ISSN: 0975-0851
Journal of Bioequivalence & Bioavailability

OPEN ACCESS Freely available online

Research Article

Bioequivalence Study of a Novel Clobetasol Propionate Topical gel 0.05% using the Vasoconstriction Bioassay

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ABSTRACT

Background: A novel propylene glycol-based gel containing Clobetasol propionate 0.05% was developed.

Methods: This phase I single center, randomized, reference-controlled, human skin vasoconstriction assay study was subdivided into 2 parts with the aim of comparing the skin blanching effect of the new Clobetasol gel versus a marketed reference cream to test their *in vivo* bioequivalence in compliance with FDA guidance. The pilot part determined the dose-duration response curve to the reference. The pivotal part was the pharmacodynamics *in vivo* bioequivalence study. Healthy volunteers received single applications on randomized sites of the forearms. In the pilot part, the reference was applied once at 8 dose durations from 0.25 to 6 h to determine the dose duration (D) at which effect is half-maximal (ED_{50}). In the pivotal part, 3 dose durations were used (ED_{50}, $D_1 \approx ½\ ED_{50}$, $D_2 \approx 2ED_{50}$). Test and reference were applied once at the ED_{50} dose duration each to 2 sites per forearm. Untreated sites acted as negative controls. Skin blanching was measured using a chromameter. The colorimetric a* variable was analyzed over time (0-24 h after product removal).

Results: In the pilot part, ED_{50}=0.52 h was defined in 12 Caucasian responders. Ninety (90) responders were enrolled in the pivotal part and 40 met the detector criterion requested for bioequivalence. a*, analyzed using Locke's method, gave 90% confidence interval of the test/reference ratio corresponding to 88.6%-101.7% (acceptance criteria: 80%-125%).

Conclusion: The test gel was bioequivalent to the reference. Registered at Clinicaltrialsregister.eu with the EudraCT number 2018-001640-59 on 27JUL2018.

Keywords: Clobetasol; Chromametry; Topical corticosteroid; Vasoconstriction bioassay

INTRODUCTION

Clobetasol propionate is a very potent (class IV in EU and I in US) topical corticosteroid, whose synthesis was patented in 1968 and has been widely used since 1973. The drug is available worldwide in various cutaneous dosage forms including creams, ointments and gels at the fixed concentration 0.05%. Therapeutic indications of topical clobetasol formulations include psoriasis (excluding widespread plaque psoriasis), recalcitrant dermatoses, lichen planus, discoid lupus erythematosus and other skin conditions which do not respond satisfactorily to less potent steroids. Treatment with cutaneous clobetasol propionate is indicated for adults, elderly and children with the recommendation to be short in duration and limited to resistant inflammatory and pruritic manifestations of steroid-responsive dermatoses, unresponsive to less potent corticosteroids.

In addition to the other known therapeutic mechanisms of action, topical corticosteroids have a vasoconstrictor effect on the peripheral dermal vessels which manifests as skin blanching. This steroidal vasoconstrictive effect is able to quickly lighten skin colour. The skin blanching effect is fully reversible and, soon after the product application is withdrawn, the skin reverts to the natural colour.

The magnitude of this pharmacodynamic effect correlates well with clinical efficacy in psoriasis and also gives a measure of

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Received: 25-Nov-2022, Manuscript No. JBB-22-19005; Editor assigned: 28-Nov-2022, PreQC No. JBB-22-19005 (PQ); **Reviewed:** 12-Dec-2022, QC No. JBB-22-19005; **Revised:** 19-Dec-2022, Manuscript No. JBB-22-19005 (R); **Published:** 26-Dec-2022, DOI:10.35248/0975-0851.22.S7.003

Citation: Queille-Roussel C, Morano F, Di Stefano AFD, Babbi P, Perego M (2022) Bioequivalence Study of a Novel Clobetasol Propionate Topical gel 0.05% using the Vasoconstriction Bioassay. J Bioequiv Availab. S7:003.

