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DESIGN AND DEVELOPMENT OF SAQUINAVIR MICROEMULSION FOR THE ORAL BIOAVAILABILITY ENHANCEMENT

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ABSTACT

PURPOSE- The present work was aimed at developing an oral microemulsion based drug delivery system for enhancing the bioavailability of Saquinavir and evaluating its in vivo potential.

METHODS- The solubility of Saquinavir was determined in various vehicles. Pseudoternary phase diagrams were developed at different Labrafac CM10: Tween 80: Polyethylene glycol 400 ratios to know the microemulsion existing zone. Solubilization capacity of the microemulsion system was determined. Saquinavir loaded microemulsion was optimized and was evaluated for its transparency, droplet size, zeta potential, viscosity, conductivity, % assay, and stability study. The optimized formulation was then subjected to stability studies as per International Conference on Harmonization (ICH) guidelines and was found to be stable over 6 months. Drug release from ME was estimated by *in vitro* studies through excised rat duodenum. *In vivo* oral absorption of Saquinavir from the microemulsion containing Labrafac CM10 (4.0%), Tween 80 (36.0%), Polyethylene glycol 400 (9.0%) and distilled water (51%) was investigated in rats.

RESULTS- Results from the data of characterization revealed that the optimized ME is transparent and having small particle size. Saquinavir, a poorly soluble drug, displayed high solubility in the developed microemulsion formulation. The *in vitro* intraduodenal diffusion and *in vivo* study revealed an increase of bioavailability (10.68 times) after oral administration of the microemulsion formulation as compared with the commercially available tablets.

CONCLUSIONS- Based on the results it could be concluded that microemulsion formulation could be used as a possible alternative to traditional oral formulations of Saquinavir to improve its bioavailability.

INTRODUCTION

Saquinavir, a potent HIV-1 and HIV-2 protease inhibitor, has been approved for use in treatments of patients with acquired immunodeficiency syndrome. This drug is effective in reducing viral load and is well tolerated. Saquinavir absorption in the gastrointestinal tract is slow, variable, and incomplete[1]. Saquinavir has a low oral bioavailability (approximately 4–8%) due to Hepatic first pass metabolism, limited absorption due to a poor water solubility and effect of the multidrug resistance transporter P-glycoprotein, which is responsible for an efflux mechanism resulting in a reduced crossing of the intestinal barrier[2-4]. Hence administering Saquinavir by the oral route appears as a formidable challenge due to its poor absorption pattern and rapid hepatic first pass metabolism. The extent of absorption and bioavailability enhancement of lipophilic drugs could be observed particularly by improving their residence time in the intestinal mucus and protecting the drug from the enzymes through intestinal lymphatic transport of drugs[5-9]. In the present study, a microemulsion formulation was prepared using Labrafac CM10 (4.0%), Tween 80 (36.0%), polyethylene glycol 400 (9.0%) and distilled water (51%) by water titration method. Pseudoternary phase diagrams were constructed to find out the zone of microemulsion at different ratios of surfactant to cosurfactant (e.g., 1:1, 2:1, 3:1 and 4:1). The effect of formulation variables on different physicochemical characteristics such as % transmittance, droplet size, and zeta potential was studied. An ex vivo diffusion study was performed using rat duodenum, and the pharmacokinetics of the optimized microemulsion were evaluated by administering it orally to rats. The relative bioavailability was also calculated after oral administration of prepared microemulsion, plain drug suspension and commercially available tablets.

