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Bioequivalence of Two Topical Formulations of Dapsone Gel 5% in Healthy Volunteers

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Biography:

Tausif Ahmed is currently working as Head-Bioanalytical,& Biopharmaceutics in the Global Clinical Management group, IPDO at Dr. Reddy's Laboratories Limited (DRL), Hyderabad. He is responsible for managing the Bioanalysis of all Bio studies and preclinical studies supporting global complex generic products at DRL. He is also involved in PK/Modeling and Simulation activities supporting global generic development. Prior to joining DRL, he was Associate Director and Head-DMPK (preclin discovery, Clinical dev. and Generic) & Dy. Test Facility Mgt. GLP toxicology dept. at Piramal Enterprises Limited, Mumbai. He has been associated with different pharmaceutical companies such as Dr. Reddy's Research Foundation (DRF), Ranbaxy Research Laboratories, Sai Life Sciences Limited and Piramal Enterprises Limited in past. He obtained MS in Pharmaceutics from NIPER and Ph.D. in Pharmaceutical Medicine (specialization: Biopharmaceutics and PK/PD) from Hamdard University (Ranbaxy Sponsored). He has been working in the field of drug discovery, development and generic BA-BE studies for more than 16 years. His area of specialization includes DMPK, metabolite-ID, population PK, PK-PD modelling and simulation, generic BA-BE studies and GLP bioanalysis. He also has experience in designing and conducting generic BA/BE studies, facing regulatory audits (GLP certification at Ranbaxy and Piramal), Phase I & Phase II studies. He has extensive experience in outsourcing preclinical and clinical studies to CROs both in India and outside. Dr. Tausif has contributed to > 12 IND filings, ~300 ANDAs and multiple Phase II/III regulatory submissions, nationally and globally. He has co-authored 2 book chapters and over 40 papers and presentations. He is a reviewer for many international journals and is on the Editorial board of Int. J. Pharma Research. He is a guest faculty at Hamdard University, NMIMS (Mumbai), NIPER and various other universities in India. He has also supervised many Master's and Ph.D. students.

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Abstract

Introduction/Background: Dapsone (Aczone®) gel 5% was approved in the United States for the topical treatment of acne vulgaris. A generic formulation is being developed by Dr Reddy's and the objective of the study was to evaluate the bioequivalence (BE) of the test product, relative to the reference product- dapsone gel 5% (RLD) in healthy human subjects under fasting conditions to meet the regulatory requirements.

Methods: An open-label, randomized, single-dose, two-sequence, four-period fully replicated crossover study separated by a washout period of 18 days. 48 subjects were randomized to receive either of the two treatment arms (test or RLD). The plasma samples were analyzed for dapsone using a validated LC-MS/MS method. Based on the estimated within-subject standard deviation of the RLD (S_{WR}) for ln-transformed pharmacokinetic (PK) parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of dapsone, the BE of test formulation with respect to RLD was determined either using average BE (ABE) or scaled average BE (SABE) criteria.

Results: The statistical analysis results demonstrated that $S_{WR} \geq 0.294$ for C_{max}, hence BE evaluated using SABE approach. The 95% upper confidence bound for $(\mu T-\mu R)^2-\theta*S^2_{WR}$ was ≤ 0, where μT and μR were least square mean of the ln-transformed PK parameters for test and reference. The T/R ratio was within the acceptance criteria of 80 to 125%. For AUCs, the $S_{WR} < 0.294$, hence BE was evaluated using ABE approach. The 90% confidence intervals (CIs) of ln-transformed data of AUC_{0-t} and $AUC_{0-\infty}$ were within regulatory acceptance limit.

Conclusion: The test product was bioequivalent to the RLD in terms of both rate and extent of absorption.

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INTRODUCTION TO CORONAVIRUS AND GENOMIC FEATURES:

Acne vulgaris is primarily seen in adolescents, involving the pilosebaceous unit. Increased sebum production, alteration in the quality of sebum lipids along with follicular hyperkeratinization and proliferation of bacteria *Propionibacterium acne* contributes to the pathogenesis of acne vulgaris[1]. The approach to treat acne vulgaris depends on its presentation and severity. Acne severity can generally be categorized into mild, moderate, or severe stages. Mild acne vulgaris is primarily characterized by non-inflammatory lesions whereas patients presenting with inflammatory lesions are generally classified as having moderate or severe acne. Various topical and systemic drugs are available for treatment of acne. Management of mild to moderate acne involves the use of topical agents such as benzoyl peroxide, topical retinoids and antibiotics as single agents or in combination. On the other hand, treatment of severe acne vulgaris involves the use of systemic antibiotics and topical agents in combination[2, 3].