corticosteroid diffusion through the outermost layer of the skin (stratum corneum) [1]. The human skin blanching or vasoconstrictor assay first described by McKenzie and Stoughton in 1962 was considered as a test suitable to predict the clinical potency of topical corticosteroid formulations [2-8]. This test was first used for ranking new formulations within the efficacy spectrum of topical corticosteroids (mild to very potent). Later, its use was extended to demonstrate the bioequivalence ("pharmacodynamics" equivalence) of a generic product versus the reference product [9]. The performance of the test was optimized using a colorimetric measurement that provides quantitative assessment of the skin blanching [9-11].

A novel gel containing clobetasol propionate 0.05% was tested for the first time in the present study in healthy men and women with the aim of proving the bioequivalence of the product vs. a marketed reference by applying the human skin blanching assay in compliance with the relevant regulatory guidelines [9]. The test formulation was a drug solution in a hydrophilic gel formed from propylene glycol and purified water, jellified with carbomer homopolimer C and neutralised with sodium hydroxide. Titanium dioxide was also present to give the gel the characteristic colour. The marketed reference product chosen for the study was a cream differing from the test product in the qualiquantitative formulation, but containing clobetasol propionate at the same concentration (0.05%) and having the same therapeutic indications.

MATERIALS AND METHODS

Study design

The present Phase I study was performed in compliance with the relevant FDA Guidance for industry about the *in vivo* bioequivalence of topical dermatologic corticosteroids [9]. The study had a single center, randomized, reference-controlled, human skin blanching/vasoconstriction assay design and was subdivided into 2 parts:

Pilot part: A pilot part was first performed to determine the dose-duration response curve of a commercially available reference product, in order to estimate the dose duration (D) at which effect is half-maximal (ED_{50}) to be used in the pivotal phase,

Pivotal part: A pivotal pharmacodynamics study part comparing the test product with the reference product (intra-individual comparison of treatments) was performed in order to document the *in vivo* bioequivalence of the new clobetasol propionate 0.05% topical gel and the reference product.

The dose duration-response approach applied to the pilot part was according to the below-represented E_{max} model:

$$E = E_0 + \frac{E_{max} \times D}{ED_{50} + D}$$

where:

E=effect elicited

E_0=baseline effect in the absence of ligand

E_{max}=maximum effect elicited

ED_{50}=dose duration (D) at which effect is half-maximal

ED_{50}, i.e. the dose duration corresponding to approximately half maximal blanching response, is chosen since it represents the portion of the dose–response relationship curve where differences can be optimally detected in order to accomplish the pivotal bioequivalence study.

The ED_{50} determined in the pilot part was used to compare test and reference in the pivotal part. Sensitivity in the pivotal part was ensured by using 2 calibrators: i.e., the reference applied at 2 dose durations: D_1 corresponding to approximately half (0.25-0.5 times) ED_{50} and D_2 corresponding to approximately twice (2-4 times) ED_{50}. Both were determined in the pilot part.

The FDA Guidance recommends that subjects must be detectors to include their data for statistical analyses in the bioequivalence assessment. Hence, subjects' responses were expected to meet the specified minimum D_2/D_1 ratio of area under the effect curve ($AUEC_{0-24}$) values in the pivotal phase as shown in the specific section below.

Study population

The study sample size was consistent with the FDA guidance recommendation [9]. In detail, in the pilot part, 12 healthy responders were planned to be enrolled. In the pivotal part, 50 healthy responders were planned to be included in order to have at least 40 evaluable subjects i.e., subjects who met the responder and detector criteria defined in the FDA guidance [9]. Responders were defined as subjects showing a visual reading of at least one unit on a 0-4 visual score scale according to 2 independent, experienced readers at 2 h following dose duration of 4-6 h during the screening period.

Eligible subjects were men and women, aged 18-45 years, in good health conditions based on medical history, physical examination, a 12-lead electrocardiogram and routine haematology and blood chemistry tests.

