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Research Article

ENHANCEMENT OF ROSUVASTATIN CALCIUM BIOAVAILABILITY APPLYING NANOCRYSTAL TECHNOLOGY AND *IN-VITRO, IN-VIVO* EVALUATIONS

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ABSTRACT

Objective: The objective of this study is to prepare efficient dosage form using nanocrystal technology and to compare with micro and marketed formulations for its *in-vitro* and the *in-vivo* behavior. The nanocrystal technology has good potential to enhance the dissolution profile of poorly soluble drugs by reducing particle size, increasing surface area, and also by raising the saturation solubility of the drug.

Methods: In this study, solubility of rosuvastatin calcium is increased by adopting nanocrystal technology. Rosuvastatin calcium nanocrystals was formulated using various stabilizers like sodium lauryl sulfate, hydroxyl propyl cellulose, hydroxyl propyl methyl cellulose, poloxamer 188, tween 80, poly vinyl pyrrolidine, by adopting two different methods top down and bottom up techniques. Formulations were compressed and physical parameters of tablets evaluated dissolution studies were performed to evaluate release and pharmacokinetic studies were done to evaluate *in-vivo* behavior.

Results: Particle size obtained by employing top down method was found to be <749.04 nm (F-1 to F-7) whereas particle size obtained by following bottom up method was higher than 2000 nm. The micronized particles possessed a size range of 61.51 µm, whereas nanoparticle formulations showed particle size of 0.509 µm, 0.399 µm respectively. Differential scanning calorimetry studies showed good compatibility between drug and excipients. Friability values ranging from 0.15±0.0565% to 0.59±0.0283% and disintegration time lies between 29±1.414 and 44±1.414 seconds for the prepared formulations. The hardness of prepared tablets was between 3.0 and 4.5±0.707 kg/cm^2 which are sufficient for maintaining the integrity of tablets. Friability, disintegration time and hardness for the micronized formulation (M-1) were 0.875±0.0919%, 50±2.828 seconds, 3.5±0.707 kg/cm^2, which are higher than the values obtained for lyophilized nanocrystals in formulation. *In-vitro* studies have shown a 36% increase in dissolution of nanocrystal formulation while *in-vivo* studies exhibited 1.87-fold increases in the bioavailability of rosuvastatin when compared to the micronized dosage form.

Conclusion: Formulations containing different constituents were prepared by following bottom up and top down techniques and evaluated *in-vitro, in-vivo*, observed significant differences between the formulations. The results proved that the bioavailability of rosuvastatin calcium has been improved considerably by applying nanocrystal technology. *In-vitro and in-vivo* evaluations showed that solubility and bioavailability have been enhanced considerably.

Keywords: Rosuvastatin calcium, Nanocrystal technology, Solubility, Bioavailability, Pharmacokinetics.

INTRODUCTION

At present about 40% of the drugs in the development pipeline and 60% of synthetic drugs are poorly soluble [1]. Nanocrystal technology possesses high potential to raise solubility of poorly soluble drugs, and the technology offers 100% industrial applicability. For a compound with poor solubility, below the mg/ml level, the increase in dissolution velocity is not sufficient to have good bioavailability. However when the particles are comminuted into nano-size range, the surface-to-volume ratio increases significantly, which leads to an increase in dissolution velocity according to the Noyes–Whitney equation [2] and subsequently an improvement in absorption in the gastrointestinal tract (GIT) [3]. It is very essential to overcome the aggregation of nano particles a way to achieve this is by employing appropriate stabilizers and dispersing the particles in hydrophilic carrier. These stabilizers were found to have effect on particle size too. The particle sizes shall be increased or decreased based on type and concentration of stabilizers used. Hence the selection [4] and concentration of stabilizer used is very important, which affects the physical stability of the final product [5,6]. The interparticulate force between nanosized particles is high resulting in high surface energy. Particles aggregate to decrease this surface free energy leading to increasing in particle size [7,8], development of nanosuspension is beneficial for water insoluble drug since it can significantly increase their dissolution velocity. The rosuvastatin calcium a synthetic lipid lowering agent with poor water solubility, which falls on bio pharmaceutical classification system (BCS) Class II with 20% bioavailability is chosen for the study. Since drugs under BCS Class II are not easily dissolved [9] they may not get absorbed from GIT [10,11]. Nanocrystal technology was adopted for improving solubility and dissolution features of nifedipine [12] and rutin [13] in various studies. Rapamune was the first successful nanocrystal product approved by FDA which showed 21% higher bioavailability than solution form. Generally, hydroxyl propyl methyl cellulose (HPMC) E3, povidone, dioctyl sulfo succinate and sodium lauryl sulfate (SLS) are used as stabilizers in nanocrystal formulation of drugs that are on market today [14]. The objective of the study is to prepare efficient dosage form using nanocrystal technology and to compare with micro and marketed formulations for its *in-vitro* and *in vivo* behavior.

