Where, \( Q_t \) is the amount of drug released in time \( t \), \( Q_0 \) is the initial amount of the drug in the solution and \( K_0 \) is the zero order release constant. The first order Eq. (2) describes the release from the system where release is concentration dependent e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

\[ \log Q_t = \log Q_0 + K_1 t /2.303 \]  

Where \( Q_t \) is the amount of drug released in time \( t \), \( Q \) is the initial amount of drug in the solution and \( K_1 \) is the first order release constant. Higuchi described the release of drug from insoluble matrix as a square root of time as given in Eq. (3)

\[ Q_t = K_H \sqrt{t} \]  

Where, \( Q_t \) is the amount of drug released in time \( t \), \( K_H \) is Higuchi’s dissolution constant.

The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model).
4.8. Mechanism of Drug release

Korsmeyer and co workers have developed a simple, semi empirical model, relating exponentially the drug release to the elapsed time (t)

\[ f_t = K t^n \]

K is a constant incorporating structural and geometrical characteristic of the drug dosage form, n is the release exponent; \( f_t \) is \( M_t / M_\infty \) (fractional release of drug).

Depending on the relative magnitude of the rate of polymer swelling to the rate of drug diffusion, various release profiles may be possible. The situation where the polymer structural rearrangement takes place rapidly in response to the swelling solvent as compared to the rate of drug diffusion generally leads to Fickian diffusion, or the so-called first order release, characterized by square root of time dependence in both the amount released and the penetrating diffusion front position in slab geometry.

In case of sorption process is completely governed by the rate of polymer relaxation, the so-called Case II transport, characterized by linear time dependence in both the amount diffused and the penetrating swelling front position, results. In most systems, the intermediate solution, which is often termed non-Fickian or anomalous diffusion, will prevail whenever the rates of diffusion and polymer relaxation are comparable. Kinetic constant incorporates structural and geometrical characters of the drug/polymer system. For non-Fickian release, the n value falls between 0.5 and 1.0 (0.5< n < 1.0), whereas in the case of Fickian diffusion, n= 0.5; for zero-order release (case transport), n=1, and for Supercase II transport, n>1. The values of n as estimated by linear regression of \( \log (M_t / M_\infty) \) vs \( \log (t) \) of different formulations were calculated.

4.9. Stability studies

Formulated solid lipid nanoparticles and PLGA nanoparticles were stored at 25°C±2°C and 60% RH ± 5% for 3 months, and average size and entrapment efficiency were determined. Stability studies for nanosuspension and lyophilized nanosuspension were conducted at two different storage
conditions, viz., room temperature and refrigerated conditions (2–8°C) for 3 months. Each one batch of nanosuspension and lyophilized nanosuspension were used for each storage condition. At periodic time intervals, the samples were withdrawn and analyzed for particle size and drug content.

5. Bioavailability Studies

Bioavailability studies of the optimized formulations were carried out in Wistar rats between the developed formulations and the pure drug. The protocol of the study was submitted to the Institutional Animal Ethical Committee and the approval for conducting the same was obtained (Proposal no. JSSCP/IAEC Ph.D/01 PH.CEUTICS/02/2011-12). A reproducible analytical technique was developed for the estimation of the drugs in the plasma samples. Various pharmacokinetic parameters such as $C_{\text{max}}$, $T_{\text{max}}$, $t_{1/2}$, $k_e$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were estimated.

Albino Wistar rats (Male) weighing 200±20g were used for bioavailability studies. All the rats were fasted for overnight before the experiments but had free access to water and the rats were divided into three groups of 6 animals each.

Group 1 Control group
Group 2 Drug suspension treated group
Group 3 Formulation treated group

Drug suspension (0.3% w/v Carboxy Methyl Cellulose) and nanoformulations were administered intravenously by causal vein at the dose of 0.18 mg which is calculated based on human dose. Blood (0.5ml) was collected by retro-orbital venous plexus puncture at 0, 0.25, 0.50, 1, 2, 3, 4, 6, 8, 12 and 24 hours after administration separately. Blood samples were placed into Eppendorf tubes containing 0.3 ml of anticoagulant (sodium citrate) solution and centrifuged immediately. After centrifugation, the plasma obtained was stored at -20°C until further analysis.
Materials and methods

The plasma samples of olanzapine were extracted using solid phase extraction (SPE) and their drug levels were quantified by using HPLC technique.