Dapsone is a synthetic sulfone derivative and highly effective in the treatment of severe acne with prominent effect in inflammatory lesions. The proposed mechanism of action of dapsone is thought to be due to combined effect of anti-inflammatory and anti-microbial properties. Anti-inflammatory actions include scavenging reactive oxygen species (ROS), inhibition of enzyme activity i.e. neutrophil myeloperoxidase, eosinophil peroxidase, suppression of activity of neutrophils, inhibition of chemoattractant induced signal transduction pathways. Antimicrobial activity is due to inhibition of bacterial dihydropterase synthase enzyme in the pathway of folic acid metabolism[4].

The oral dose of dapsone for severe acne ranges from 25 mg/day to 300 mg/week. This dose accounts for the development of dose dependent hemolysis and methemoglobinemia particularly in individuals with glucose-6 phosphate-dehydrogenase (G6PD) deficiency. These unwanted adverse events results from increased oxidative stress on erythrocyte due to metabolite, dapsone hydroxylase[5].

To minimize the hematological adverse events associated with oral dapsone, topical formulation of dapsone 5% gel has been developed to act efficaciously on the more affected areas and to reduce the systemic side effects. In 2015, Dapsone 5% gel (Aczone® gel 5%) was approved for the treatment of severe acne vulgaris by United States Food and Drug Administration (USFDA) based on two randomized controlled trials[6, 7]. FDA's nonbinding recommendations, draft guidance on dapsone provides the following two options for a generic manufacturer to obtain marketing approval in the US market. i) a combination of *in vitro* and *in vivo* studies with pharmacokinetic endpoints, or ii) an *in vivo* study with clinical endpoints[8]. A generic version of dapsone gel 5% was developed by Dr. Reddy's Laboratories Ltd. To the best of our knowledge there is no published data on comparative bioavailability in healthy subjects for dapsone gel 5%. Further, pharmacokinetic data for dapsone gel 7.5% and dapsone 100 mg oral tablets in healthy volunteers only is available in literature and there is a paucity of pharmacokinetic data following topical application of dapsone gel 5%. Hence, a PK study was conducted to compare the relative bioavailability of test and reference formulations of dapsone gel 5% in normal healthy adult human volunteers under fasting conditions for the purpose of obtaining marketing approval from USFDA. This could be the first such publication on detailed pharmacokinetics of dapsone gel 5%.

MATERIALS AND METHODS

In vitro experiments (*In vitro* drug release test (IVRT) and *in vitro* permeation test (IVPT)):

A pivotal *in vitro* drug release test was carried out according to the SUPAC guidelines, using Franz diffusion cells to assess the release profiles of dapsone across a synthetic membrane from test and reference formulation of dapsone gel 5%. Comparison of the *in vitro* release of dapsone from the blinded formulations (representing reference formulation, Aczone and test formulation 5% dapsone gel, respectively) and was performed using a validated methodology based on the principles of the FDA's SUPAC-SS guidelines and the FDA draft guidance for acyclovir and dapsone[9]. An appropriately developed and validated *in vitro* model (vertical diffusion cells) was used for performing *in vitro* skin permeation experiments. For the pivotal IVPT study, the formulations were blinded (labelled A or B, formulation A (ACZONE® (dapsone) Gel, 5%) and formulation B (Dr. Reddy's test product)) and the dosing randomisation was performed per the FDA draft guidance on dapsone gel. The cumulative amount of dapsone delivered to the receptor solution for each formulation was determined for three skin donors with n=4 per skin donor. For each formulation, a total of 4 repetitions per formulation per skin donor (total of 3 skin donors) were performed (for a total of n=12 replicates). The flux over each sampling point (e.g. 0-2 h, 2-4 h, etc.) was calculated for each formulation.

In vivo bioequivalence (BE) study: Study design and participants:

This was an open-label, randomized, two-treatment, two-sequence, four-period, single-dose, fully replicated crossover bioequivalence study conducted under fasting conditions. Subjects were enrolled in the study, if they were healthy males aged between 18 to 45 years (both inclusive); had body mass index (BMI) between 18.5 to 30.0 kg/m^2; did not have any significant diseases or clinically significant abnormal findings in medical history, physical examination, laboratory evaluations, electrocardiogram (ECG) and chest X-ray recordings at screening, non-smokers and non-alcoholic.

Subjects were excluded from study if they had known history of hypersensitivity to dapsone or related class of drugs, subjects with G6PD deficiency and/or with methemoglobinemia, use of sunscreens, cosmetics, lotions, moisturizers, or other topical medications within 24 hours before initial drug application, donation of blood or participation in a clinical study within 90 days prior to the first dosing. Subjects who met all the inclusion criteria and none of the exclusion criteria were dosed in the study. A sample size of 48 subjects (including dropouts) was determined to be sufficient to establish bioequivalence between the dapsone formulations under fasting conditions with adequate power considering the following estimates: test/reference ratio ~ 90% to 111.1%, intra-subject %CV ~ 30% for C_{max}, power ≥ 80%, significance level (α): 5% and bioequivalence limits 80 % - 125%.

Test product used in the present study was dapsone gel 5% of Dr.