MATERIALS

Rosuvastatin calcium (pure) gifted sample from Microlabs Ltd., hydroxy propyl methyl cellulose LR, pluronic 168 LR, microcrystalline cellulose 102 LR, tri calcium phosphate LR, D-mannitol AR, sodium di hydrogen ortho phosphate purchased from Himedia Ltd. Mumbai, hydroxy propyl cellulose LR, povidone LR, sodium lauryl sulfate LR, tween 80 LR, lactose LR purchased from S.D. Fine Chemicals Ltd., acetonitrile high performance liquid chromatography (HPLC) grade obtained from Ranbaxy Fine Chemicals Ltd. New Delhi, trisodium citrate from AR Spectrum Reagent and Chemicals Cochin, sodium hydroxide obtained from LR Loba Chemie Ltd., citric acid AR purchased from Hi Pure Fine Chemicals Industry Chennai.

METHODS

Estimation of rosuvastatin calcium by ultraviolet (UV)

Absorbance maximum was determined by using drug solution diluted suitably and in UV 1650 PC Shimadzu scan showed maximum absorbance at wavelength of 244 nm for rosuvastatin calcium. Calibration curve plotted at different concentrations was linear, and correlation coefficient was found to be r^2=0.9997.

Study of physical interaction between drug and excipients

Interaction between drug and excipients was studied by using infrared (IR) – potassium bromide (KBR) method KBR pellets were prepared for drug + KBR, and excipients used + KBR by employing sufficient pressure obtained pellets were analyzed using Fourier transform IR (FT-IR) 8400 S Make-Shimadzu.

Preparation of rosuvastatin nanocrystal formulation

Formulations were prepared by using two technologies for study purpose.

1. Top down technique.
2. Bottom up technique.

Procedures used for preparing nanocrystals of rosuvastatin calcium described below.

1. Preparation of rosuvastatin nanocrystals by top down technique [15]: Rosuvastatin calcium was dispersed in a 20 ml aqueous surfactant solution with magnetic stirring at 500 rpm, further size reduction done by using homogenizer (Make - Polytron, Model - PT3 1000, 230±10 v) at 8000 rpm for 10 minutes and RPM was increased to 24,000 rpm (10 minutes) for further size reduction. Temperature of 12°C was maintained throughout the procedure. Table 1 summarizes the ingredients used in formulations.
2. Preparation of rosuvastatin nanocrystals by bottom up technique [16]: Rosuvastatin calcium was dissolved in 6 ml of acetonitrile which constitutes (organic phase). The aqueous phase used is 0.2% of the surfactant/polymeric stabilizer/0.1% surfactant and 0.1% polymeric stabilizer in 1:1 ratio. The organic phase containing 1% of the drug was slowly added to the aqueous phase, under magnetic stirring (500 rpm). The mixer was then homogenized at 8000 rpm for 10 minutes, followed by 18,000 rpm at 25°C for 20 minutes. Table 2 summarize the ingredients used in formulations.