MATERIALS AND METHODS

MATERIALS: Saquinavir (sqv) was received as a gift sample from Cipla pharmaceutical ltd. (Mumbai, India). The following materials were donated by Gattefosse (Mumbai, India) and were used as received: Labrafac

CM10 (C 8 -C 10 polyglycolized glycerides), Maisine 35-1 (glyceryl monolinoleate), Lauroglycol FCC (propylene glycol laurate), Labrafil 1944 CS (apricot kernel oil polyethylene glycol [PEG] 6 esters), and Labrafac PG (propylene glycol caprylate/caprate). Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil), Cremophor EL (polyethoxylated castor oil) were obtained from Colorcon Asia (Mumbai, India). Span 20 (sorbitan monolaurate), Tween 80 (polyoxyethylene sorbitan monooleate), PEG 600 and PEG 400 were bought from Fine chemicals (Mumbai, India). Acetonitrile and methanol used in the present study were of high performance liquid chromatography (HPLC) grade. All other chemicals were reagent grade.

ANIMALS: Male Albino rats (250 ± 20 g) were used for the comparative *in vivo* studies. The animals were maintained at a constant light (14L: 10D), temperature (24°C-25°C), and humidity (60%) and were supplied with food and water ad libitum. Animal experiments were approved by Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India, with the permission number of 404/01/a/CPCSEA. Male albino rats were obtained from Zydus Health care, Ahmadabad, India.

METHODS

1. Solubility Studies: The solubility of Saquinavir in various components (oils, surfactants, and cosurfactants) was determined as follows (10); 10 ml of each of the selected vehicles was added to each cap vial containing an excess of Saquinavir (1 gm). After sealing, the mixture was heated at 40ºC in a water bath to facilitate the solubilization. Mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a shaker at 25ºC for 48 hours. Then each vial was centrifuged at 3000 rpm for 5 minutes, and excess insoluble Saquinavir was discarded by filtration using a membrane filter (0.45 µm, 13 mm, Whatman, Mumbai, India). The concentration of Saquinavir was then quantified by HPLC.

2. Pseudoternary Phase Diagrams: Pseudoternary phase diagrams of oil, surfactant/ cosurfactant (S/CoS), and water were developed using the water titration method[10]. The mixtures of oil and S/CoS at certain weight ratios were diluted with water in a dropwise manner. For each phase diagram at a specific ratio of S/CoS (ie, 1:1, 2:1, 3:1, and 4:1 wt/wt), a transparent and homogenous mixture of oil and S/CoS was formed by vortexing for 5 minutes. Then each mixture was titrated with water and visually observed for phase clarity and flowability. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. To determine the effect of drug on the microemulsion existing zone, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the hydrophobic component. Phase diagrams were then constructed using Chemix software.

3. Preparation of Microemulsion: Microemulsion formulations were prepared by water titration method varying the ratio of oil, surfactant, co-surfactant, and water keeping the concentration of Saquinavir constant in each case (11). Drug (Saquinavir) was mixed in accurate quantity of oil (Labrafac CM10) and to that surfactant (Tween 80) and co-surfactant (Polyethylene glycol 400) at the fixed ratio were mixed gently for 10 minutes with the help of magnetic stirrer at room temperature. The mixture was then finally titrated with distilled water until a stable and transparent microemulsion was obtained. Microemulsion formulation was optimized through formulation (oil-surfactant, surfactant-co-surfactant and oil–water ratio) and process variables (Time and rpm). Particle size and percentage transmittance were the parameters evaluated during the optimization. Dilution study was also performed with the optimized microemulsion.

4. Characterization and Evaluation of microemulsion:

4.1. Percentage Transmittance: Transparency of microemulsion formulation was determined by measuring percentage transmittance through U.V. Spectrophotometer (UV-1601-220X. SHIMADZU). Percentage transmittance of samples was measured at 650 nm with purified water taken as blank and three replicates were performed for each sample.

4.2. Droplet Size Analysis and Zeta-Potential Determination: Droplet size and zeta potential measurement of optimized ME formulations was carried out by dynamic light scattering through Zetasizer HAS 3000 (Malvern Instruments Ltd., Malvern, UK).

4.3. Viscosity and Conductivity Measurements: The viscosity of the optimized ME was evaluated by a Brookfield LVDV 111 + CP viscometer (Stoughton, MA) at 30^0C using a CPE 42 spindle at 5 rpm. Experiments were performed in triplicate for each sample, and results were presented as average ± standard deviation. Electrical conductivity of microemulsion was measured using a conductometer (CM 180 conductivity meter. Elico, India) at ambient temperature.