Reddy's Laboratories Ltd,, batch number: EF16005, expiry date: April 2018 and the reference product was Aczone® (dapsone) gel 5% of Allergan Inc, USA, lot number: 95315, expiry date: March 2019). Same batch numbers of test and reference were tested in the IVRT, IVPT and human BE study.

Drug Administration and Study Restrictions:

All the eligible subjects were randomized to either of the two sequences (RTRT and TRTR). After an overnight fast of at least 10 hours, a single dose (2 gm dose equivalent to 100 mg dapsone) of either test or reference product, as per the randomization schedule, was applied on the subject's upper back and spread across a pre-determined marked surface of 900 cm^2 to have a thin film using a stainless steel spatula to all the subjects in sitting position. Subjects received alternate treatment in subsequent periods and the same procedure was followed. A washout period of 18 days was maintained between the subsequent periods. This interval of 18 days was chosen based on the available literature[10]. Subjects were instructed to avoid any strenuous activity throughout their housing period. Standard meals were served at appropriate intervals during the study periods. It was ensured that study drug was not applied to open wounds or any skin condition such as cuts, scars, scratches, abrasions, moles, uneven skin texture, etc., All the subjects were requested to take shower at 1 hour pre-dose and allowed to wash the application site gently with non-medicated soap or cleanser to clean away any oil, grease, or bacteria, during shower. Subjects were not allowed to take shower or bathe for at least 18 hours after study drug administration. Further subjects were instructed not to cover the application area with clothing for at least 4 hours after drug application. Subjects were instructed to abstain from consuming any alcoholic products, food and drugs containing xanthine, grapefruit containing food and beverages for 48 hours prior to drug administration and throughout their stay in the clinical facility. Urine scan for drugs of abuse and breath alcohol test was carried out prior to check in of each period.

Blood Sampling and Analysis:

A total of 18 blood samples (5 ml each) were collected from each subject in each period. The venous blood samples were collected at 0 (pre-dose), 4, 8, 12, 18, 24, 30, 36, 42, 48, 72, 84, 96, 108, 120, 144, 168, and 192 hours from the start of drug application. After collection, all the collected blood samples were kept in ice water bath until centrifugation. The samples were centrifuged at 3500 rpm for 10 min at 5°C±3°C to separate plasma. The resultant plasma samples were stored at -70°C±10°C until analysis.

Bioanalytical Method:

Bioanalytical method was validated as per USFDA guidelines[11]. Solid phase method was used for extraction. The linearity range, 50.162 pg/ml to 24980.894 pg/ml was found adequate to quantify the expected concentration range of drug from subject's plasma with the proposed dose of dapsone gel 5%. Quality control (QC) samples at 4 levels were used during routine sample analysis. The precision and accuracy of QC samples during analysis of samples ranged from 2.32% to 3.56% and 95.97% to 101.14%, respectively. The precision and accuracy for calibration curve standards and quality control samples met the acceptance criteria as per USFDA guidelines[11].

The plasma concentrations of dapsone were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) bioanalytical method. The analysts involved in sample analysis were kept blinded of the sequence of administration of the test and reference products during the entire study period. The method used a Shimadzu HPLC system, equipped with a quaternary pump, degasser, autosampler, and thermostatted column compartment. The compounds were analyzed on a Chromolith Performance RP-18e (Merck KGaA, Germany) chromatographic column (4.6 × 100 mm) maintained at ambient temperature (40°C). The mobile phase, consisting of acetonitrile and 2 mM ammonium acetate (80:20 v/v), was pumped at a flow rate of 0.6 ml/min. Dapsone D8 was used as the internal standard (IS). Mass detection was performed on an API 5500 Triple Quad instrument (Applied Biosystems MDS SCIEX, Toronto, Canada) using a turbo electrospray interface in positive ionization mode. All the study related activities like study drug dispensing, dosing, blood sample collection, sample handling, processing and bioanalysis were carried out under sodium vapour lamp.

Pharmacokinetic Analysis:

The pharmacokinetic parameters (C_{max}, $AUC_{0-t,}$ $AUC_{0-\infty,}$ $T_{max,}$ Residual area, K_{el} and $T_{½}$) were calculated individually for each analyzed subject from the plasma concentration-time profile using non-compartmental model by using statistical package SAS® 9.2 or higher version for dapsone. Subjects who completed at least two periods and received reference products twice were considered for calculation of S_{WR}. Subjects who completed all the periods or at least one test and one reference periods, were included in the analysis of average bioequivalence.

Statistical Analysis:

IVRT data

The release rate of dapsone from the formulation(s) tested was calculated as slope of the linear portion of the profile (cumulative amount of drug released per cm^2 against square root of time (t), as recommend per SUPAC guidelines)[9].

In vivo bioequivalence study

Statistical estimation was performed for the log transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ to calculate the averages μT and μR of test and reference product respectively and to ascertain within-subject SD by using the data of reference product (S_{WR}) administered twice in different periods.

