



EVALUATION OF TOPICAL BIOAVAILABILITY OF CLOTRIMAZOLE USING DERMATO PHARMACO KINETIC METHOD

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ABSTRACT

In this single-dose-one arm, open label two way parallel design, pharmacokinetic study of two marketed formulations of Clotrimazole using 12 healthy Indian male subjects the pharmacokinetic parameters of two marketed Clotrimazole topical formulations were compared. Marketed Clotrimazole topical formulations (A & B) were applied on the pre-marked forearms of the subjects as per the dosing schedule. Treatment sample B was used as a reference sample. Subjects received treatment A & treatment B on both the arms simultaneously, following open label two way parallel design. Skin Stratum Corneum samples were collected in sterile glass test tubes during the study period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, & 6.0 hours post-dose application. The Stratum Corneum samples were analysed for Clotrimazole concentrations only. Pharmacokinetic parameters of Clotrimazole were calculated as C_{max}, t_{max}, AUC (0-t) and AUC (0-∞). Clotrimazole was estimated in Stratum Corneum using a validated Spectroscopic method. If the point estimate of the geometric mean ratio and the confidence intervals for the entire log transformed pharmacokinetic parameters [C_{max}, AUC (0-t) and AUC (0-∞)] were entirely included in the range of 80-125%, then the treatments were claimed to be bio-equivalent.

Keywords: Dermatopharmacokinetic, Stratum Corneum, Methyl Salicylate, Bioequivalence, Skin stripping.

INTRODUCTION

Bioequivalence is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration profiles will be identical without significant statistical differences. Thus in the case of topical formulations the drug has to penetrate through the layers of skin to reach the local site of action which is a complex process only due to the rate limiting barrier of the Stratum corneum. [1] The penetration of a drug through the skin is a complex process typically rate-limited by the stratum corneum (SC). This external layer of the skin is composed of terminally differentiated corneocytes embedded in a complex lipid matrix comprising primarily ceramides, cholesterol, and free fatty acids. Delivery of drug by passive diffusion and the pharmacological effect elicited are dose-related: the better the drug permeates the skin, the greater the therapeutic effect. It follows, therefore, that formulation plays an important role in topical drug delivery as the composition of the vehicle will influence the partitioning and/or the diffusivity of the drug and hence the absolute amount delivered. [1-2] The determination of the Bioequivalence of topical products involves the Dermatopharmacokinetic (DPK) approach. The DPK approach includes any measure of drug concentration in the skin, whether directly or indirectly related to the drug's therapeutic action, which can be determined continuously or intermittently for a period of time. This may include the measurement of either drug concentration in Stratum corneum over time and/or drug concentration in serial biopsy samples. The measurement of the change in the Stratum corneum drug concentration as a function of time is the objective of DPK approach and thus is a valid means of comparing a generic and innovator product for their ability to deliver drug to the deeper layers of the skin. [3] DPK studies offer certain advantages as it is painless, the active drug substances (moieties) are protected from gastric enzymes, it avoids first pass effect, and it is simple to terminate if any adverse or undesired effect is observed. [2-4]

Various Techniques and Methods Practiced in DermatoPharmacokinetic:

There are many in-vitro, in-vivo methods for pharmacokinetic assessment of the dermal products, of which the most important and easy method is in-vivo tape stripping technique, which and some other techniques are as mentioned below:

- ❖ Tape Stripping Technique
- ❖ Microdialysis [2, 4]
- ❖ In Vitro Permeation Assessment [4, 6]
- ❖ Confocal Laser Scanning [4]
- ❖ Cadaver Skin Permeation [5]
- ❖ Vasoconstrictor Assay [5]

Tape Stripping Technique:

The method consists of the standardized protocol of repeated applications and removal of adhesive tape on the skin surface, whereby consecutive layers of Stratum Corneum cells are sampled. As discussed by J Lademann et al; Tape stripping is a standard measuring method for the investigation of the Dermatopharmacokinetic of topically applied substances using adhesive films. These tape strips are successively applied and removed from the skin after application and penetration of topically applied substances; thus, the layers of the corneocytes and certain amount of topically applied substances are removed. The amount of the substances and the amount of Stratum corneum removed with the single tape strip is to be determined for calculation of the penetration profile. The topically applied substances removed from the skin can be thus determined by various analytical methods like HPLC, Mass Spectroscopy and other spectroscopic measurements. [4-5] Clotrimazole or Menthol is a covalent organic compound made synthetically or obtained from peppermint or other mint oils. It is a waxy, crystalline substance, clear or white in color, which is solid at room temperature and melts slightly above. The main form of menthol occurring in nature is (-)-menthol, which is assigned the (1R,2S,5R) configuration. Menthol has local anesthetic and counterirritant qualities, and it is widely used to relieve minor throat irritation. [6]