4.4. Stability Studies: The optimized ME was stored at three different temperature ranges for 6 months i.e., refrigerating condition (2^0C – 8^0C), room temperature and elevated temperature (50 ± 2^0C) and shelf life of the stored microemulsion system was evaluated by visual inspection (phase separation), % transmittance, Particle size and % Assay[12]. In order to estimate the metastable systems, the optimized microemulsion formulation was also centrifuged (Remi Laboratories, Mumbai, India) at different rpm like 5,000, 10,000 and 15,000 for 30

minutes at room temperature and observed for any change in homogeneity of microemulsion.

4.5. Determination of Saquinavir Content in the microemulsion: Microemulsion formulation was analyzed for drug content by U.V. spectrophotometer at 239 nm.

4.6. *In Vitro* Release Studies: The methods employed were modified from experimental procedures well described in the literature[13]. Male albino rats (230-270 g) were killed by overdose with pentobarbitone administered by intravenous injection. To check the intraduodenal permeability, the duodenal part of the small intestine was isolated and taken for the in vitro diffusion study. Then this tissue was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. The microemulsion sample was diluted with 1 mL of distilled water (outside mixing for 1 minute by vortex mixer), and for the tablet sample a suspension of tablet was made in distilled water. The resultant sample (2 mg/mL) was injected into the lumen of the duodenum using a syringe, and the 2 sides of the intestine were tightly closed. Then the tissue was placed in a chamber of organ bath with continuous aeration and a constant temperature of 37^0C. The receiver compartment was filled with 23 mL of phosphate-buffered saline (pH 5.5). The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 239 nm, keeping the respective blank. The percent diffusion of drug was calculated against time and plotted on a graph.

4.7. *In Vivo* Absorption Studies: Absorption studies were performed in male albino rats weighing 230 to 270 gms. Animal experiments were approved by Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India, with the permission number of 404/01/a/CPCSEA. Male albino rats were obtained from Zydus Health care, Ahmadabad, India. The animals were fasted overnight prior to the experiment but had free access to water. The microemulsion was administered by oral snode in an equivalent dose of 25 mg/kg of Saquinavir to first group of rats (6 rats). The tablet suspension was administered in the same manner to the second group of rats. Oral administration of the PDS (same dose) was also given to the third group of rats. The blood samples (approximately 400-500 µL) were collected from the retro-orbital vein using a heparinized needle (18-20 size) at after 0.5, 1, 2, 3, 4, 6, 12, and 24 hours after oral administration. The blood samples were collected into a heparinized microcentrifuge tube. Then the samples were subjected to centrifugation on a laboratory centrifuge (Sigma, 3K30) at 10,000 rpm for 10 minutes at 0^0C, and supernatant plasma was collected into another microcentrifuge tube and kept at -20^0C until analysis[14].

4.8.1. HPLC Analysis of Plasma Sample: The concentration of Saquinavir in plasma samples was determined by HPLC analysis[15]. The HPLC system with Hewlett-Packard (Agilent) 1100 series components, a quaternary pump, auto sampler, and variable wavelength UV detector (Palo Alto, CA) was used to analyze Saquinavir at 240 nm. Chromatographic separations were achieved using an Inertsil ODS-3V column (250 ×4.6 mm, 5 µm) (GL Science, Tokyo, Japan). The mobile phase used for the plasma sample was Acetonitrile and methanol of HPLC grade (70:30 V/V). Eppendorfs tubes, previously treated with 0.5 ml of 2% sodium citrate solution were used for the collection of blood to prevent blood clotting. Then the blood mixture was centrifuged at 10,000 rpm at 5^0C for 10 min to separate plasma proteins. Supernatant was taken in separate Eppendorf tubes and equal volumes methanol was added. Then this mixture was centrifuged at 5,000 rpm at 5^0C for 10 min. After centrifugation, 0.2 ml of supernatant was transferred to 2 ml Eppendorf tubes. To the solutions, 0.8 ml of mobile phase was added and mixed well with a vortex mixer. The samples were stored at - 20^0C before analysis. A volume of 20 µl of each sample were injected on to HPLC column. The samples were analysed at ambient temperature. The samples were analysed at 240 nm with flow rate at 1.0 ml/min. The mobile phase and all solvents were passed from 0.22 µm filter paper and sonicated before use. The blank was run on a chromatograph to check the impurity level in solvents. Various pharmacokinetic parameters like C_{max}, T_{max} were calculated using Quick Cal Software. The area under curve (AUC $0 \rightarrow 24$) for $0 \rightarrow 24$ hrs was calculated and extrapolated to (AUC $0 \rightarrow \infty$) infinity. The linearity of the method was found suitable in the range of 0.05 to 10 µg/mL ($R^2 = 0.9999$).