Volunteers were excluded if they used any dermatological product on the ventral forearms or used any topical or systemic corticosteroid or any other vasoactive drug that could interfere with the blanching reaction for one month prior to enrolment.

Other exclusion criteria included: skin pigmentation or differing between arms, hypersensitivity to any component of the investigational products, previous or planned exposure to UV, any systemic, cutaneous or circulatory disorders, history of drug, caffeine (>5 cups coffee/tea/day), tobacco (≥ 5 cigarettes/day) or alcohol abuse and unwillingness to use highly effective contraceptive methods during the study and for at least one month after study end, in case of women of child-bearing potential.

Before entering the study, each screened subject received a single 10 µl application of reference cream to test their responsiveness as a detectable clear-cut blanching response.

The duration of the study for each subject was of 2 days excluding the screening phase. During each study Part, the subjects underwent the interventional study phase from the morning of Day 1 to midday of Day 2.

The study was performed at the CPCAD Phase I Unit (Centre de Pharmacologie Clinique Appliquée à la Dermatologie), CHU Nice, France.

Investigational treatments

The test clobetasol propionate 0.05% gel (pilot and pivotal part) and the reference Clobesol® 0.05% cream (pivotal part only) were applied to the skin, to predefined sites on the ventral side of forearms, without occlusion, according to a randomized application list. Each circular test site was 2.2-cm in diameter corresponding to the external edge of the chromameter probe. Ten (10) µl of test or

reference were applied once to the center of the site with calibrated pipettes and then gently spread on the skin with a gloved finger. According to the FDA guidance, suitable doses should range between 2 and 10 mg of formulation per cm^2 of skin surface [9]. The dose selected for this study was 10 µl (≈ 10 mg) per test site (3.8 cm^2) i.e., ≈ 2.6 mg/cm^2. During the dose duration period, the test sites were protected by an original non-occlusive CPCAD device in order to avoid any lateral spreading of the formulation and to prevent any kind of external skin irritation.

A staggered application with synchronized removal was chosen for the study: the investigational products were applied to skin sites at different times and removed at the same time from all the sites. Removal time was defined as time 0 h (T0). Product removal was performed by 3 consecutive gentle swabs with a dry disposable tissue and without washing. The test sites were left undisturbed for a further 10 minutes before skin blanching assessment.

Treatment application was randomised per volunteer to predefined forearm test sites. Each volunteer received a unique randomization number that determined the application scheme. The randomization lists (one for the pilot part and one for the pivotal part) were computer-generated by the biometry unit of CROSS Research, Switzerland, using the PLAN procedure of the SAS® system version 9.3 (TS1M1).

Pilot part: In the pilot part the reference only was applied. On Day 1, on each forearm, 4 sites were treated, while the 5^{th} and 6^{th} sites remained untreated as negative controls. Allocation of each test site to one of the 8 predefined dose durations (6, 4, 2, 1.5, 1, 0.75, 0.5 and 0.25 h) or to untreated control was performed according to the pilot part randomised application scheme.

Pivotal part: Both test gel and reference cream were applied on Day 1. Of the 8 test sites of each forearm, 6 were treated, while the 7^{th} and 8^{th} sites remained untreated. For each subject, the treatments were randomly assigned to each test site of each forearm as follows:

- Two (2) sites received the test at ED_{50}.
- Two (2) sites received the reference at ED_{50}.
- One site received the reference at D_1.
- One site received the reference at D_2.
- Two (2) sites were left untreated.

Allocation of each test was performed according to the pivotal part randomised application scheme.

Randomization

On study Day 1, each enrolled subject was assigned a randomisation number, dispensed in chronological order. No number could be omitted or skipped.

The clinical site was provided with opaque sealed envelopes labeled with a randomisation number. Each envelope contained the assigned application scheme and was opened only after subject's allocation to the related randomisation number.

The randomisation list of study Part 1 was disclosed only after randomisation of the last subject of the pilot phase.