SUBJECT AND METHOD

Study Subjects:

Sufficient numbers of healthy Indian male human subjects was screened, out of those 09 male subjects were enrolled in the study and 03 male subjects were taken as standby. A total of 12 male subjects were applied with the study medication in the beginning of the study. The screening consent & study consent was taken respectively before drug application. Thereafter, subject's medical records were documented and physical examination was conducted. Inclusion eligibility was also based on successful completion of a clinical health evaluation, which consisted of a personal interview; a complete physical examination (BP, pulse, weight, temperature, and respiratory rate); diagnostic testing that included a 12-lead electrocardiogram and chest radiograph; a laboratory testing that included a complete blood cell count, metabolic and hepatic tests (alanine amino transferase [reference range, 5-55 U/L], aspartate amino transferase [5- 34 U/L]), urine analysis, pregnancy test (for female subjects), blood chemistry for glucose (70-109 mg/dL), blood urea nitrogen (7-23 mg/dL), and creatinine (0.-1.3 mg/dL), as well as serologic tests for hepatitis (B and C), and HIV antibodies. Testing was performed by Central Pathology Laboratory, N-6 CIDCO, Aurangabad, (MS) INDIA 431005. Subjects were excluded if laboratory values were significantly above or below the reference range and/or if all tests had not been performed. In addition, the laboratory data were reviewed by the investigators of the clinical unit prior to the enrollment of the subjects. Subjects were compensated for participation.

Study Design:

This study was carried out as per the ICH (Step 5), 'Guidance for Good Clinical Practices (GCP)' and the principles of Declaration of Helsinki (Scotland, October 2000). The Independent Ethics Committee shall review the protocol and the informed consent form for this study. A single-dose-one arm, open label two way parallel design was used. Subjects were admitted and housed in the clinical facility at least 2 hour before the application of the dose during the study. Informed consent (Appendix 5) for the dosing / sampling procedure was obtained from each subject on admission to the clinical facility. Each of the marketed Clotrimazole formulation was applied on the forearm of the study subjects as per the dosing schedule. Treatment sample B was used as a reference sample [7]. The dosing procedure was as mentioned below:

- ❖ Both the forearms were washed with mild soap and copious amount of water and dried in air.
- ❖ Both the forearms were marked for total of 08 application sites of 1 sq.cm area each.
- ❖ 5 mm length product (semisolid dosage forms) or sufficient amount of drug sample was applied on all the sites so that the product completely and smoothly covers the site area.
- ❖ The stratum corneum samples were collected from the sites on the desired pre decided time.

Stratum Corneum Sampling:

Skin Stratum Corneum samples were collected in sterile glass test tubes during the study period. The samples were collected pre-dose and at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 hours post-dose application. The stratum corneum samples were analysed for Clotrimazole concentrations only. For each subject the total number of blood draws were 02 (01 for screening and another during post study assessment); the total volume of blood withdrawn (10 ml for the pre-study evaluation and 10 ml for the post study) through the vein puncture were not exceed 20 ml.

Procedure:

Study samples were collected as follows. The pre-dose samples were collected within one hour prior to drug application. The post-dose samples were collected within 2 minutes of the scheduled time where the end time of collection to the nearest minute would be recorded.

- ❖ Before sampling the drug remained on the site was removed by mild force using two cotton swabs to ensure the complete removal of residual drug from the site.
- ❖ The pre cut (1 sq. cm) adhesion tape was applied on the site and the mild force was applied to ensure the proper adhesion of the tape on the site area. The tape was removed and discarded.
- ❖ Eight adhesion tape pieces were applied on the site area in the same manner and each tape was removed from the site before the next one is applied. The removal was done using the forceps and the removal should be done by one stroke to ensure the complete removal of stratum corneum.

- ❖ All 8 samples tapes were collected in a single test tube which were then sealed and stored in the refrigerator at -20°C till analysed.