Pharmacokinetic parameters namely, $C_{\text{max}}$, $T_{\text{max}}$ and $k_{\text{el}}$ were determined for individual drug treatments from the observed plasma concentration-time data. AUC were calculated by trapezoidal rule from time zero to the last observed concentration.

Pharmacokinetic parameters such as peak plasma concentration ($C_{\text{max}}$), time to peak concentration ($T_{\text{max}}$), Area under the plasma concentration - time curve ($\text{AUC}_{0-t}$ & $\text{AUC}_{0-\infty}$), elimination rate constant ($k_{\text{el}}$) and Elimination half-life ($t_{1/2}$) were calculated separately and the blood level data of the nano formulations and the pure drug were compared statistically.

6. Estimation of drugs

Proper selection of the chromatographic method depends upon the nature of the sample (ionic or neutral molecule), its molecular weight and solubility. The drug selected for the present study is polar in nature and hence either reverse phase or ion pair or ion exchange chromatography can be used. For the present study reverse phase HPLC method is considered to be more suitable because it is extremely specific, linear, precise, accurate, sensitive and rapid method.

6.1. Optimized chromatographic conditions

The following chromatographic conditions were selected for the estimation of selected drug in plasma samples.

6.1.1. Chromatographic Conditions for Olanzapine

- Stationary phase: Hibar C$_{18}$ (250 x 4.6 mm i.d., 5µ)
- Mobile Phase: Acetonitrile: 25mM of Phosphate buffer of pH 6.5
- Mobile phase ratio: 40:60 % v/v
- Flow rate: 1.0 ml/min
Materials and methods

Sample volume : 20 µl using Rheodyne 7725i injector
Detection : 258 nm
Data station : LC-20AD
Internal Standard (IS) : Atorvastatin
Retention Time : 6.9 (Drug), 14.4 (IS)

6.1.2. Selection of detection wavelength for olanzapine

100 µg/ml of olanzapine in acetonitrile was prepared, scanned in the UV region of 200 - 400 nm and the UV spectrum was recorded. From the spectra, detection wavelength at 258 nm was selected for olanzapine.

6.1.3. Preparation of standard and sample olanzapine solutions

a. Standard stock solution of olanzapine

10 mg of olanzapine working standard was accurately weighed and transferred into a 10 ml volumetric flask and dissolved in acetonitrile and made up to the volume with the same solvent to produce a 1mg/ml of olanzapine. The stock solution was stored in refrigerator at –20 ± 2ºC until analysis.

The stock solution was diluted to suitable concentrations to obtain calibration curve (CC) standards and quality control (QC) samples.

b. Calibration Curve Standards and Quality Control Samples

Working solutions for calibration were prepared from the stock solution by an adequate dilution using acetonitrile: water. Calibration standards curve was prepared for the concentration from 11 µg/ml to 220 µg/ml.

c. Standard stock solution of atorvastatin (Internal Standard)

10 mg of atorvastatin internal standard was accurately weighed and transferred into a 10 ml volumetric flask, dissolved in acetonitrile - water mixture (1:1) and made up to the volume with the same solvent to produce a 1mg/ml of atorvastatin. The stock solution was stored in refrigerator at 20 ± 20 C until analysis. The stock solution was diluted to suitable concentration (100 µg/ml) with HPLC grade water, prior to use as internal standard.
d. Plasma samples

Calibration standards, validation QC samples and rat plasma samples were prepared by adding 0.5 ml plasma to Eppendorf tube followed by adding internal standard solution. All samples were mixed by vortexer for 30 s. After these procedures, Samprep SPE Column C_{18} (50µm, 70Å) 100mg/1ml solid phase extraction cartridge was conditioned with methanol, water sequentially and sample was loaded. The cartridge was washed with 2.0 ml of water. The drug and internal standard were eluted from the cartridge using 0.5 ml of acetonitrile and water in the ratio (50:50). The resulting solution was used for the analysis.

6.1.4. Method of analysis

The bio analytical calibration curve samples and plasma sample solutions were injected with above chromatographic conditions and the chromatograms were recorded. The quantification of the chromatogram was performed using peak area.

6.1.5. Statistical analysis

Statistical analysis was performed using a Graphpad Prism software, V.5.01, USA. The pharmacokinetic parameters like C_{max}, T_{max}, t_{1/2}, K_{el}, AUC_{0-t} and AUC_{0-∞} of all the formulations are presented in Mean ± S.D. A p<0.05 was considered statistically significant.