Blinding

The study was not blinded as the primary variable was an objective measurement that could not be influenced by un-blinded experimentation. However, in order to safeguard the allocation concealment, the personnel and the subjects were blinded to the randomized application schemes until they were disclosed by opening the opaque sealed envelopes.

Ethical procedures

The documentation of the study was reviewed by the competent Ethics committee and approved in October 2018. The French Competent Authority authorized the study in August 2018. The study was conducted in accordance with the Declaration of Helsinki and the general principles of ICH Harmonized Guidelines for GCP and in compliance with local regulations. Study subjects did not undergo any study procedure before signing the written informed consent form.

Pharmacodynamic parameters: Skin blanching

Colorimetric vasoconstriction measurements were performed at 10 min and 2, 4, 6, 19 and 24 h after product removal (T0). Baseline measurement occurred within 1 h prior to application. Colorimetric measurements were performed with a chromameter (Konica Minolta CR 400) using the L* a* b* system, common for evaluation of the human skin blanching test [10,12,13]. According to the FDA guidance, only a* data, which represent the balance between red (positive values) and green (negative values) are to be used to calculate the AUEC. L* (luminance) and b*, which is not modified by the blanching phenomenon, were measured and collected but not analyzed. Before use, the chromameter was calibrated to a standard white calibration plate (CR-A); calibration was performed each time the instrument was switched on.

Safety and tolerability

Adverse events, intake of concomitant medications and evaluations of local tolerability were recorded throughout the study.

Adverse events were defined as any untoward medical occurrence to study subjects receiving the study treatment and which did not necessarily have to have a causal relationship with the study treatment. For each reported adverse event, relationship with study treatment, when assessable, was classified as reasonably possible or not reasonably possible and intensity as mild, moderate or severe.

The local tolerability was scored before applications and 10 min, 2, 4, 6, 19 and 24 h after removal using a 6-grade scale: 0 (No reaction), 0.5 (Only slight erythema), 1 (Only erythema), 2 (Erythema with papules or oedema), 3 (Erythema, oedema with papules, oedema with vesicle) and 4 (Blisters). Signs and symptoms observed at the local tolerability evaluation could also be recorded as adverse events.

Data analysis

For statistical analysis, the subjects were grouped into the following analysis sets: enrolled set, per protocol set, safety set and pharmacodynamic set, the last one being used only in the pilot part. Reasons for exclusion of subjects from the per protocol set included: subjects enrolled but not randomized, lack of compliance, missing primary colorimetric data, failure to satisfy any inclusion/exclusion criteria (eligibility deviations) and intake of prohibited medications. Reason for exclusion of subjects from the safety set was missing application of at least one dose of the test or reference product. The pharmacodynamic set included the per protocol subjects who were detectors, i.e. subjects whose $AUEC_{0\text{-}24}$ at D_1 and D_2 was negative and also met the following criterion:

$$\frac{AUEC_{0-24}atD_2}{AUEC_{0-24}atD_1} \geq 1.25$$

Where:

$D_1 \approx ½\ ED_{50}$,

$D_2 \approx 2ED_{50}$

$AUEC_{0-24}$ at D_2=0.5[$AUEC_{0-24}$ at D_2 (left arm)+$AUEC_{0-24}$ at D_2 (right arm)]

$AUEC_{0-24}$ at D_1=0.5[$AUEC_{0-24}$ at D_1 (left arm)+$AUEC_{0-24}$ at D_1 (right arm)]