4.8.2. Pharmacokinetic Data Analysis: For oral administration, the area under the drug concentration-time curve from 0 to 24 hours (AUC) was calculated using the 2-compartment mode of Quick Cal Software for pharmacokinetic analysis.

4.9. Statistical Analysis: All data are expressed as the mean ± SD and comparison of the mean values was performed using either Student's *t*-test or ANOVA. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION:

Solubility Study: The solubility data of Saquinavir in various vehicles is given in Table 1. Labrafac CM10 (HLB Value=10) showed higher solubilizing capacity compared to other vehicles. Hence, Labrafac CM10 was selected as oil phase. Tween 80 and polyethylene glycol 400 were selected as surfactant and co-surfactant respectively for the preparation of optimized microemulsion.

Solubility of drug was determined in optimized ME and its individual components. Fig 1 shows the solubility of drug that optimized ME has higher solubilizing capacity (151.83 mg/ml) than its oil phase (Labrafac

CM10→52.89 mg/ml), surfactant (Tween 80→31.45 mg/ml) and co-surfactant (PEG 400→34.26 mg/ml) taken individually. So Labrafac CM10 based microemulsion formulation can increase the solubility of lipophilic drug i.e., Saquinavir.

Pseudo Ternary Phase Diagram Study: Pseudo-ternary phase diagrams were constructed as shown in Figure 2 to identify the microemulsion existing zone from which appropriate concentration ranges of different components of microemulsion can be obtained. 4:1 and 3:1 ratios of Tween 80 & PEG 400 gave clear microemulsion and the microemulsion existing region with 3:1 ratio was comparatively larger than 4:1, hence 3:1 ratio of surfactant and co-surfactant was selected. Added advantage of 3:1 ratio is less % of surfactant.

Characterization results: The physicochemical characteristics of the developed microemulsion appear in Table 1. It was clear from the physicochemical data that the developed system had low viscosity (~27.5 cP). From the viscosity and electroconductive study it can be concluded that the system is of the o/w type.[16] The refractive index of the developed system (1.331) was similar to the refractive index of water (1.333). In addition, the developed system showed percent transmittance 99.4%. The refractive index and percent transmittance data prove the transparency of the system. The nanometric size range of the particle was retained even after 100 times dilution with water, which proves the system's compatibility with excess water. Aggregation of the system will not take place due to negative charge of the droplets. Data of the stability study was indicated that the optimized Labrafac CM10 based microemulsion was stable up to 6 months.

In Vitro Intestinal Permeability Study: In vitro intestinal permeability data are shown in Figure 3. The drug diffused at a faster rate from the microemulsion system than from the tablet dosage form. The total percentage diffusion was much higher for the microemulsion system than for the tablet dosage form. After 5 hours of diffusion, 87.42% of the drug was diffused from the microemulsion system, as compared with 67.13% diffused from the commercially available tablets.

