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COMPARATIVE BIOAVAILABILITY EVALUATION OF FLUVASTATIN SODIUM AFTER ORAL AND TRANSDERMAL ADMINISTRATION IN RABBITS

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Abstract:

Membrane-moderated transdermal systems of Fluvastatin Sodium liposomes were prepared by incorporating the drug reservoir within a shallow compartment moulded from a drug-impermeable backing membrane and 2% w/v cellulose acetate rate-controlling membrane . The pharmacokinetic performance of Fluvastatin Sodium following transdermal administration was compared with that of oral administration. This study was carried out in a randomized cross-over design in male New Zealand albino rabbits. The estimation of Fluvastatin Sodiumin plasma was carried out by LC-MS/MS method. The parameters such as maximum plasma concentration (C max), time for peak plasma concentration (t max), mean residence time (MRT) and area under curve (AUC 0 - ∞) were significantly (P< 0.001) differed following transdermal administration compared to oral administration. The relative bioavailability of Fluvastatin Sodium was increased about nine fold after transdermal administration as compared to oral delivery. This may be due to the avoidance of first pass effect of Fluvastatin Sodium. The concentration of Fluvastatin Sodium in plasma was found to be stabilized and maintained in a narrow range over the study period up to 24 hrs for transdermal formulation where as the concentration was decreased rapidly up on oral administration. It was concluded that the relative rate of extensive first pass metabolism was significantly reduced in transdermal administration, resulted in increased relative bioavailability and reduced frequency of administration.

Keywords: *Fluvastatin Sodium, Liposomes, Transdermal systems, LC-MS/MS, In-vivo studies.*

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INTRODUCTION:

The transdermal route of drug delivery has gained great interest of pharmaceutical research, as it circumvents number of problems associated with oral route of drug administration. The barrier nature of skin inhibits the penetration of most drugs. The use of lipid vesicles as delivery system for skin treatment has gained attention in recent years [1]. Liposomes are microscopic or submicroscopic particles and are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids.Liposomes are microscopic vesicles that contain amphipathic phospholipids arranged in one or more concentric bilayers enclosing an equal number of aqueous compartments. The thermodynamically stable, lamellar structures form spontaneously when a lipid is brought into contact with an aqueous phase [2].

The aim of the present study was to develop and evaluate the potential use of liposome vesicles in the transdermal drug delivery for delivery of Fluvastatin Sodium . Fluvastatin Sodium is an effective drug in the treatment of hyperlipidemic patients, Fluvastatin Sodium is a methylated derivative of lovastatin that acts by competitively inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) reductase, the enzyme that catalyzes the rate limiting step in cholesterol biosynthesis. Administration of conventional tablets of Fluvastatin Sodium has been reported to exhibit fluctuations in plasma drug levels, results either in manifestation of side effects or reduction in drug concentration at the receptor sites also is a cholesterol-lowering agent and is structurally similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase. Fluvastatin Sodium lowers hepatic cholesterol synthesis by competitively inhibiting HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthesis pathway via the mevalonic acid pathway [3]. Due to short biological half life (5.3 hours) and low bioavailability (5%) due to extensive first pass metabolism makes it suitable candidate for transdermal drug delivery system. An in vivo evaluation study was conducted to ascertain pharmacokinetic parameters in rabbits after oral and transdermal administration of of Fluvastatin Sodium in rabbits.

MATERIALS AND METHODS:

The *in vivo* study of the optimized formulations were performed as per the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social Justice and Empowerment, Government of India. Prior approval by Institutional animals ethics committee was obtained for conduction of experiments (Ref: IAEC/IX/10/VGOI/ CPCSEA, Dated 21-12-23).