Analytical Method:

Specific and sensitive analytical methods for the quantitative evaluation of drugs are decisive for the successful execution of pre-clinical and clinical trials. To demonstrate the suitability of a bio-analytical method for the intended use and to ensure reliable and reproducible results, validation of methods is required. The HPLC method was developed and validated using Waters Agilent chromatographic system. The linearity of the response of drug was verified from 1 µg/ml to 10 µg/ml concentrations. The calibration graphs were obtained by plotting the response versus the concentration. 1.36 gm of potassium dihydrogen orthophosphate was dissolved in 1000 ml of water. The pH was adjusted to 3.0 (+ 0.05) with orthophosphoric acid and the solution was filtered through 0.45 µ membrane filter. A mixture of buffer (pH 3.0) and Acetonitrile was prepared in the ratio of (30:70) v/v and degassed. A mixture of water and methanol was prepared in the ratio of (30:70) v/v and degassed. Accurately weighed 50 mg of Clotrimazole working standard was transferred into 100 ml volumetric flask. To this about 50 ml of diluent was added and the solution was sonicated for 15 minutes for dissolution. The volume was made up with the diluents. Further 5 ml of this solution was diluted to 50 ml. 5 ml of the resulting solution was again diluted to 50 ml to have concentration of 5 µg/ml. The solution was filtered through 0.45 µ membrane filter or finer porosity membrane filter.

Preparation of sample solution for Clotrimazole CANDID 1 % Cream Formulation:

Accurately weighed 500 mg of Clotrimazole Candid 1 % Cream was taken. Blank skin strips were collected from application sites on the forearm of a human volunteer. These stratum corneum samples were taken in a volumetric flask and the Clotrimazole Cream weighed earlier was added to it. To this about 20 ml of diluent was added. The mixture was shaken well, sonicated for about 15 minutes and the filtered through 0.45 µ membrane filter. The filtrate was taken in a 50 ml separating funnel and acidified by adding 1 ml of 0.1 N HCl. To this solution, 10 ml of cyclohexane was added and the flask was stoppered and shaken vigorously for 10 minutes. The organic layer was separated and dried under vacuum. The dried residue was then dissolved in 50 ml of diluent in a 100 ml volumetric flask, sonicated for 30 min with intermittent shaking and cooled to room temperature. The volume was made-up to 100 ml with diluents. Further, 5 ml of the resulting solution was diluted to 50 ml with diluents and mixed. The solution was centrifuged at 5000 rpm for 10 minutes and then filtered through 0.45 µ membrane filter.

Preparation of sample solution for Clotrimazole Canesten 1 % Cream Formulation:

Accurately weighed 500 mg of Clotrimazole Canesten 1 % Cream was taken. Blank skin strips were collected from application sites on the forearm of a human volunteer. These stratum corneum samples were taken in a volumetric flask and the Clotrimazole Canesten 1 % Cream weighed earlier was added to it. To this about 20 ml of diluent was added. The mixture was shaken well, sonicated for about 15 minutes and the filtered through 0.45 μ membrane filter. The filtrate was taken in a 50 ml separating funnel and acidified by adding 1 ml of 0.1 N HCl. To this solution, 10 ml of cyclohexane was added and the flask was stoppered and shaken vigorously for 10 minutes. The organic layer was separated and dried under vacuum. The dried residue was then dissolved in 50 ml of diluent in a 100 ml volumetric flask, sonicated for 30 min with intermittent shaking and cooled to room temperature. The volume was made-up to 100 ml with diluents. Further, 5 ml of the resulting solution was diluted to 50 ml with diluents and mixed. The solution was centrifuged at 5000 rpm for 10 minutes and then filtered through 0.45 μ membrane filter.

Evaluation of system suitability:

- ❖ The column efficiency determined for the Clotrimazole peak from the standard preparation should not be less than 5000 theoretical plates and tailing factor for the same peak should not be more than 2.0.
- ❖ The percentage relative standard deviation for five replicate injections of standard preparations should not be more than 2.0.