Characterization of nano crystal formulation

Particle size analysis

The particle size of preparations were analyzed using phase contrast microscope (Make - Leica, Model - S 40, 230±10 v) and laser diffraction (LD) using microtac particle size analyzer (Blue wave model S 4521).

Solubility

To perform solubility study, formulation equivalent to 50 mg of drug was added to 50 ml water in an iodine flask and maintained at 37°C for 24 h. The samples collected were collected and incubated at 37°C, filtered using Whatman filter paper and analyzed with UV-spectrophotometer at 244 nm [17].

Lyophilization of nanosuspension

Lyophilization of prepared formulations was done to enhance the stability and to achieve desirable features formulations were mixed with mannitol in (1:1) ration and freeze dried at −80°C for 24 h (in Lyodel freeze dryer).

Differential scanning calorimetry (DSC)

Thermal properties of the preparations were investigated by DSC Q20 V24.4 Build 116 DSC/TAC-7 thermal analysis controller with an intracooler-2 cooling system. The product to be analyzed was placed on perforated aluminum sealed pans. Thermal behavior of samples was investigated at a scanning rate of 5°C/minutes covering a temperature range of 40-200°C.

Chromatography condition for assay [18]

The chromatographic system used was Shimadzu SPD-M 10 AVP with the analytical column of C18-250 A, 4.6 mm×250 mm, 5 µm particle size. The mobile phase was 585 ml ammonium acetate buffer solution pH 4.0:360 ml acetonitrile (HPLC grade): 50 ml tetrahydrofuran. The detector wavelength was set at 244 nm, and the flow rate was 1.2 ml/minutes.

Preparation of granules [13]

Dry granulation method was employed for preparing granules. All ingredients including prepared nanocrystals were blended together and passed through 40 mesh (#), followed by slugging. The slugs were de slugged and passed through 25 mesh (#) magnesium stearate was added as a lubricant prior to the final compression.

Table 1: Formula for formulations F1-F7

Formulation code	SLS	Polaxmer 188	PVP	HPC	HPMC	Tween80+HPC	Tween80+PVP	Followed by homogenization technique
F1	√	-	-	-	-	-	-	8000 rpm for 10 minutes+24,000 rpm for 10 minutes
F2	-	√	-	-	-	-	-	
F3	-	-	√	-	-	-	-	
F4	-	-	-	√	-	-	-	
F5	-	-	-	-	√	-	-	
F6	-	-	-	-	-	√	-	
F7	-	-	-	-	-	-	√	

SLS: Sodium lauryl sulfate, PVP: Poly vinyl pyrrolidine, HPC: Hydroxyl propyl cellulose, HPMC: Hydroxyl propyl methyl cellulose

Table 2: Formula for formulations F8-F15

Formulation code	SLS	Polaxmer 188	PVP	HPC	HPMC	Tween 80+ HPC	Tween 80+ PVP	Solvent and anti-solvent technique	Followed by homogenization technique
F8	√	-	-	-	-	-	-	500 mg drug dissolve in acetone	8000 rpm for10 minutes+
F9	-	√	-	-	-	-	-	6 ml+42 ml surfactant stabilizer	24,000 rpm for 20 minutes
F10	-	-	√	-	-	-	-		
F11	-	-	-	√	-	-	-		
F12	-	-	-	-	√	-	-		
F13	-	-	-	-	-	√	-	500 mg drug+acetone 6 ml+	
F14	-	-	-	-	-	-	√	42 ml polymeric stabilizer+ surfactant stabilizer	
F15	√	-	-	-	-	-	√	500 mg drug+acetone 6 ml+ 20 ml HPMC (0.2%)+SLS (0.2%) 20 ml	8000 rpm for 10 minutes+ 18,000 rpm for 10 minutes

SLS: Sodium lauryl sulfate, PVP: Poly vinyl pyrrolidine, HPC: Hydroxyl propyl cellulose, HPMC: Hydroxyl propyl methyl cellulose

Compaction and evaluation of nanocrystal rosuvastatin calcium tablet

Compression of tablets was carried out by using 10 station rotary press Make - Rimek. Prepared tablets were stored in air tight container prior to further evaluations. Tablets were evaluated for critical quality attributes like weight variation, hardness (monsanto hardness tester), friability (Roche friabilator), disintegration and dissolution.