Preparation of Liposomes by thin film hydration technique:

Liposomes were prepared by rotary evaporation-sonication method.[4]The lipid mixture (500mg) consist of phospholipid (Soya Lecithin), edge activator (Tween 80) and drug (10 mg/ml) in different ratios was dissolved in an organic solvent mixture consist of chloroform and methanol (2:1, v/v) then placed in a clean, dry round bottom flask. The organic solvent was carefully evaporated by rotary evaporation (Buchi rotavapor R-3000,Switzerland) under reduced pressure above the lipid transition temperature(at 600c for 1 hr) to form a lipid film on the wall of the flask. The final traces of the solvents were removed by subjecting the flask to vacuum over night. The dried thin lipid film deposited on the wall of the flask was hydrated with a phosphate buffer solution (pH 6.4) by rotation for 1hr at room temperature at 60 rpm. The resulting vesicles were swollen for 2 hrs at room temperature to getlarge multilamellar vesicles . To prepare small Liposome vesicles, the resulting vesicles were sonicated at 100 kHz, 80 Amp for 30 minutes at pulse on 30sec and pulse off 50 sec using a probe sonicator (OrchidScientifics,Nasik).The obtained suspension was passed through a series of 0.45µ and0.22µ polycarbonate filters and then stored at 4°C.

Preparation of rate controlling membrane

Solvent evaporation technique was employed in the present work for the preparation of Cellulose acetate films. The polymer solutions were prepared by dissolving the polymer (2% w/w Cellulose acetate) in 50 ml of Ethyl acetate Methanol (8:2). Dibutyl phthalate at a concentration of 40% w/w of the polymer was used as a plasticizer. 20 ml of the polymer solution was poured in a Petri plate (9.4 cm diameter) placed on a horizontal flat surface. The rate of evaporation was controlled by inverting a funnel over the Petriplate. After 24 hours the dried films were taken out and stored in a desiccators[5].

Preparation of Liposomes loaded gels:

Accurately weighed quantity of 500 mg of Hydroxy propyl methyl cellulose was dispersed in 5 ml of distilled water and was allowed for swelling over night. The swollen carbopol was stirred for 60 minutes at 800 rpm. The previously prepared required Fluvastatin Sodium equivalent Liposomes, methylparaben and propylparaben were incorporated into the polymer dispersion with stirring at 500 rpm

by a magnetic stirrer for 1 hour. The pHof above mixture was adjusted to 7.4 with tri ethanolamine (0.5%). The gel was transferred in to a measuring cylinder and the volume was made up to 10ml with distilled water [6] .

Design of membrane moderated transdermal therapeutic system:

A circular silicon rubber ring with an internal diameter of 2.5 cm and a thickness of 3mm was fixed on to a backing membrane (an imperforated adhesive strip was supplied by Johnson and Johnson Limited ,Mumbai). This serves as a compartment for drug reservoir. Gel equivalent to 40 mg of Fluvastatin Sodiumwas taken into the compartment as a drug reservoir. Cellulose acetate membrane of known thickness was fixed onthe ring with glue to form a membrane moderated therapeutic systems. A double sided adhesive strip was fixed on the rim of the ring above Cellulose acetate membrane[7].

***In Vivo*Evaluation:**

Subject selection: Twelve New Zealand healthy rabbits with a mean age of 10±2 weeks and with a mean body weight of 3±0.2 kg were used in this study. Each group consisted of six rabbits (n=6) each and were subjected for overnight fasting, it was taken care that there was no stress on the animals. Rabbits were randomly divided into two groups for different sampling time and each group was housed in one cage. Food and water were available ad libitum at all times during the experiment. The study was conducted in a crossover design with 2 weeks washout periods in between the two experiments. The animal dose of Fluvastatin Sodium was calculated relevant to human dose by using the following formula[8].

Human dose of Fluvastatin Sodium = 40 mg.

$$\text{Animal dose} = \frac{\text{Human Dose} \times \text{Animal Weight}}{\text{Human Weight}}$$

= 40x3/70= 1.71mg /kg

Blood sampling: About 1 ml of blood samples were collected from the tracheal lobular vein of the rabbit using and the blood was stored in screw top heparinized plastic tubes, the sampling time for blood was done at 0 min (predose), 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 24 hr. The plasma was immediately separated by aspiration after centrifugation at 4000 rpm for 5 minutes and frozen at -20 ºC until analysed by LC-MS/MS method.[9]

Determination of Pharmacokinetic Parameters:

Various pharmacokinetic parameters such as peak plasma concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half-life ($t_{½}$) and mean residence time (MRT) were calculated using the noncompartmental pharmacokinetics data analysis software PK Solutions 2.0™(Summit Research Services, Montrose, CO, USA). [10]

Statistical analysis of the pharmacokinetic parameters:

The pharmacokinetic parameters of the tested formulations were statistically analyzed using paired sample's t-test for normal distributed results of C_{max}, K_a, K_e, MRT and $AUC_{0-\alpha}$ values. All tests were performed at 0.001 level of significance. [10]

RESULTS AND DISCUSSION:

The *in vivo* experiments were conducted as per the protocol and procedure described earlier. The ability of optimized formulation as a drug delivery system to release drugs in a predetermined time release manner was investigated in rabbits after oral administrations was investigated. Bioanalytical methods employed for the quantitative determination of drugs and their metabolites in biological matrix (plasma, urine, saliva, serum etc) play a significant role in evaluation and interpretation of pharmacokinetic data. For the successful conduct of pharmacokinetic study, the development of selective and sensitive bioanalytical methods plays an important role for the quantitative evaluation of drugs and their metabolites (analytes). The LC-MS/MS methods were highly sensitive and suitable for the detection of drug in plasma even in low concentrations. Various pharmacokinetic parameters such as peak plasma concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC), elimination rate constant (K_{el}), biological half-life ($t_{½}$) and mean residence time (MRT) were calculated using the noncompartmental pharmacokinetics data analysis software PK Solutions 2.0™(Summit Research Services, Montrose, CO, USA).

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Calibration curves were constructed from blank sample (plasma sample processed without IS), blank+IS samples and eight point calibration standards for Fluvastatin Sodium in plasma. Plasma concentrations of Fluvastatin Sodium at different times were calculated and are shown in Fig 4&5. Pharmacokinetic parameters such as absorption rate constant, elimination rate constant, half life, AUC,

and MRT were calculated from the plot of time versus plasma concentration and subjected to statistical analysis and the results were shown in Table 2. Plasma Concentration of Fluvastatin Sodium following oral and transdermal administration in rabbits at different times were calculated . The results from the oral administration of Fluvastatin sodium indicated the maximum plasma concentration (C_{max}) 26.7 ± 0.24 ng/ml at 3 hrs (t_{max}) while transdermal administration exhibited the maximum plasma concentration (C_{max}) of 30.3 ± 0.12 ng/ml at 12 hrs (t_{max}). The oral administration of Fluvastatin sodium resulted in a low and quite variable AUC of 98.3± 1.22 ng.hr/ml, where as the transdermal resulted in AUC of 1012.5 ±4.05 ng.hr/ml. The mean residence time of transdermal administration (24.3± 0.15 hrs) was found to be more than oral administration (4.1 ± 0.12 hrs). The results indicated that the parameters significantly differed following transdermal administration, compared to oral administration. The concentration of selected drugs in plasma was found to be stabilized and maintained in a narrow range over the study period up to 24 hrs for transdermal formulation where as the concentration was decreased rapidly up on oral administration. The maximum plasma concentration (C_{max}) was attained at 3 hrs after oral administration and it was observed after 12 h upon application of transdermal formulation of same dose.

Table 1 :Analyte Concentrations of Stock Dilutions of Standard Fluvastatin Sodium Solution with Plasma

S.No	Sample Name	Analyte Concentration (ng/ml)	Analyte peak area	IS Peak Area	Area Ratio	Calculated Concentration (ng/ml)	Accuracy (%)
1	Aqueous mixture	N/A	2247	1940259	0.001160	0.094	N/A
2	Plasma Blank	0	0	0	0	N/A	N/A
3	Blank+ ISTD	0	0	1217732	0	N/A	N/A
4	CC1	0.101	1325	1102974	0.0012	0.098	97.03
5	CC2	0.202	2696	1132350	0.00238	0.197	97.52
6	CC3	0.506	7223	1258131	0.005740	0.481	95.06
7	CC4	2.022	30281	1277746	0.0237	1.995	98.66
8	CC5	10.11	150940	1281233	0.118	9.933	98.25
9	CC6	40.443	584605	1221929	0.478	40.351	99.77
10	CC7	101.107	1494406	1242395	1.2	101.455	100.34
11	CC8	202.213	2956131	1271509	2.32	196.099	96.98