Pharmacokinetic Analysis:

Individual plasma concentration– time curves were constructed; C_{max} and T_{max} were directly obtained from these curves. AUC from time 0 (baseline) to 6 hours (AUC_{0-6}) was calculated using the trapezoidal rule (Chow and Liu, 2000; Chow and Liu, 2007). From the terminal log-decay phase, elimination rate constant (k_e) was estimated using linear regression, and $t_{1/2}$ was estimated using the following equation: $t_{1/2} = \ln 2/k_e$ where \ln was defined as the natural logarithm. Extrapolation of AUC from baseline to infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-6} + (C_6/k_e)$ where C_6 was defined as concentration at 6 hours. To compare the bioavailability of the formulations tested, C_{max} , AUC from baseline to time t (AUC_{0-t}), and $AUC_{0-\infty}$ was carried out for each study. Ratios of C_{max} , AUC_{0-t} , and $AUC_{0-\infty}$ for all formulations were calculated and 90% CIs were obtained. The 90% CIs for the corresponding ratios of C_{max} , t_{max} , AUC_{0-t} , and $AUC_{0-\infty}$ should be within the 80% to 125% range.

RESULTS

Twelve subjects were enrolled in the comparison between two formulations of Clotrimazole (mean age, 25.16 years). The bioequivalence values of the test drug A were C_{max} of 20.107 ± 2.398 $\mu\text{g/ml}$, t_{max} of 1.48 ± 0.261 h, AUC_{0-t} of 84.997 ± 10.455 h. $\mu\text{g/ml}$, $AUC_{0-\infty}$ of 150.756 ± 22.859 h. $\mu\text{g/ml}$; of the test drug B C_{max} of 26.3084 ± 2.216 $\mu\text{g/ml}$, t_{max} of 1.48 ± 0.261 h, AUC_{0-t} of 87.576 ± 11.15 h. $\mu\text{g/ml}$, $AUC_{0-\infty}$ of 141.207 ± 21.553 h. $\mu\text{g/ml}$.

Formulation		C_{max}	T_{max}	AUC_{last}	AUC_{INF_obs}
Candid Cream A	Number of Subjects	12	12	12	12
	Mean	20.10	1.48	84.99	150.76
	SD	2.398	0.261	10.455	22.859
	Min	19.48	1.5	84.32	145.56
	Median	24.39	1.75	100.9	178.33
	Max	26.24	2	118.25	219.47
	CV%	10.1	14.9	10.4	12.8
	Geometric Mean	23.651	1.732	100.009	176.955

Table 1: Statistical Data for Formulation A

Formulation		C_{max}	T_{max}	AUC_{last}	AUC_{INF_obs}
Canesten Cream B	Number of Subjects	12	12	12	12
	Mean	26.30	1.48	87.57	141.20
	SD	2.216	0.261	11.15	21.553
	Min	20.13	1.5	83.58	143.6
	Median	24.46	1.75	103.1	186.69
	Max	26.61	2	118.45	212.43
	CV%	9.2	14.9	11.1	12
	Geometric Mean	23.986	1.732	100.011	178.642

Table 2: Statistical Data for Formulation B

Dependent	Test	FormRef	RefGeoLSM	TestGeoLSM	Ratio[%Ref]	CI_90_Lower	CI_90_Upper
Ln(AUCINF_obs)	A	B	178.64	176.96	99.06	80.04	102.05
Ln(AUClast)	A	B	100.01	100.01	100	84.87	98.06
Ln(C _{max})	A	B	23.99	23.65	98.60	84.15	96.16