Based on the within-subject SD for the pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the reference product as (S_{WR}), the assessment of bioequivalence for that parameters of test product to the reference product was determined using ABE or SABE approach[12]. Accordingly, analysis of variance (ANOVA) was performed at the α level of 0.05 using PROC MIXED procedure and the 90% CI on the geometric mean Test-to-Reference ratio calculated for dapsone. For any log-transformed parameter where the $S_{WR} \geq 0.294$, the SABE method was used. Accordingly, 95% Upper confidence bound for $(\mu T-\mu R)^2-(\theta*S^2_{WR})$ calculated for dapsone, where: μT and μR were least square mean of the ln-transformed pharmacokinetic parameters for the test and reference product. The upper 95% confidence bound on the linearized

SABE statistic was calculated for dapsone. SABE was concluded for the test product to the reference product if both these criteria were satisfied for ln-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for dapsone. i) The point estimate (test/reference geometric mean ratio) falls within the interval 80 -125 % and ii) The 95% upper confidence bound for $(\mu T-\mu R)^2$- $(\theta*S^2_{WR}) \leq 0$, where $\theta= (\ln(1.25)^2/(S^2_{w0})$, and $S_{w0} = 0.25$ (regulatory limit)[8].

If $S_{WR} < 0.294$ for any of the log transformed primary pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$), then bioequivalence assessment was determined using the conventional ABE method. The 90% CI of the relative mean C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the test to reference formulation for Ln-transformed data should be within 80 % to 125% for dapsone to establish bioequivalence[8].

Safety Assessment:

Safety was assessed throughout the study. Assessments were performed through medical history, physical examination, vital signs assessment, 12-lead ECG, X-ray (postero-anterior view) recording, clinical laboratory parameters (e.g. haemogram, biochemistry, serology, Glucose-6-phosphate dehydrogenase (G6PD), Methemoglobin and urine analysis) at the time of screening and monitoring of adverse events, vitals measurement, and subjective symptomology during the study.

RESULTS

***In vitro* Release Test (IVRT):**

Fig. 1 depicts the cumulative amount of dapsone released per unit area of cellulose membrane from tested formulations (Dapsone Gel A and Dapsone Gel B representing Aczone and test formulation 5% dapsone gel, respectively) against the square root of time, along with the steady state release calculated between 1.5 and 4 h. As demonstrated in **Table 1**, sameness in steady state release was observed between test and reference formulation according to the SUPAC statistical analysis. The 90 % confidence interval is determined according to the SUPAC guidelines, where limits ranging between 75 and 133.33 % demonstrate sameness between test and reference formulation.

Figure 1: Mean cumulative amount of dapsone (ng/cm²) delivered to receptor solution 48 h post-application of formulations A and B. Data points represent the cumulative amount of dapsone from 4 replicates per skin donor, 3 skin donors, (n=12). Error bars represent one standard error of the mean.

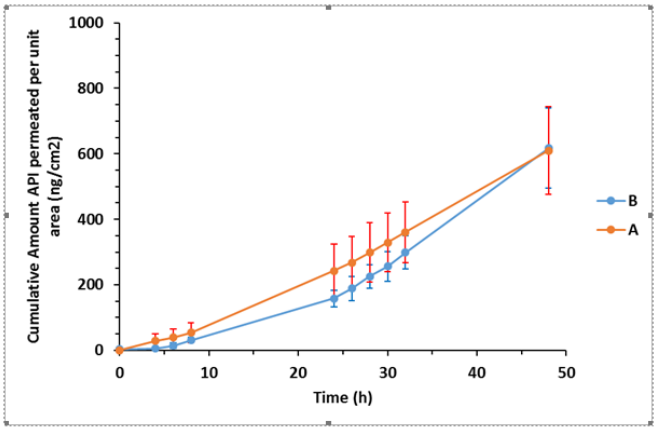

TABLE 1: Statistical analysis of the steady state release slopes comparing the in vitro release profile (from T=1.5 to 4 h) of Dapsone from the blinded test formulations.

Reference formulation	Test formulation	90% confidence interval	Result
Dapsone Gel A (Aczone gel 5%)	Dapsone Gel B (Test formulation 5% dapsone gel)	87.83 - 104.53%	Sameness
Dapsone Gel B (Test formulation 5% dapsone gel)	Dapsone Gel A (Aczone gel 5%)	95.67 - 113.85%	Sameness

Note: The 90% confidence interval is determined according to the SUPAC guidelines where limits ranging between 75 and 113.33% demonstrate sameness between conditions.