Dissolution study [19]

Dissolution study was performed using USP Type II (paddle type) dissolution apparatus (Make - Labindia Model-Disso 2000) at 50 rpm with 900 ml of citrate buffer. Samples were withdrawn at specified time intervals and sink condition was maintained throughout the study by adding fresh dissolution medium after each withdrawal the sample withdrawn were filtered by using Whatman filter paper no: 41 and analyzed by using UV-spectrophotometer at 244 nm.

***In-vivo* study [18,20]**

In-vivo studies were performed by using rabbits the study was performed after obtaining the approval from PSG Institutional Animal Ethics Committee Registration No.: 158/99/CPCSEA. The calculated animal dose was administered orally and blood samples (0.5 ml) were collected at specified time intervals (1, 2, 4, 6, 8, 10, 12 hrs) through marginal ear vein from rabbits and stored in micro centrifuge tubes containing 10 µl of 30% sodium citrate plasma separations were carried out and separated plasma was stored in frozen condition (80°C) [18] and samples were analyzed by HPLC with column of C 18-250 A, 4.6 mm×250 mm, 5 µm particle size. The mobile phase consists of acetonitrile: Formic acid (60:40) with flow rate of 1.0 ml/minute and wavelength 244 nm used for detection. The retention time of internal standard and rosuvastatin calcium was 2.2 minutes and 4.6 minutes respectively.

Pharmacokinetic analysis

Pharmacokinetic analysis performed to determine relative bioavailability non compartmental study was conducted to determine the pharmacokinetic parameters. The relative bioavailability of rosuvastatin calcium was calculated using the formula:

Relative bioavailability = AUC combined/AUC control × 100

RESULTS

FT-IR studies

Obtained IR spectrum clearly showed that the functional group of drug and polymers were present in the formulations, physical mixture unaltered.

Particle size analysis

The particle size of the formulations prepared by employing two techniques were as follows, the particle size obtained by following top down method was found to be <749.04 nm (F-1 to F-7) whereas particle size obtained by following bottom up method was higher than 2000 nm (F-8 to F-15). Fig. 1 shows particle size distribution in formulations and Fig. 2 shows particle size distribution of plain drug. The formulation prepared by top down technique was selected for further studies.

Particle size distribution

The size distribution of the particles in nanocrystal and microcrystal suspension formulation was determined through LD and diameters were calculated by distribution volume. The micronized particles possessed a size range of 61.51 µm whereas nanoparticle formulations F-3 and F-5 showed particle size of 0.509 µm, 0.399 µm, respectively. Fig. 3 shows percentile drug distribution of formulation F-5. The formulations prepared showed good redispersibility.

DSC studies

Thermal properties of the formulations were investigated by DSC to study the interactions and to determine the incorporation pattern of rosuvastatin calcium in formulations. Formulations F-3 and F-5 showed an endothermic peak at 121.12, 166.89, 165.29, 157.37, and 166.74°C (Fig. 3).

Saturation solubility

The saturation solubility for lyophilized nanocrystals is clearly depicted in Fig. 4 which shows formulation F-3, F-4, and F-5 possessed saturation solubility of 509.4, 527.1 542.3 µg/ml and for micronized formulation M-1 it is 164.2 µg/ml, which shows increase in the saturation solubility of nanocrystal formulation.