***In Vivo* Studies:** Figure 4 shows plasma drug levels vs. time curve for Saquinavir microemulsion and plain drug suspension (PDS) after oral administration in male albino rats. Pharmacokinetic parameters are recorded in Table 3. Optimized microemulsion formulation of Saquinavir shows higher Cmax in blood as compared to plain drug suspension. The Cmax of tablet formulation was 0.96 µg/mL after 0.5 hrs, whereas it was 1.55 µg/mL for the microemulsion formulation after 3 hours. This may have been due to the slow diffusion of Saquinavir from the dispersed oil globules to the continuous medium. But the release of drug was greater and more sustained from the microemulsion formulation than from tablets. The enhanced absorption may be explained in terms of (1) the huge specific surface area of the microemulsion droplets (mean droplet size ~27.9 nm), (2) improved permeation of the Saquinavir because of the presence of surfactant, which reduces the interfacial tension to nearly 0; and (3) the stability of the microemulsion in the gastrointestinal tract. A statistically significant difference ($P < 0.05$) between the two formulations was found from the ANOVA analysis. Higher relative bioavailability may be due to the avoidance of first-pass hepatic metabolism by intestinal lymphatic transport, which circumvents the liver.

CONCLUSION

The developed microemulsion containing Labrafac CM10 (4.0%), Tween 80 (36.0%), polyethylene glycol 400 (9.0%) and distilled water (51%) was found to be transparent, with a particle size of 22.7 nm. Microemulsion showed higher *in vitro* drug release as compared to plain drug suspension. Results of *in vivo* studies showed significantly higher AUC with microemulsion than with plain drug suspension. The relative bioavailability of drug from microemulsion was found to be 57.68%, which is 10.68 times higher than that of plain drug. Hence, it can be concluded that the microemulsion formulation can be employed to improve the bioavailability of a poorly absorbed drug. However, further studies in higher animals and human being need to be performed before this formulation can be commercially exploited.

Table 1: Physicochemical Parameters of developed Microemulsion

Parameters	Value
Particle Size (nm)	22.7±1.28
Particle size after dilution (nm)	23.5±1.54
Zetapotential (mV)	-2.66
Electroconductivity (µΩ)	247±3
Viscosity (cP)	27.5 ± 1
% Transmittance	99.4 ± 0.4
% Assay	98.76±0.87

Table 2: Results of stability studies.

Temperature (^{0}C)	Phase separation		% transmittance		Particle size (nm)		% of Assay	
	After 4 month	After 6 months	After 4 month	After 6 months	After 4 month	After 6 month	After 4 month	After 6 months
2^0C-8^0C	No	No	98.8±0.8	97.8±2.4	24.9± 2.4	25.3±1.8	98.3±1.5	97.8±2.9
Room Temp	No	No	99.2±1.2	98.4±1.3	24.2 ± 2.1	24.9 ± 3.1	99.4±1.9	99.1±2.1
Elevated Temp (50 ± 2^0C)	No	No	98.5±0.8	97.9±1.5	25.2 ± 2.6	26.7 ± 3.2	99.2±0.9	98.6±1.7

Table 3: Pharmacokinetic profile of Saquinavir after administration in rat:

Pharmacokinetic Parameter	Group I	Group II	Group III
C_{max} (µg/ml)	1.55	0.96	0.48
T_{max} (Hrs)	3.0	0.5	0.5
$T_{1/2(}$Hr)	10.784	0.584	0.512
AUC $_{0\text{-}t}$ (µg hr ml^{-1})	13.51	1.69	1.23
AUC $_{0\text{-}\infty}$ (µg hr ml^{-1})	15.07	1. 41	1.22
K_{el}	0.0116	0.0129	0.0131
*Relative Bioavailability (%)	10.68	--	--

*Relative bioavailability = $(AUC_{ME} / AUC_{Tab}) \times (Dose_{Tab}/Dose_{ME})$., Mean ± standard deviation, n = 3-4.

Fig 1: Solubility of drug in various vehicles.