***In vitro* Permeation Test (IVPT):**

A pivotal IVPT permeation study on ACZONE® (dapsone) Gel, 5% and 5% dapsone gel of Dr. Reddy's was performed. The cumulative amount of dapsone delivered to the receptor solution for each formulation was determined for three skin donors with n=4 per skin donor. The mean cumulative amounts of dapsone permeated per unit area at 48 h post-application of formulations A and B were 609 ng/cm² and 617 ng/cm², respectively. The cumulative amount of dapsone that permeated into the receptor solution over 48 h is shown in Fig. 1 and the flux is shown in **Fig. 2 (Table 2).**

The flux over each sampling point (e.g. 0-2 h, 2-4 h, etc.) was calculated for each formulation. The flux for both formulations appears to follow similar trends over the 48 h time course. There is a plateau in the flux for both formulations at 32 h until the completion of the experiment; therefore, the maximum flux (C_{max}) could not be accurately determined during the 48 h time frame.

***In vivo* Bioequivalence Study:**

Subjects Demographics

Forty-eight healthy male subjects were enrolled in this study. The mean (±SD) age of subjects was 29.4 (±6.31) years, body weight was 67.73 (±9.49) kg and body mass index (BMI) was 24.07 (±2.75) kg/m².

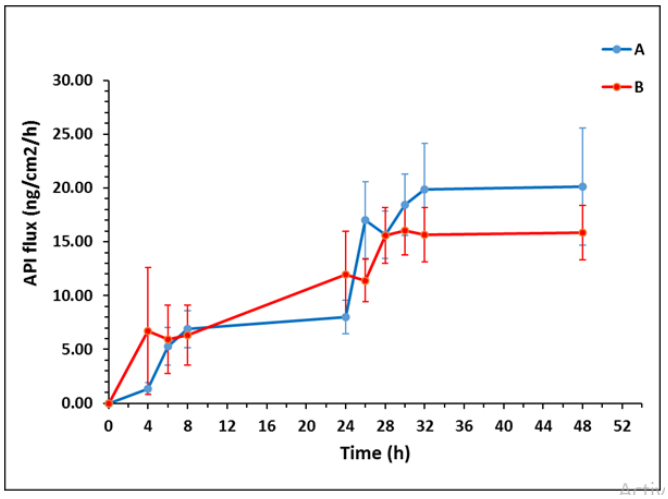

Figure 2: Mean flux of dapsone (ng/cm²/h) calculated for each formulation. Data points represent the flux of dapsone from 4 replicates per donor, 3 donors (n=12). Error bars one standard error of the mean.

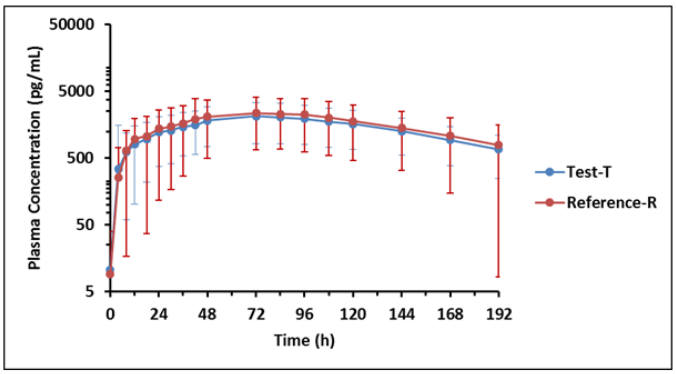

Figure 3: Mean (SD) plasma concentration versus time profile of dapsone following single topical dose administration of reference R and test T formulations.

TABLE 2: Mean cumulative amount of Dapsone (ng/cm2) delivered to the receptor solution 48 h post application of formulations A and B.

Formulation	N	Mean, Cumulative Amount API Permeated per unit area (ng/cm²)	Standard Error, Cumulative Amount API Permeated per unit area (ng/cm²)
A	12	609.86	133.45
B	12	617.5	121.5

Note: For the formulations, there were 4 replicates per skin donor, 3 skin donors (n=12) and 1 replicate per skin donor, 3 skin donors, (N=0) for the blank

TABLE 3: Descriptive statistics of formulation means for Dapsone.

Formulations	Test Product T		Reference Product R	
PK Parameters	**N**	**Mean ± SD (%CV)**	**N**	**Mean ± SD (%CV)**
C_{max} (pg/ml)	79	2463.545 ± 1610.330 (65.366)	76	2996.523 ± 2297.532 (76.673)
AUC_{0-t} (pg*h/ml)	79	271725.237 ± 148883.342 (54.792)	76	307539.472 ± 213692.079 (69.484)
AUC_{0-inf} (pg*h/ml)	76	336015.921 ± 182326.271 (54.261)	74	382487.346 ± 331394.513 (86.642)
$^{\#}T_{max}$ (h)	79	72.650 (4.000 - 145.020)	76	72.525 (4.000 - 168.000)
K_{el} (h^{-1})	76	0.014 ± 0.006 (42.116)	74	0.014 ± 0.005 (39.212)
$t_{1/2}$ (h)	76	60.947 ± 29.764 (48.836)	74	58.974 ± 27.355 (46.385)
Residual Area %	76	18.151 ± 10.820 (59.614)	74	17.294 ± 10.588 (61.223)
AUC_{0-t}/ AUC_{0-inf} Ratio	76	81.849 ± 10.820 (13.220)	74	82.706 ± 10.588 (12.802)

