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Oral Bioavailability of Simvastatin Novel Formulation in Albino Rats

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Abstract: The aim of this study was to compare the single dose oral bioavailability of two formulations of Simvastatin in albino rats. Plasma was analyzed for simvastatin using a sensitive, reproducible, accurate and validated LC-MS/MS method. Pharmacokinetic parameters including AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, and $t_{1/2}$ were determined from plasma concentration for both formulations. Self-emulsifying Formulation of simvastatin showed a significant improvement in bioavailability (1.5 fold) as compared with the conventional tablets.
Key words: Bioavailability, Pharmacokinetics, LC-MS/MS, Simvastatin

Introduction:

As the tendency of the poorly water soluble drugs to enter development pipeline increases, the challenges to find innovative methods of developing stable and bioavailable dosage forms increases **(1, 2, 3, 4)** Drug delivery approaches aim to develop a carrier system which can hold the molecule effectively and can navigate them towards the right destination without affecting the route and at the same time modify the drug release characteristics. For water insoluble drugs with high permeability, drug absorption by GIT is limited by drug dissolution rate (**5, 6) s**olubility/dissolution are good pointer and major contributor to drug bioavailability. Self micro emulsifying systems have drawn greater attention because of their solubilisation and transport properties. The currently available statins generally possess a low systemic bioavailability coupled with extensive first pass hepatic metabolism **(7)**.Simvastatin (Butanoic acid, 2, 2 dimethyl-1, 2, 3, 7, 8, 8a hexahydro 3, 7-dimethyl-8-(2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)-ethyl)-1-napthalenyl ester) which is a potent and effective lipid lowering agent from the family of statins with a good tolerability profile (**8**) has systemic bioavailability only 5%.The objective of the study is to develop a self emulsifying formulation(**9,10)** of simvastatin and to assess the bioavailability in comparison to simvastatin tablets in albino rats.

Materials and Methods:

Simvastatin was obtained from Ranbaxy Labs (Gurgaon, India). Transcutol, Labrasol, Plurol-oleique, and Maisine 35-1 oil were procured from Gattefosse, France. Acetonitrile, Methanol, Propylene Glycol were purchased from CDH Fine Chem. Simvastatin (Zocor™) tablets 40 mg from Merck and self-micro emulsifying formulation was made in our lab.

Preparation of self-emulsifying system of Simvastatin:

Various compositions of Labrasol (surfactant), Plurol-oleique (co-surfactant), transcutol (solubility enhancer) and Maisine oil were prepared and determined for micro emulsion zone. Optimized composition of self micro-emulsifying system is given below (**Table 1**)

Table 1: Composition of self emulsifying formulation filled in capsules

Ingredients	%w/w
Simvastatin	7.7
Labrasol	69.6
Plurol oleique	10.4
Transcutol	1.2
Maisine oil	11.1

Characterization Selected self micro emulsifying system as per table 1 was characterized for thermodynamic stability, viscosity and droplet size. Tendency to form micro emulsion and appearance of globule size was assessed under microscope (Olympus, Japan). Mean droplet size, zeta potential and polydispersity index was determined by Zetasizer (Malvern, UK). Viscosity was determined using Brookfield DVIII Ultra V 6.0 RV viscometer. *in vitro* dissolution studies were performed according to USP32.

Study design and sampling schedule: A single dose, balanced, 2-treatment, 2-period, cross-over bioavailability study was designed in albino rats under fasting conditions. 18 animals weighing 150-225g were taken for study. Each animal received dose equivalent to 1mg of simvastatin. Simvastatin formulations (Tablets 40mg and self emulsifying system 40mg) were suitably suspended in fixed volume of purified water to provide 1mg/ml dose accurately in a cross over design, observing a wash out period of 7 days. Blood samples were collected at a period of 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hours post dose. 1.5 ml of blood was withdrawn in eppendroff tube previously containing ACD buffer (0.2 ml) as an anticoagulant. The blood samples were mixed gently with the anticoagulant and kept for 2 hrs. The blood samples were centrifuged under refrigeration at 4000 rpm for 15 minutes. The plasma was transferred to another eppendroff tube sealed and stored at -20^{o}C and drug analysis was carried out using LC-MS/MS.