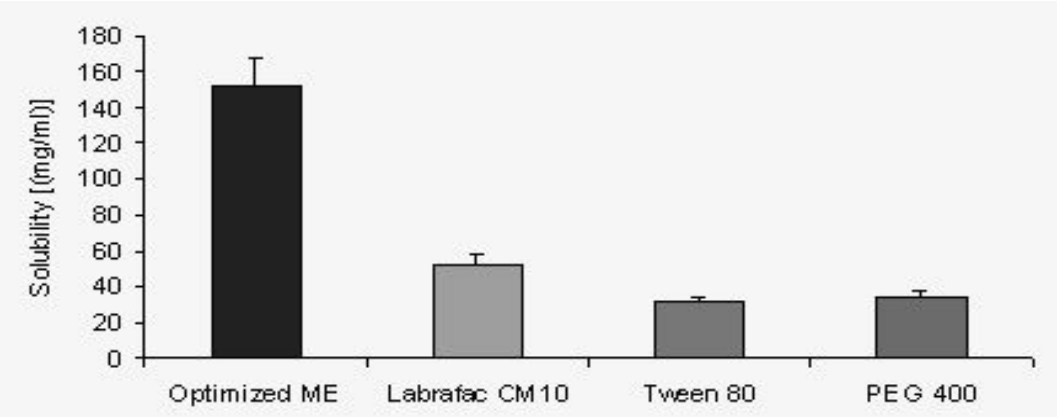
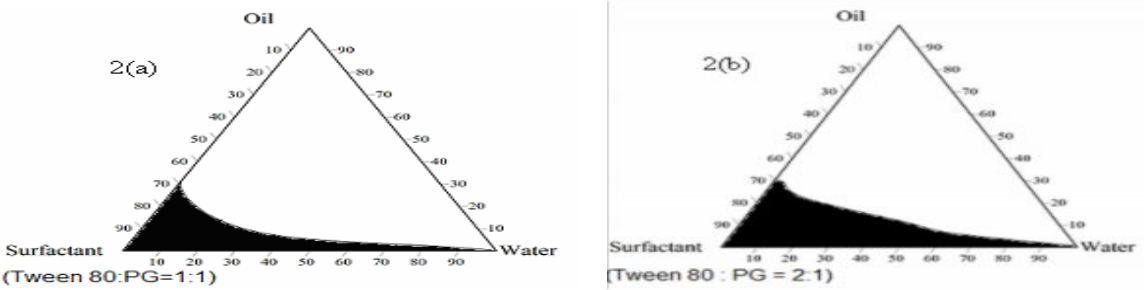


Figure 2: Pseudo-ternary diagrams



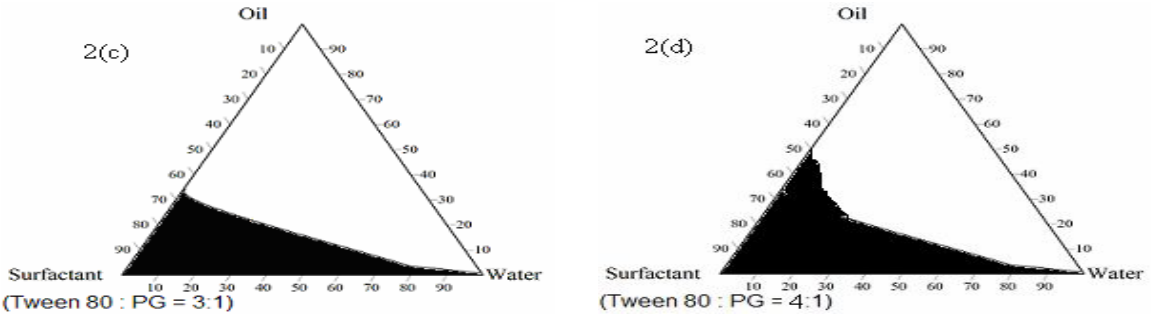


Figure 3: Comparative *in vitro* diffusion profile of Saquinavir.