Note: SD, Standard deviation; CV, coefficient of variation

TABLE 4: Summary statistics of pharmacokinetic parameters of Dapsone after a single topical dose administration of Test T and Reference R formulations (N=33)

Parameter	Reference Sigma S_{WR} (%)	(T/R) Ratio (%)	95% Upper Confidence bound
Ln C_{max}	0.5069	92.9944	- 0.1380
Ln AUC_{0-t}	0.2728	Not applicable	
Ln $AUC_{0-\infty}$	0.2511	Not applicable	

TABLE 5: Summary statistics of pharmacokinetic parameters of Dapsone after a single topical dose administration of Test T and Reference R formulations

Pharmacokinetic Parameter (units)	Log-transformed geometric least square means		(T/R) Ratio (%)	90% Confidence Intervals	Intra-subject CV (%)
	Test T*	Reference R#			
AUC_{0-t} (ng.h/ml)	294466.518	294571.313	99.96	93.16 – 107.27	25.19
$AUC_{0-\infty}$ (ng.h/ml)	232810.517	239634.2329	97.15	90.47 – 104.33	26.51

*N=79 for Test T; #N=74 for Reference R

Pharmacokinetics and bioequivalence assessment

Pharmacokinetic analysis was performed on all subjects included in the pharmacokinetics data set. The pharmacokinetic parameters were derived individually for each analyzed subject from the plasma concentration vs. time profiles. Actual sampling time points were used for the estimation of pharmacokinetic parameters. Among 48 enrolled subjects, the data of 33 subjects who completed at least two periods successfully and who received reference product twice were considered for calculation of within in subject reference variability (S_{WR}). The data of 31 subjects who completed all the study periods successfully were considered for calculations of 95% upper bound. The data of 44 subjects who completed at least two periods successfully with one test and one reference product were considered for ABE approach. Descriptive statistics of pharmacokinetic parameters for dapsone were calculated and reported for the test and reference formulation in Table 3. The pharmacokinetic parameters calculated for test formulation were similar to those of the reference formulation. The mean (±SD) plasma concentration versus time profile for dapsone after single dose administration of test and reference formulations are presented in Fig. 3. The curves were superimposed on each other.

Outlier test was performed on log-transformed parameters $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$. Lund's method was used for outlier detection. One subject was detected as an outlier for log-transformed parameters $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$ of dapsone. This subject's data was investigated retrospectively to explore for the abnormal plasma concentrations. All the observations noted during screening, enrollment, dosing, safety monitoring, discharge and post study evaluation were investigated. There was no significant observation found. The clinical phase of the study was conducted in compliance to the protocol, SOPs and GCP. The bioanalytical phase was conducted in compliance to the protocol, SOPs and GLP. Hence, the data of this subject was considered for final pharmacokinetic and statistical analysis.

The S_{WR} for C_{max} was 0.50686 which was found to be greater than 0.294 (Table 4), hence BE assessed using SABE approach for C_{max} parameter. Accordingly, when analyzed using SABE approach for C_{max} parameter, the 95% upper confidence bound for $(\mu T-\mu R)^2-\theta\sigma^2_{WR}$ was -0.1380 for C_{max}, which was less than "0". Further the geometric mean test-to-reference ratio was 92.99% for C_{max} which was well within the acceptance range of 80 to 125 % (Table 4). Thus both the criterion of SABE was met for C_{max} parameter as set by USFDA.

On the other hand, the S_{WR} of reference product for AUCs (AUC_{0-t} and $AUC_{0-\infty}$) were 0.273 and 0.248 respectively which was <0.294, hence BE was assessed using conventional average BE approach. The summary statistical results, test-to-reference ratio (%) and 90% CI for geometric least square means of ln-transformed pharmacokinetic parameters for dapsone (AUC_{0-t} and $AUC_{0-\infty}$) were provided in Table 5. The 90% CI of ln-transformed data for the AUCs (AUC_{0-t} and $AUC_{0-\infty}$) for test and reference formulations were found to be 90.50 – 104.36 and 92.72 – 106.71 respectively. The values were within the regulatory acceptance limits of 80 -125 %. In addition to 90% CI approach, a Schuirmann test was applied to assess the bioequivalence between two formulations for dapsone [13]. The power for log transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ was more than 80 % for dapsone. From the ANOVA comparisons using SABE and ABE approach, it was found that no significant sequence, period and treatment effects observed for log transformed pharmacokinetic parameter C_{max}. There was no significant sequence and treatment effects observed for log transformed pharmacokinetic parameters AUC_{0-t} and $AUC_{0-\infty}$. However, significant period effect was observed for log transformed pharmacokinetic parameters- AUC_{0-t} and $AUC_{0-\infty}$.