Table 3: Pharmacokinetic Comparison between the formulations A and B

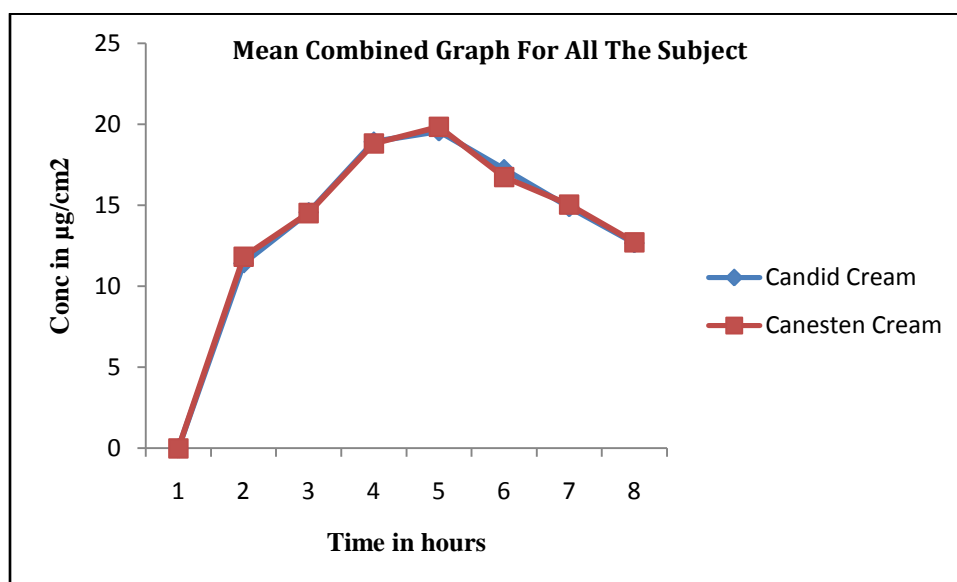


Figure 1: Combined Mean Graph for all the Subject

Pharmacokinetic parameters:

Mean and SD values of t_{max} , C_{max} , AUC_{0-T} , and $AUC_{0-\infty}$ for each formulation are shown in Table 1-3 and depicted in Figure 1.

Table 3 shows the 90% CIs of the ratios (two formulations) for the transformed values of C_{max} (as an index of rate of absorption), AUC_{0-t} , and $AUC_{0-\infty}$ (as an index of the extent of absorption); The 90% CIs for the corresponding ratios of C_{max} , AUC_{0-6} , and $AUC_{0-\infty}$ were within the 80% to 125% range. T_{max} values were obtained, but there were not compared in the bioequivalence analysis because this parameter is not considered as a bioequivalence criteria.

DISCUSSION

The results of our study suggest that the treatment A and B formulations of diclofenac were not statistically different in terms of their PK parameters (C_{max} and AUC). Considering that all 90% CIs of the ratios of the PK parameters (C_{max} and AUC) were found to be within the predetermined range (80% -125%). Based on the above observations, this study suggests that the formulations of treatment A and B are bioequivalent. No moderate or serious AEs were reported by the investigators. Potential recall bias of AEs in this study was not likely because only one dose of each formulation was administered during each treatment; subjects were under medical surveillance in the clinical unit.

CONCLUSION

This study has demonstration that all the pharmacokinetic parameters calculated for test formulations A were close to those of the reference formulation B and there were no statistically significant difference between the two formulations. Test formulation A and Reference formulations B were bioequivalent with respect to the rate and extent of Diclofenac absorption, which was expressed by similar values for C_{max}, AUC_{0-t} and AUC_{0-∞} which clearly indicated that these values were within the acceptable bioequivalence limits of 80-125%. Thus, it can be assumed that the two formulations were therapeutically equivalent and interchangeable in clinical practice. Both the formulations were generally well tolerated. In summary, as the measurement of the change in the Stratum Corneum drug concentration as a function of time is the objective of DPK approach and thus from the above results it is clear that DPK is a valid means of comparing a test and reference product for their ability to deliver drug to the deeper layers of the skin.

REFERENCES

1. Center for Drug Evaluation and Research, Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations. United States Food and Drug Administration 2003.
2. Benfeld E, Hansen SH, Volund A, Menne T, Shah VP. Bioequivalence of Topical Formulations in Humans: Evaluation By Dermal Microdialysis Sampling and the Dermatopharmacokinetic Method. *Journal of Investigative Dermatology* 2007; 127: 170-178.
3. Shah VP, Glynn GL, Yacobi Y. Bioequivalence of Topical Dermatological Dosage Forms—Method of Evaluation of Bioequivalence. *Pharmaceutical Research* 1998; 15(2): 167-171.
4. Shah VP. Progress in Methodologies for Evaluating Bioequivalence of Topical Formulations. *American Journal of Clinical Dermatology* 2001; 2(5): 280.

5. Thomas J, Franz MD. Assessing The Validity Of Stratum Corneum Tape Stripping To Determine The Bioavailability / Bioequivalence Of Topical Drug Products. A letter to Roger Williams, FDA-CDER 1999.
6. Herkenne C, Naik A, Kalia YN, Hadgraft J, Guy RH. Ibuprofen Transport into and through skin from topical formulations: In vitro- In vivo Comparison. Journal of Investigative Dermatology 2007; 127: 135-142.
7. Sanna V, Peana AT, Moretti MDL. Effect of Vehicle on Clotrimazole Permeation from New Topical Formulations: In Vitro and In Vivo Studies. Current Drug Delivery 2009; 6: 93-100.