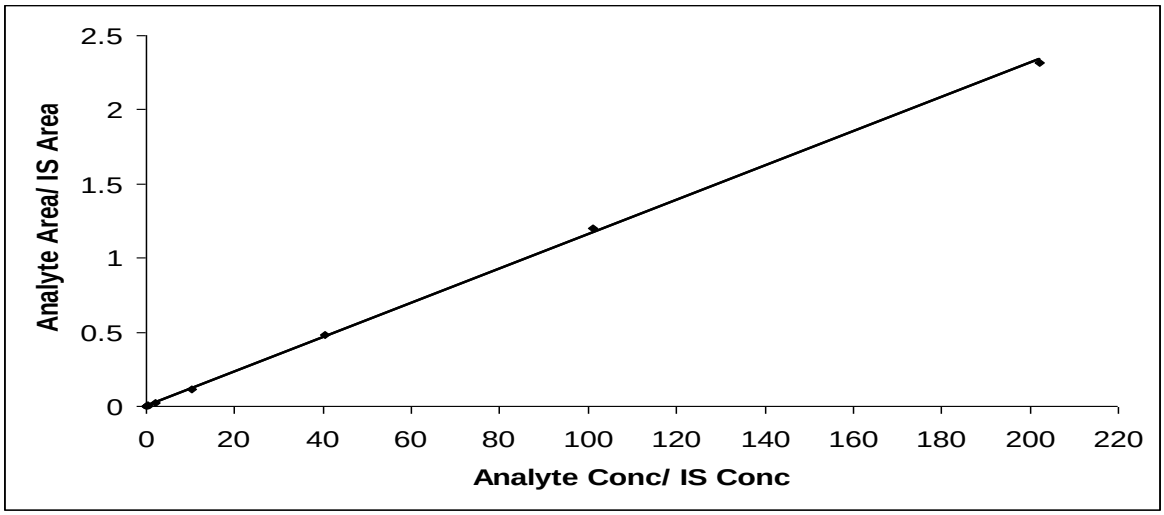


Figure 1 : Calibration Curve for Estimation of Fluvastatin Sodium in Plasma

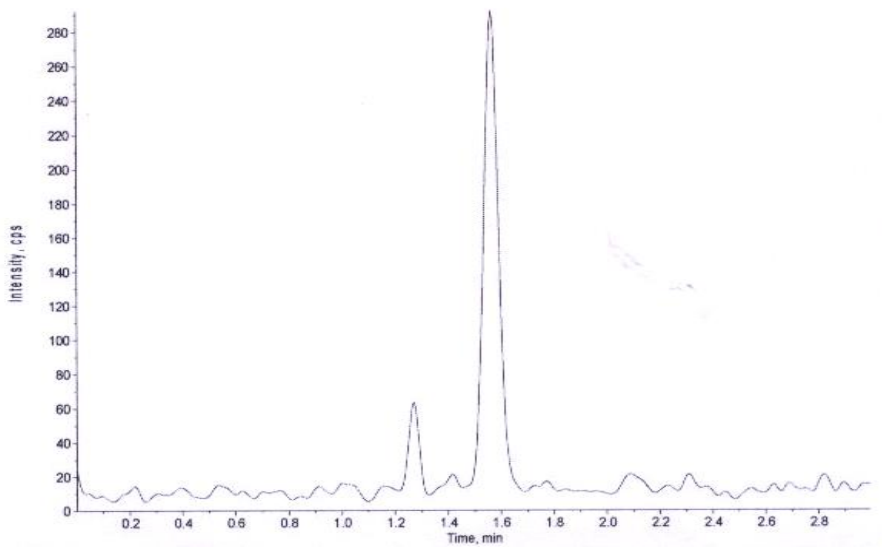
Sample Name: Plasma blank+ISTD Mass(es):415.3/178 amu Concentration: 0.000 ng/ml Calculated conc:N/A ng/ml Proc.Algorithm: Analyst classic Bunching factor: 2 Noise Threshold: 10.00 Area Threshold :100.00 Num Smooths :10 Sep .Width: 0.20 Sep. Height: 0.01 Exp .Peak Ratio: 5.00 Exp.Adj Ratio: 4.00 Exp .Val Ratio :3.00 RT Window :30.0 Expected RT: 2.00 Use Relative RT:False Int. Type: No Peak Retention Time:0.00 Area: 0 Height: 0 Area Ratio: 0.00 Start Time:0.00 End Time:0.00	