In both study parts, for the calculation of the primary parameter, 2 sets of a* were measured at each time point and the arithmetic mean of the 2 consecutive measurements was calculated. Baseline-corrected colorimetric parameter Δ a* was calculated by subtracting the baseline a* from each a* value measured at each post-dose measurement of each site (including non-treated sites) of each subject. Mean control Δ a* for each post-dose time point was calculated as the arithmetic mean of the Δ a* values obtained at the 2 untreated sites of each arm of each subject. The effect E at each post-dose time point at each treated site was calculated by subtracting the mean control Δ a* from the Δ a* calculated for each post-dose time point at each treated site. Since the application was staggered with synchronized removal, AUEC was calculated using the E values from T0 to T24h ($AUEC_{0-24}$) by the trapezoidal method for each individual treated site obtained at each time point. For the aim of $AUEC_{0-24}$ calculation, the effect at T0 (that cannot be determined by the colorimetric measures) was set to zero (0). The dose duration-response analysis in the pilot part was performed by fitting the individual $AUEC_{0-24}$ with a non-linear least square regression for the following E_{max} model pooling individual observations from all the subjects (naïve pooled data method) to determine the population ED_{50} according to the equation below. The observed ED_{50} could be rounded by up to 15 min to obtain the ED_{50} value used in the pivotal study.

$$AUEC_{0-24}(D) = E_0 + \frac{E_{max} \times D}{E_{max} + D}$$

D_1 and D_2 were calculated as approximately half ED_{50} ($D1 \approx ½\ ED50$) and twice ED_{50} ($D_2 \approx 2ED50$), respectively. These values bracket ED_{50}, corresponding to approximately 33% and 67% respectively of the maximal response and represent the sensitive portion of the dose-duration response curve.

The pharmacodynamic analysis of the pivotal part was performed on subjects included in the pharmacodynamic set. The bioequivalence test of pivotal part required the use of untransformed data. The presence of both positive and negative data prevented the use of conventional statistical transformations. Therefore, Locke's method, able to provide an exact confidence interval from untransformed data, was applied [14]. The 90% confidence interval was calculated for the ratio of mean $AUEC_{0-24}$ response to the test product (mean of 4 replicates at ED_{50} dose duration) to mean $AUEC_{0-24}$ response to the reference product (mean of 4 replicates at ED_{50} dose duration). According to the FDA guidance, no reference interval has yet been defined for bioequivalence acceptance [9]. However, the acceptance interval 80%-125% was taken into account, pending evaluation of data submitted to the agency.

RESULTS

Disposition of subjects

The study took place between late October 2018 and late June 2019. In the pilot part, 12 healthy responders (9 female and 3 male Caucasians) with a median age of 31.0 years (range 26-41) were included and completed the study as planned. In the pivotal part, 90 healthy responders were included and completed the study as per protocol. No enrolled subject discontinued prematurely the study. All enrolled subjects had post-randomisation data, attended all the study visits, fulfilled all of the inclusion criteria and did not meet any exclusion criteria at enrolment or during the study. No major protocol deviations were observed. Therefore, all the subjects were included in the per protocol analysis set in both study Parts. In the pivotal part, out of the 90 enrolled responders, 40 subjects (27 women and 13 men with a median age 36.0 range 19-45) met the criteria for detectors and were included in the pharmacodynamic analysis set. Baseline demographic data are summarized in Tables 1 and 2.

Table 1: Pilot part demography-Enrolled, per protocol and safety sets.

			Enrolled, per protocol and safety sets N=12
Sex	Female	n (%)	9 (75.0)
	Male	n (%)	3 (25.0)
Race	Caucasian	n (%)	12 (100.0)
Age (years)		N	12
		Mean (SD)	32.1 (4.5)
		Median	31
		(Min, Max)	(26,41)
Height (cm)		N	12
		Mean (SD)	169.2 (12.1)
		Median	166.5
		(Min, Max)	-155,192
Body weight (kg)		N	12
		Mean (SD)	63.83 (14.35)
		Median	57.5
		(Min, Max)	(48.0,90.0)
Body mass index (kg/m^2)		N	12
		Mean (SD)	22.10 (2.90)
		Median	21.71
		(Min, Max)	(18.8,27.2)

Note: N: Sample; n (%): Number and percentage of subjects; SD: Standard Deviation; Min: Minimum; Max: Maximum.

Table 2: Pivotal part demography-Enrolled, per protocol and safety sets.