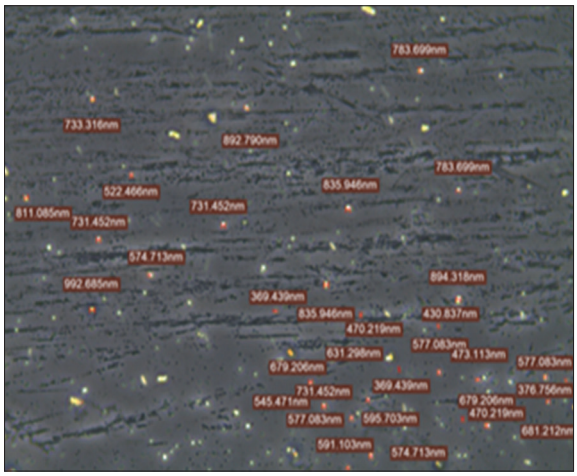

Fig. 1: Particle size distribution in formulations

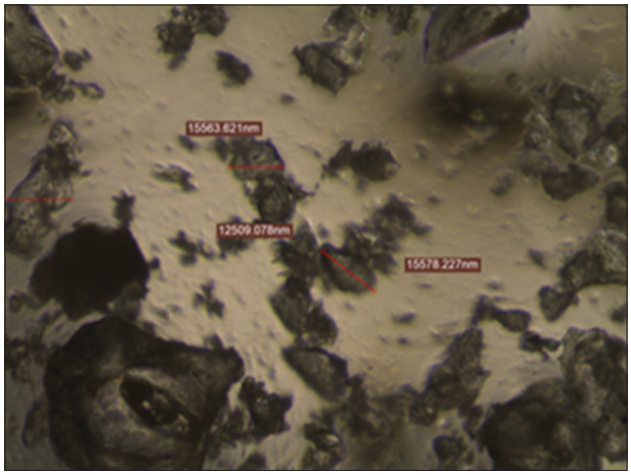

Fig. 2: Particle size distribution of drug

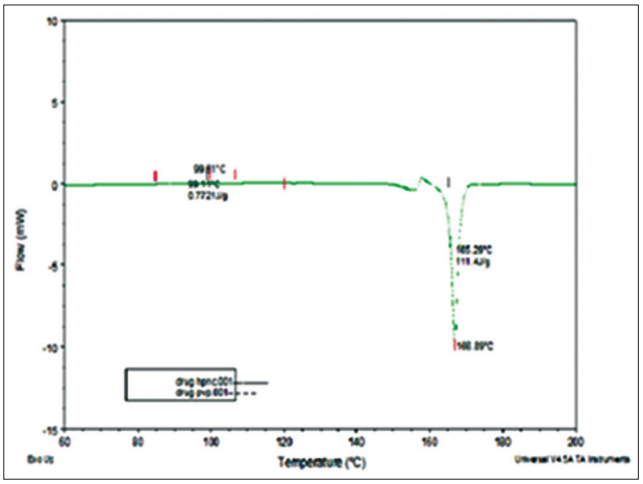

Fig. 3: DSC graph

Drug content

The formulation F-1 with (SLS: Drug: Mannitol) possessed higher drug content 67.6% and drug content was found to be low for formulation F-3 (poly vinyl pyrrolidine [PVP]: Drug: Mannitol) 35.12%.

Evaluation of prepared tablets

Friability values ranging from 0.15±0.0565% to 0.59±0.0283% and disintegration time lies between 29±1.414 and 44±1.414 seconds for the prepared formulations. The hardness of prepared tablets was between 3.0 and 4.5±0.707 kg/cm^2 which are sufficient for maintaining the integrity of tablets. Friability, disintegration time and hardness for the micronized formulation (M-1) were 0.875±0.0919%, 50±2.828 seconds, 3.5±0.707 kg/cm^2, which are higher than the values obtained for lyophilized nanocrystals in formulation.

***In-vitro* release study**

In-vitro release study for formulations F-1 to F-7 and M-1 was found to be 90.9±0.89%, 80.1±0.72%, 96.3±0.29%, 98.1±0.34%, 98.1±0.51%, 84.6±0.73%, 81.0±0.87% and 72.0±0.077% at the end of 45 minutes respectively. The drug release at various time intervals is summarized in Table 3.