Figure 2.Chromatograms of blank Plasma

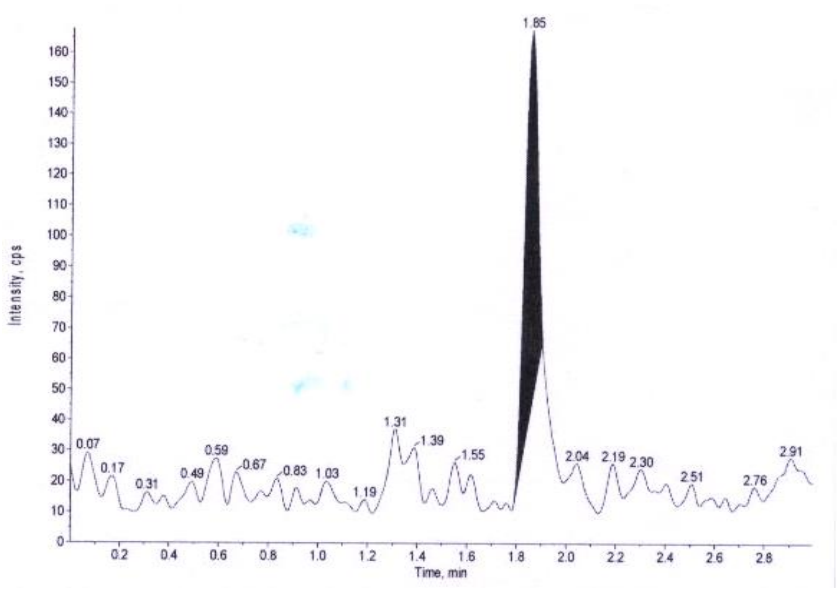
Sample Name: Plasma blank-Fluvastatin sodium Mass(es):455.5/165.1 amu Concentration: N/A ng/ml Calculated conc: N/A ng/ml Proc.Algorithm: Analyst classic Bunching factor:1 Noise Threshold:20.00 Area Threshold :200.00 Num Smooths :10 Sep .Width: 0.20 Sep. Height:0.01 Exp .Peak Ratio: 5.00 Exp.Adj Ratio: 4.00 Exp .Val Ratio :3.00 RT Window : 30.0 Expected RT: 1.80 Use Relative RT:False Int. Type: Base To Base Retention Time:1.85 Area:416 Height:125 Start Time:1.79 End Time:1.90	

Figure 3. Chromatogram of stock solution of Standard Fluvastatin Sodium Solution with Plasma

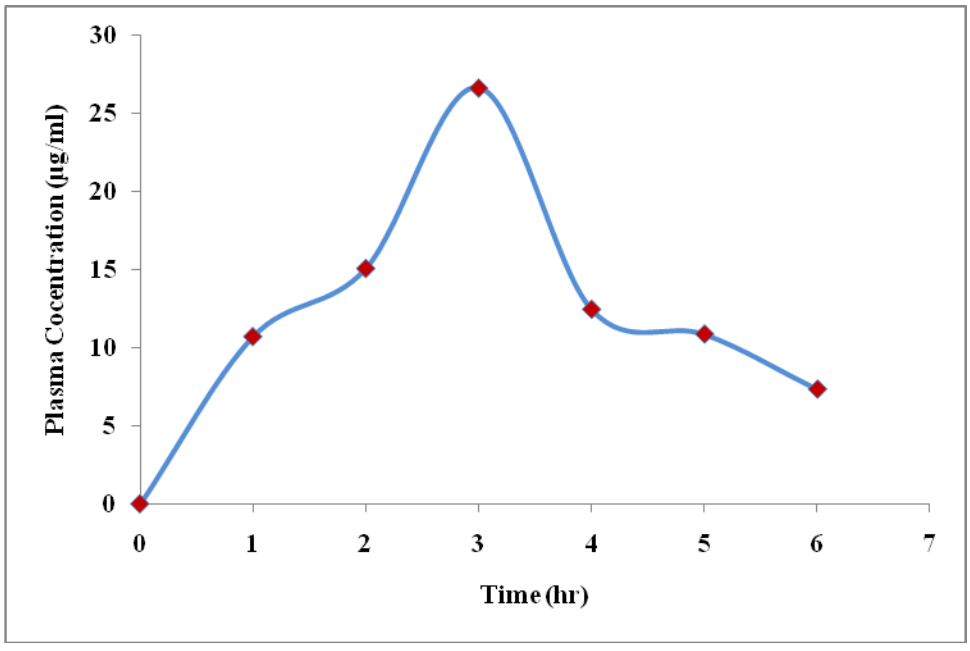


Figure 4: Plasma Concentration-Time Curve of Fluvastatin Sodium following oral administration of oral suspension