Safety was assessed throughout the study. A total of 3 adverse events were reported during the clinical phase of the study. Out of which, 2 adverse events of vomiting were reported in one subject after receipt of reference product and 1 adverse event of headache in another subject after receipt of test product. The adverse events were mild to moderate in severity and were expected and probably related to the study drug. All adverse events were resolved. No serious adverse event was observed during entire study period.

DISCUSSION

Dapsone (Aczone) 5% gel is approved by the U.S. Food and Drug Administration (FDA) for the treatment of acne vulgaris in adults and children older than 12 years [10]. The use of generic preparation of a therapeutically well-established active drug principle has to be justified by the appropriate bioequivalence study, because the proof of bioequivalence of the test and reference products assures the equal therapeutic efficacy. As per the FDA's nonbinding recommendations, draft guidance of dapsone, a combination of *in vitro* and *in vivo* studies with pharmacokinetic endpoints was chosen for obtaining marketing approval in the US market for Dr. Reddy's generic product. Accordingly pivotal IVRT and IVPT studies were performed using a test formulation against US reference formulation, Aczone,5% w/w dapsone gel.

The purpose of this IVRT study was to investigate the product sameness between the generic formulation developed by the Dr.Reddy's and the comparator, Aczone gel (containing 5% dapsone), by performing an *in vitro* drug release experiment. The SUPAC statistical analysis demonstrated the absence of significant differences in the steady state release of dapsone (from 1.5 and 6 h) between the test formulation and the US reference product, Aczone gel 5% w/w.

The aim of the IVPT study was to determine whether the formulation developed by the Dr. Reddy's provided levels of the drug (dapsone) into and across the skin similar to those achieved with the application of the generic product, Aczone gel. In order to achieve this aim, a validated the *in vitro* model was used to perform the *in vitro* skin permeation experiments. The study results demonstrated that Aczone gel and the Sponsor's prototype formulations provide comparable ($p > 0.05$) delivery of the drug in the receiver fluid (over 48 h).

Based on the available literature dapsone seemed to be highly variable when applied topically [10]. Considering this, and to establish variability a full replicate design was selected based on the following grounds: i) test replicates improves average and equivalent to getting another data point (Test/Reference), ii) reducing the sample size, iii) allows to avoid compounding sequence effects, and iv) more balanced design (i.e., use of only two sequences). The subject population was selected with the aim to minimize variability and permit detection differences between pharmaceutical products.

Safety of the dapsone 5% gel in acne and 100 mg oral tablet in healthy volunteers has been well established [14,15]. Each g of dapsone 5% gel contains 50 mg of dapsone. Accordingly 2 gm of dapsone 5% gel dose proposed in the current bioequivalence study is equivalent to 100 mg dose of dapsone[16]. Therefore, the published studies on dapsone 100 mg tablet and dapsone 5% gel supports the safety of the proposed dose of 2 gm of dapsone gel 5% in healthy volunteers. Thus the choice of the dose used (2 gm equivalent to 100 mg) was justified based on analytical and safety grounds.

Subject's upper back was selected as the drug application site on the basis of large and uniform surface area. Further this was one of the application site as per the prescribing information of dapsone gel 5%. Multiple blood samples were collected prior to dose administration (0.0 hour) and up to 192 hours according to the study protocol. This sampling was planned in order to provide a reliable estimate of the extent of absorption.

The plasma concentration profile shows slow absorption over 72 hours post dose period. The concentration achieved at individual time-points post dose is very low. This may be attributed to route of administration. Dapsone gel 5% administered topically penetrates slowly and appear in small quantities in the systemic circulation. Hence bioavailability and maximal plasma dapsone concentration after topical application are generally less than 1% of the applied dose compared with equivalent oral dose administration [5,16]. The observed study data is in line with concentration profile seen in literature with topical preparation maintaining low plasma concentration [5].

Lower the rate and extent of absorption in systemic circulation, lesser will be the systemic toxicity. Thus by ensuring low plasma concentration with topical preparation, numerous potential benefits to patients could be provided, including avoidance of dose dependent hemolysis and methemoglobinemia particularly in individuals with G6PD deficiency, as well as reduced risk of serious adverse drug reactions related to elevated blood dapsone concentrations.

No statistically significant differences were observed between the treatments suggesting that the two formulations (test and reference) are interchangeable when Schuirmann test was applied for dapsone for primary pharmacokinetic parameters. ANOVA comparisons using ABE approach revealed significant period effect for log transformed pharmacokinetic parameters AUC_{0-t} and $AUC_{0-\infty}$. During the entire duration of study, clinical conditions were kept equivalent in all periods of study. A significant period effect could conceivably reflect different positioning, timing and degree of physical activity in the four periods. To our knowledge, none is applicable in the current study. Nevertheless, period effects are not expected to influence the comparison of formulations and bioequivalence is demonstrated. Therefore, significant period effect appears to be insignificant in nature and can be ignored.