***In-vivo* studies**

The pharmacokinetic profiles were calculated using non compartmental model, the pharmacokinetic profile for formulations F-3, F-5, and M-1 is summarized in Table 4. The animal dose of 0.76 mg of rosuvastatin calcium was administered and C_{max} was found to be 8.4 µg/ml, 10.3 µg/ml and 6.1 µg/ml for the formulations F-3, F-5 and M-1 respectively. The area under curve$_{0-t^*}$ of formulations F-3, F-5, and M-1 were 56.3, 46.0, 30.1 µg/hrs/ml. The half-life of F-3, F-5 and M-1 were found to be 13.29, 7.786 and 16.525. The K_{el} was calculated as 0.05152, 0.08902, and 0.04194, for the formulations F-3, F-5, and M-1 respectively. The mean residual time and drug distribution in the body for formulations F-3, F-5 and M-1 was 5.565, 5.3978 and 5.314 hrs/ml respectively, which was found to be higher than micronized formulation M-1. The relative bioavailability of nanocrystal formulations F-3, F-5 and M-1 (micronized formulation) was found to be 153.00, 187.04, and 100.00%.

DISCUSSION

The results obtained from IR studies clearly showed that there is no interaction between the drug and other excipients in the formulation thus proves the compatibility between excipient, drug and drug preparation. Particle size is one of the important parameter as physicochemical properties influences the biopharmaceutical features of the nanoformulation significantly. As smaller particle size possesses high surface area, there is an increase in the solubility property. The particle size of formulations prepared by top down technique was found to be <749.04 nm (F-1 to F-7) and the particle size of bottom up technique were found to be more than 2000 nm (F-8 to F-15). Particle sizes were found to be influenced by stabilizers used and method of preparation. Usage of stabilizers like HPMC, PVP and hydroxyl propyl cellulose (HPC) prevented aggregation of nano particles. Formulations with HPC as stabilizer produced smaller particles than other stabilizers used in top down technique and formulation with HPMC as stabilizer produced smaller particles in the bottom up technique. The prepared formulations after lyophilization were free-flowing and non-sticky with no aggregation of particles. The formulations showed increased solubility and possessed good redispersibility. Results obtained after DSC studies showed that the peak intensity of formulations F-3 and F-5 was same, and the difference in the peak area of F-3 and F-5 is due to changes in nature of the drug [20]. The results obtained after the evaluation of critical quality attributes of tablets were found satisfactory. The prepared tablets were found to possess sufficient hardness and the friability the obtained values after evaluations indicated that tablets were strong enough to withstand shocks during handling, and the surface of the tablets remained stable after friability studies. The release study results indicate that the dissolution rate of formulation F-1 to F-7 was higher than micronized formulation M-1. Among formulations prepared formulations F-3, F-4, F-5, containing PVP, HPC, and HPMC as stabilizers showed the maximum dissolution rate of nearly 98.0%, that is, 36.0% increase in the dissolution rate of formulation containing lyophilized nanocrystals of rosuvastatin calcium when compared with the micronized formulation. When compared to the marketed formulation M-1, Formulations F-3, F-5, shows 38.0% and 69.0% increase in the peak plasma concentration at the end of 4 hrs. The half-life of the F-3 and F-5 were reduced when compared with M-1, and the reduction in half life may be due to rapid excretion of rosuvastatin calcium nanocrystals The maximum plasma concentration was achieved by formulation F-5 which contains (HPMC: Drug: Mannitol) as its constituents, when compared to formulation F-3 which contains (PVP: Drug: Mannitol). Although the plasma concentration was high, the half-life was found to be less for formulation F-5 than formulation F-3 this shows less rate of elimination for formulation F-3 with (PVP: Drug: Mannitol). However, obtained results clearly indicated increased bioavailability in nanocrystal formulations F-3 and F-5 than micronized formulation M-1. The bioavailability increased up to 1.87-fold for formulation F-5 and 1.53-fold for the formulation F-3 prepared by nanocrystal technology. This clearly shows good applicability of nanocrystal technology for enhancing solubility and bio availability for poorly soluble drugs.