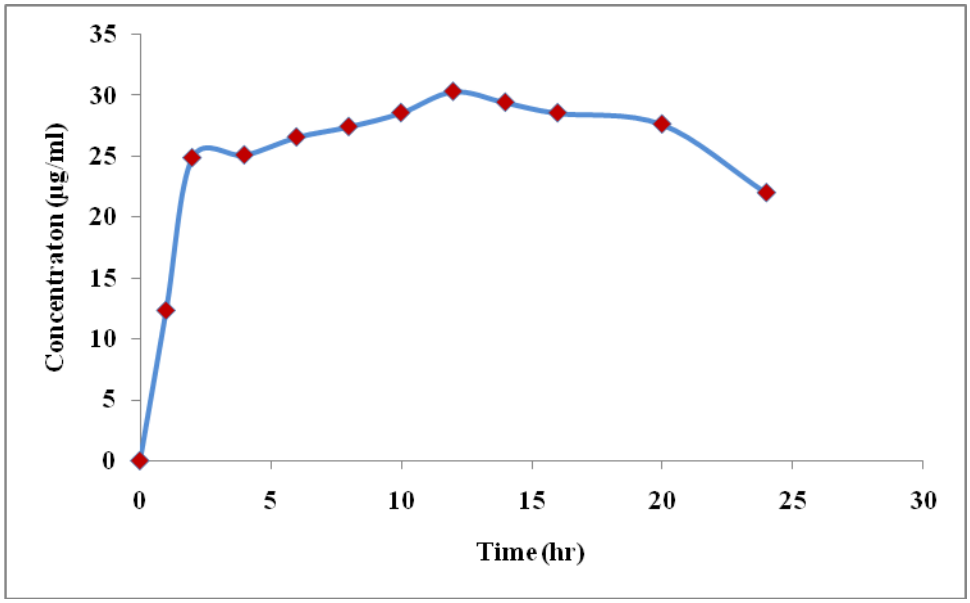


Figure 5: Plasma Concentration-Time Curve of Fluvastatin Sodium following topical administration of optimized transdermal formulation

Table 2: Statistical Treatment of Pharmacokinetic Parameters (Mean ± S.D.) of following oral administration of oral suspension and optimized transdermal formulation of Fluvastatin Sodium

Pharmacokinetic parameter	Oral suspension	Optimized transdermal formulation	Calculated value of 't'
C_{max} (ng/ml)	26.7 ± 0.24	30.3 ± 0.12	13.50***
Tmax (h)	3.0± 0.15	12.0± 0.21	5. 36***
MRT (h)	4.1 ± 0.12	23.6 ± 0.4	23.40***
$t_{1/2}$ (h)	1.76 ± 0.03	12.15 ± 0.06	6. 45***
K_{el} (h^{-1})	0.39 ± 0.08	0.05 ± 0.07	2.67***
K_a (h^{-1})	0.65 ± 0.07	0.21 ± 0.02	14.45***
$AUC_{0-\infty}$ (ng h/ml)	98.3±. 1.22	1012.5.±4.05	137.56***
Null hypothesis (H_o): There is no significant difference between the pharmacokinetic parameters of oral administration of oral suspension and optimized transdermal formulations of Fluvastatin Sodium . Table value of 't' with 10 DF at the 0.001 level is 4.587.			
Result: H_o is not accepted as the calculated 't' value more than the table Value of 't' with 10 DF at 0.001 levels of significance. It was therefore concluded that there was significant difference between the pharmacokinetic parameters of oral administration of oral suspension and optimized transdermal formulations of Fluvastatin Sodium .			

Values are presented in Mean ± SD

CONCLUSION:

The *in vivo* pharmacokinetic studies revealed that the transdermal formulations of Fluvastatin Sodium exhibited controlled release and absorption kinetics over longer periods of time which in turn maintained the desired plasma concentrations over longer periods of time.

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