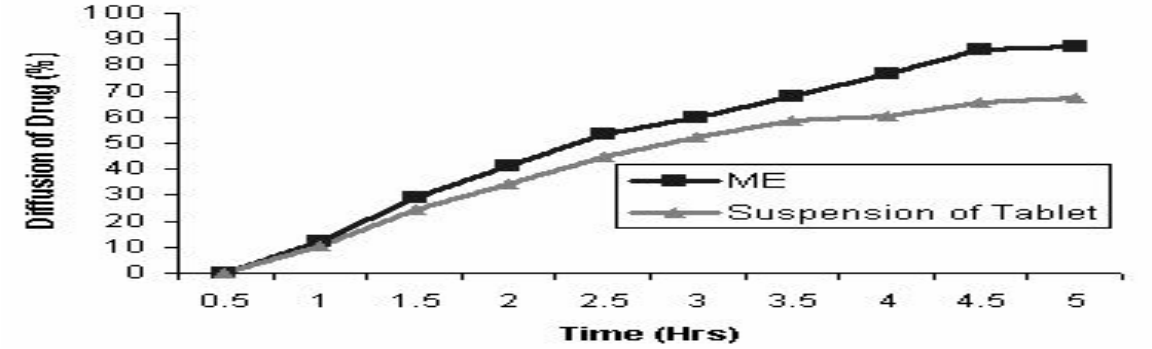
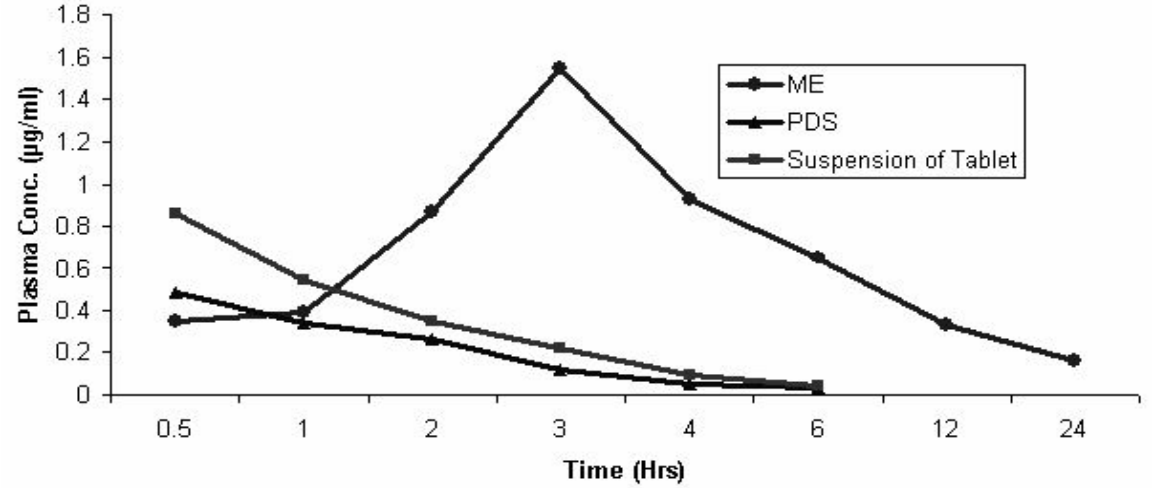


Figure 4: Comparative plasma concentration of Saquinavir



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REFERENCES

1. K Singh, L Dickinson, A Chaikan, D Back, C Fletcher. Pharmacokinetics and Safety of Saquinavir/Ritonavir and Omeprazole in HIV-infected Subjects. Clinical Pharmacology & Therapeutics, 2008; 83(6): 867–872.

2. Molla, A., Granneman, G.R., Sue, E., Kempf, D.J. Recent developments in HIV protease inhibitor therapy. Antivir. Res, 1998; 39: 1–23.

3. Williams, G.C., Sinko, P.J. Oral absorption of the HIV protease inhibitors: a current update. Adv. Drug Deliv. Rev, 1999; 39: 211–238.