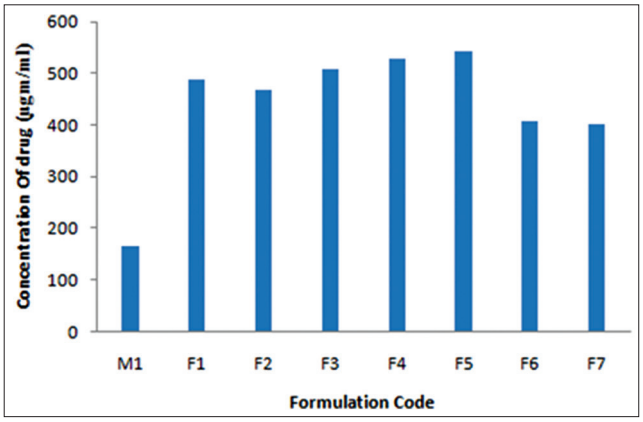

Fig. 4: Saturation solubility (µg/ml)

CONCLUSION

Formulations containing different constituents were prepared by following bottom up and top down techniques and evaluated *in-vitro, in-vivo*, observed significant differences between the formulations. The results proved that the bioavailability of rosuvastatin calcium has been improved considerably by applying nanocrystal technology. *In-vitro and in-vivo* evaluations showed that solubility and bioavailability have been enhanced considerably. *In-vitro* studies have shown a 36% increase in dissolution of nanocrystal formulation while *in-vivo* studies exhibited 1.87-fold increases in the bioavailability of rosuvastatin when compared to the micronized dosage form.

Table 3: *In-vitro* release profile of formulations F1-F7 and M-1

Time	F-1 (%)	F-2 (%)	F-3 (%)	F-4 (%)	F-5 (%)	F-6 (%)	F-7 (%)	M-1 (%)
5 minutes	56.7±0.72	39.6±0.24	61.2±0.13	62.1±0.64	64.8±1.22	54.0±0.51	50.4±0.49	23.4±0.17
10 minutes	68.4±0.34	51.3±0.48	73.8±0.79	78.3±0.79	74.7±0.98	57.6±0.58	56.7±0.36	37.81±0.18
20 minutes	78.3±0.65	64.8±0.56	77.4±0.58	83.7±0.68	83.7±0.89	64.8±0.89	67.5±0.69	48.6±0.23
30 minutes	80.1±1.3	67.5±0.68	85.5±0.61	91.8±0.41	90.9±0.39	72.9±0.21	77.4±0.39	56.7±0.44
45 minutes	90.9±0.89	80.1±0.72	96.3±0.29	98.1±0.34	98.1±0.51	84.6±0.73	81.0±0.87	72.0±0.77

Table 4: Pharmacokinetic parameters

Pharmacokinetic parameters	Observations		
	F-3	F-5	M-1
Cmax (µg/ml)	08.40	10.30	06.10
Tmax (hrs/ml)	4th hr	4th hr	4th hr
AUC (0-t*) µg/hrs/ml	46.0	56.3	30.1
AUMC (0-t*) µg/hr2/ml	255.6	303.9	159.95
AUC (0−∞) hr/ml	78.59723	73.14901	56.32487
MRT (hrs/ml)	5.556522	5.397869	5.313953
Kel (hrs)	0.052152	0.089026	0.041945
Half-life t½ (hr)	13.29	7.785896	16.52517
Relative bioavailability %	153.00	187.04	100.00

C_{max}: Maximum concentration of drug in plasma, T_{max}: Time of maximum concentration of drug in plasma, AUC: Area under the curve of the plot of C_{max} versus T_{max}, AUMC: Area under moment curve, MRT: Mean residence time, Kel: Rate constant of elimination

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