Based on the results obtained from the statistical analysis of log-transformed primary pharmacokinetic parameter C_{max} where $S_{WR} > 0.294$, bioequivalence assessment was carried out using the SABE. The point estimate (test/reference geometric mean ratio) was within the acceptance range of 80% - 125% for primary pharmacokinetic parameter C_{max}. Further the 95% upper confidence bound for the mean of test and reference formulations was less than zero for primary pharmacokinetic parameter C_{max}. Thus both the criterion of scaled average BE were met for C_{max} parameter as set by FDA. On the other hand, as S_{WR} of reference product for AUCs (AUC_{0-t} and $AUC_{0-\infty}$) is less than 0.294, the 90% CI for geometric least square mean ratio of ln-transformed data for the AUC_{0-t} and $AUC_{0-\infty}$ for test and reference formulations was within the

acceptance limit of 80-125% for dapsone, thus permitting to conclude bioequivalence.

This may be the first published study to demonstrate the variability of dapsone gel 5% in healthy subjects. The study results revealed high intra-reference variability for C_{max} (S_{WR} > 0.294), thereby allowing to use FDA recommended RSABE approach[12]. The high variability observed for C_{max} may be attributed to marked individual variability for percutaneous absorption. This may be strongly influenced by and related to difference in individual skin properties. The safety profile observed in the study was expected and as per the prescribing information of dapsone gel 5%[7,15,16]. Given this information, the study drug appeared to be well-tolerated and there were no safety concerns observed.

CONCLUSION

In summary, the analysis of both *in vitro* release data and *in vitro* skin permeation and penetration data demonstrated the comparability in the performance of the US reference formulation Aczone gel 5% w/w and Dr. Reddy's test formulations, including 5% w/w of dapsone. The results of this single dose bioequivalence study indicated that the test and reference formulations of dapsone gel 5% met the USFDA set criteria of bioequivalence in healthy subjects under fasting conditions. Data from the study demonstrated that both the test and reference products were well tolerated by all the subjects in the study.

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References

1. Makrantonaki E, Ganceviciene R, Zouboulis C. An update on the role of the sebaceous gland in the pathogenesis of acne. Dermatoendocrinol. 2011; 3(1): 41-49.

2. Nast A, Dréno B, Bettoli V, et al. European Dermatology Forum. European evidence-based (S3) guidelines for the treatment of acne. J Eur Acad Dermatol Venereol. 2012; 26(1): 1-29.

3. 3. Tan J. Dapsone 5% Gel: A New Option in Topical Therapy for Acne. Skin Therapy Lett. 2012; 17(8): 1-3.

4. Strauss JS, Krowchuk DP, Leyden JJ, Lucky AW, Shalita AR, Siegfried EC, et al. Guidelines of care for acne vulgaris management. J Am Acad Dermatol. 2007; 56(4): 651-663.

5. Pickert A, Raimer S. An evaluation of dapsone gel 5% in the treatment of acne vulgaris. Expert Opin Pharmacother. 2009; 10(9): 1515-1521.

6. Thiboutot DM, Willmer J, Sharata H, Halder R, Garrett S. Pharmacokinetics of dapsone gel, 5% for the treatment of acne vulgaris. Clin Pharmacokinet. 2007; 46(8): 697-712.

7. Stotland M, Shalita AR, Kissling RF. Dapsone 5% gel: a review of its efficacy and safety in the treatment of acne vulgaris. Am J Clin Dermatol. 2009; 10(4): 221-227.

8. Draft Guidance on Dapsone. United States Food and Drug Administration (USFDA). 2017;

9. SUPAC-SS: Nonsterile Semisolid Dosage Forms; Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), 1997.

10. Clinical, Pharmacology and Biopharmaceutics Review(s), NDA 21-794. Aczone. Center for Drug Evaluation and Research, FDA, 2008.

11. Bioanalytical Method Validation. Guidance for Industry. U.S. Department of Health and Human Services. Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM).

12. Davit BM, Chen ML, Conner DP, Haidar SH, Kim S, Lee CH, et al. Implementation of a reference-scaled average bioequivalence approach for highly variable generic drug products by the US Food and Drug Administration. AAPS J. 2012; 14(4): 915-924.

13. Schuirmann DJ. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. J Pharmacokinet Biopharm. 1987; 15(6): 657-80.

14. Coutinho B. (2010). Dapsone (Aczone) 5% gel for the treatment of acne. Am Fam Physician. 81(4): 451-452.

15. Del Rosso JQ, Kircik L, Gallagher CJ. Comparative efficacy and tolerability of dapsone 5% gel in adult versus adolescent females with acne vulgaris. J Clin Aesthet Dermatol.2015; 8: 31-37.

16. Pieters FA, Zuidema J. The pharmacokinetics of dapsone after oral administration to healthy volunteers Br J Clin Pharmacol. 1986; 22(4): 491-94.