4. Noble, S., Faulds, D. Saquinavir A review of its pharmacology and clinical potential in the management of HIV infection. Drugs, 1996; 52: 93–112.

5. Charman S, Charman W, Rogge M, Wilson T, Dutko F, Pouton C. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm Res, 1992; 9: 87-93.
6. Constantinides P, Scalart J, Lancaster C, et al. Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. Pharm Res, 1994; 11: 1385-1390.
7. Kovarik J, Muelle E, Van Bree J, Tetzloff W, Kutz K. Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. J Pharm Sci, 1994; 83: 444-446.
8. Shah N, Carvajal M, Patel C, Infeld M, Malick A. Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. Int J Pharm, 1994; 106: 15-23.
9. Matuszewska B, Hettrick L, Bondi J, Storey D. Comparative bioavailability of L-683,453, a 5a-reductase inhibitor, from a self-emulsifying drug delivery system in beagle dogs. Int J Pharm, 1996; 136: 147-154.
10. Pradip Kumar Ghosh, Rita J. Majithiya, Manish L. Umrethia, and Rayasa S. R. Murthy. Design and Development of Microemulsion Drug Delivery System of Acyclovir for Improvement of Oral Bioavailability. AAPS PharmSci Tech, 2006; 7 (3): 1-6.
11. Hung-Jang G., Shou-Nan Chen, Jenn-Shyong Hwang, C. Tien, M. T. Kuo. Studies of Formation and Interface of Oil-Water Microemulsion. Chinese Journal of physics, 1992; 30 (5): 665-678.
12. A. Radomska, R. Dobrucki; The use of some ingredients for microemulsion preparation containing retinol and its esters. International journal of Pharmaceutics, 2000; 196(2): 131-134.
13. Smith P. Methods for evaluating intestinal permeability and metabolism in vitro. Pharm Biotechnol, 1996; 8: 13-34.
14. Charles M. Buchanan, Norma L. Buchanan, Kevin J. Edgar, James L. Pharmacokinetics of Saquinavir after Intravenous and Oral Dosing of Saquinavir: Hydroxybutenyl-β-Cyclodextrin Formulations. Biomacromolecules, 2008; 9 (1): 305–313.
12. Andrea Heinz, Clare J. Strachan, Faraj Atassi, Keith C. Gordon and Thomas Rades†. Characterizing an Amorphous System Exhibiting Trace Crystallinity: A Case Study with Saquinavir. Cryst. Growth Des., 2008; 8 (1): 119–127.
13. McEvoy G. AHFS Drugs Information, American Society of Health System Pharmacists. Bethesda, MD: Atlantic Books, 1998; 471-480.
14. Ghosh PK, Umrethia M, Majithiya RJ, Murthy RSR. Preparation and physicochemical characterisation of caprylocapryl macrogol -8-glycerides microemulsion for oral drug delivery. Ars Pharm, 2004; 45: 353-372.
15. M. A. Campanero , M. Escolar , M. A. Arangoa , B. Sádaba , J. R. Azanza. Development of a chromatographic method for the determination of saquinavir in plasma samples of HIV patients. Biomedical Chromatography, 2001; 16(1): 7-12.
16. Gibaldi M, Perrier D. Pharmacokinetics. New York, NY: Marcel-Dekker; Volume15, second edition, 1982.
17. V. Jain, V. Prasad, P. Jadhav, P. R. Mishra. Preparation and performance evaluation of saquinavir laden cationic submicron emulsions. Drug delivery, 2009; 16(1): 37-44.
18. Dimitrios G Fatouros, Ditte M Karpf, Flemming S Nielsen, and Anette Mullertz. Clinical studies with oral lipid based formulations of poorly soluble compounds. Ther Clin Risk Manag, 2007; 3(4): 591–604.
19. Bidyut K. Paul and Satya P. Moulik. Uses and applications of microemulsions. Current Science, 2001; 80 (8): 990-1001.

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