Analysis of Plasma samples**:** A method of determining simvastatin in rat plasma was evaluated using LC-MS/MS system with Shimadzu Controller integrated system. The analyte was quantitated using a solid phase extraction procedure. Quality control samples at concentration of low 0.510 ng/ml (LQC), medium 8 ng/ml and 22 ng/ml (MQC) and high 58 ng/ml (HQC) were prepared in rat plasma and analyzed with each assay validation run to ensure acceptable assay precision and accuracy. Calibration curve for simvastatin was prepared in the range of 0.2 ng-80 ng/ml. For sample preparation, 50 µl of internal standard, Lovastatin (250 ng/ml) was taken in a micro centrifuge tube and 500 µl plasma was added. Solid-phase extraction was carried out using refrigerated centrifuge set at temperature range of 4-8^{o}C. The cartridges were conditioned using 1 ml methanol and 1 ml HPLC grade water in centrifuge for 1 minute. Samples were loaded, centrifuged for 3 minutes at 1000-1200 rpm followed by washing with 1 ml methanol solution for 2 minutes at same rpm. The sample was eluted twice with 0.5 ml methanol by running the centrifuge at 400-600 rpm for 1 min. The elute was evaporated to dryness at 40^{o}C and at 15 psi under nitrogen. The residue was reconstituted in 300 µl mobile phase. The data were acquired and calculated on shimadzu controlled using software analyst 1.4. Linear regression was used to obtain the best fit of data for the calibration curves. The Lower Limit of Quantitation (LLOQ) was 0.5ng/ml and the Upper Limit of Quantitation (ULOQ) was 80ng/ml. In addition, the stability of Simvastatin in plasma during freeze thaw cycles, extracted samples in the refrigerators and on bench top, in biological matrix at room temperature, stock solution at room temperature were also studied.

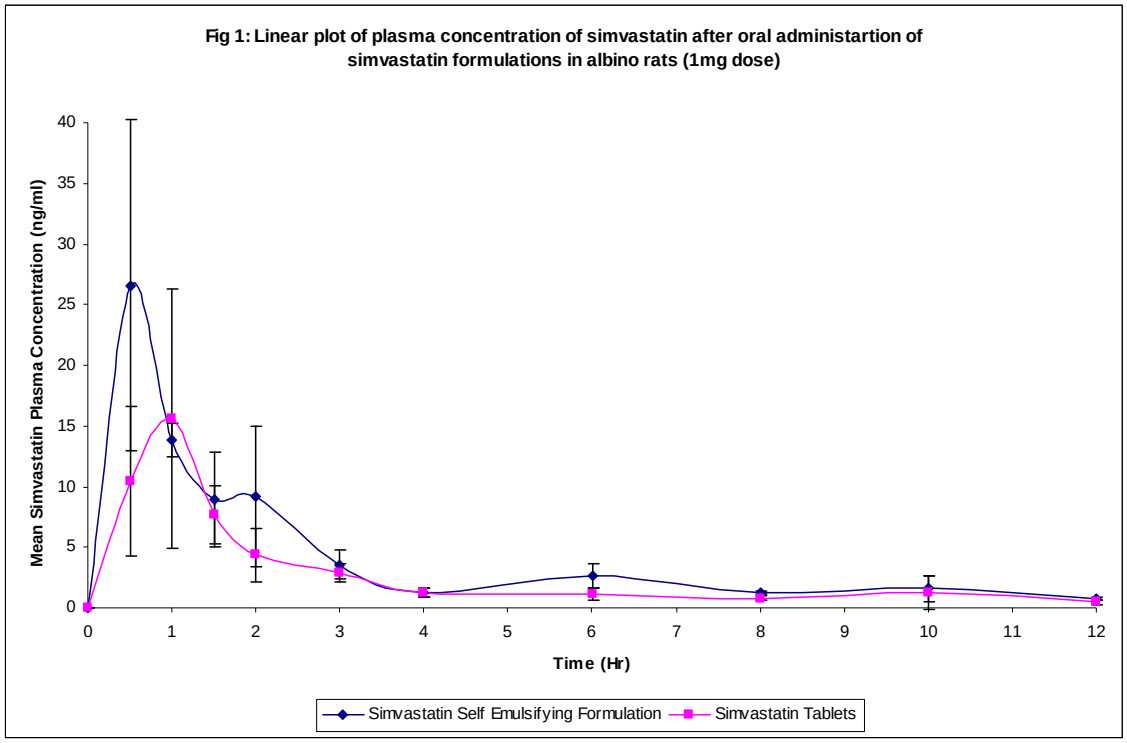
Pharmacokinetic Analysis**:** The pharmacokinetic (PK) parameters were determined from plasma concentration data of Simvastatin by non-compartmental methods. Concentration below LOQ limit (limit of quantitation) was assigned a zero value during estimation of PK parameters. The maximum plasma concentration (Cmax) and the time taken to reach the maximum plasma concentration (Tmax) were taken directly from the observed data. The AUC 0-t, the area under the plasma concentration time curve from 0 hr to the last measurable concentration (Clast) was calculated by a combination of trapezoidal methods. The AUC extrapolated to infinity ($AUC_{0-\infty}$)was calculated by the following equation:

$$AUC_{0-\infty} = AUC_{0-t} + C_{last}\,\lambda/Z$$

Where λZ is the terminal elimination rate constant. The λZ was estimated by performing least square regression analysis using at least 3 points in the terminal log linear phase. The elimination rate constant was estimated from the equation $0.693/\lambda Z$. The relative bioavailability of simvastatin self emulsifying formulation vs the

Table 2: Pharmacokinetic parameters of the maximum plasma concentration (C_{max}) and area under the curve (AUC_{0-t}) for simvastatin following oral administration in albino rats.

Pharmacokinetic parameters	Simvastatin self-emulsifying (Test)	Simvastain tablets (Reference)	T/R Ratio (test/reference)
AUC_{0-t} (ng h/ml)	47.63 ± 11.39	31.58 ± 4.88	1.51
C_{max} (ng/ml)	24.83 ± 12.88	16.84 ± 9.49	1.47
T_{max} (h)	0.58 ± 0.20	0.92 ± 0.38	
AUC_{0-inf} (ng/ml x h)	52.61 ± 10.92	34.33 ± 6.25	
$T_{1/2}$ (h)	3.42 ± 1.37	3.27 ± 1.42	
Kel	0.233±0.116	0.257±.077	



conventional formulation after oral administration was calculated as follows:

F%= AUC sample x Dose oral X 100
AUC oral Dose sample

Statistical Analysis**:** The analysis was performed using Win-Nonlin software (Pharsight Corporation Main, Mountain View, USA). Analysis of variance was performed on the log transformed AUC $_{0-t}$, $AUC_{0-\infty}$ and Cmax using a mixed effect model. All effects were tested at the 0.05 level of significance against the residual error (mean square error) from the ANOVA model.

Results and Discussion**:** The study presented here was conducted in 18 rats. The described LC-MS/MS method to quantify simvastatin in rat plasma was validated and is specific and sensitive for simvastatin. Simvastatin was measurable at the first sampling time (0.5 hr) in test formulation (self-emulsifying) and the standard formulation (tablets). Plasma concentration profile of simvastatin attained after administration of test and reference products are shown in Fig (1). Figure clearly indicates that the mean plasma concentration profile for self emulsifying

formulation of simvastatin showed greater improvement of drug absorption than oral tablet formulation. The pharmacokinetic parameter of simvastatin for the test and reference treatment is shown in Table 2.Peak concentration of 24.83ng/ml and 16.84ng/ml were attained at 0.58 hr and 0.92 hr after administration of test and reference products respectively.

Conclusion:

Based on the above results, the test product Simvastatin 40 mg self emulsifying formulation is found to be super-bioavailable as compared to the reference product, Zocor 40 mg tablets under testing condition in rats.

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