			Enrolled, per protocol and safety sets N=40
Sex	Female	n (%)	27 (67.5)
	Male	n (%)	13 (32.5)
Race	Caucasian	n (%)	40 (100.0)
Age (years)		N	40
		Mean (SD)	34.3 (7.5)
		Median	36
		(Min, Max)	(19,45)

Height (cm)	N	40
	Mean (SD)	168.9 (10.0)
	Median	166.5
	(Min, Max)	-155,193
Body weight (kg)	N	40
	Mean (SD)	65.72 (12.87)
	Median	63.5
	(Min, Max)	(43.0,100.0)
Body mass index (kg/m^2)	N	40
	Mean (SD)	22.93 (3.36)
	Median	22.63
	(Min, Max)	(17.3,32.4)

Note: N: Sample; n (%): Number and percentage of subjects; SD: Standard Deviation; Min: Minimum; Max: Maximum.

Pharmacodynamic data

Mean $AUEC_{0\text{-}24}$ obtained for each tested dose-duration in the per protocol analysis set in the pilot part is presented in Table 3.

Under the study conditions, analysis of pilot part data produced the following results see Table 4: ED_{50} was approximately 0.50 h (30 min), D_1 which corresponds to approximately half ED_{50}, was 0.25 h (15 min), and D_2 which corresponds to approximately twice ED_{50} was 1 h.

Mean values of $AUEC_{0\text{-}24}$ calculated in the pivotal part are summarized in Table 5.

The outcome of the bioequivalence analysis is presented in Table 6. As the calculated parameter G was less than 1, the study met the bioequivalence requirements and the 90% confidence interval of the test product/reference product ratio could be calculated. Based on these data, the calculated 90% confidence interval was 88.6% and 101.7%. This interval is totally included in the reference interval 80%-125%, thus it can be concluded that the test and the reference product are bioequivalent.

Safety data

During the pilot part, all the subjects received a total amount of 80 µl of reference cream (i.e., 10 µl on 8 test sites). During the pivotal part, all the subjects received a total amount of 80 µl of reference cream (i.e., 10 µl on 8 test sites) and 40 µl of test gel (i.e., 10 µl on 4 test sites).

No adverse events and no local irritation occurred during the pilot part. During the pivotal part, 14/90 subjects (15.6%) experienced a total of 15 events of mild (11) to moderate (4) severity. Eight (8) of these events were headaches. No reasonably possible causal relationship was found between the events and the study treatment. There were no serious adverse events and no event led any subject to discontinuation. The frequency of the adverse events is reported in Table 7. Only 3 episodes of local slight erythema (i.e., score of 0.5) were observed in 2 of the 90 subjects (2.2%) on the sites treated with the reference product. Two (2) reactions were observed 10 min after product removal on the sites treated at the dose durations ED_{50} and D_2 and the third one was observed 6 h after product removal on the site treated at the dose duration D_1. These mild reactions were transient and resolved spontaneously. One local slight erythema was also observed on a non-treated site at T0. As per protocol, the observed reactions being of mild intensity were not reported as adverse events. No local signs of irritation were reported with the test product.

Table 3: Pilot phase: Average values of $AUEC_{0\text{-}24}$ by dose-duration-Per protocol set (N=12).

		Reference product: Clobetasol propionate 0.05% cream							
		0.25 hours	0.5 hours	0.75 hours	1 hour	1.5 hours	2 hours	4 hours	6 hours
$AUEC_{0\text{-}24}$	N	12	12	12	12	12	12	12	12
	Mean	-15.63	-26.741	-20.607	-19.385	-26.859	-29.138	-24.316	-32.068
	SD	19.907	17.422	10.756	11.584	14.407	12.234	7.341	13.983
	CV%	-127.36	-65.15	-52.2	-59.76	-53.64	-41.99	-30.19	-43.6
	Min	-48.37	-71.55	-39.09	-37.46	-55.85	-49.5	-39.3	-58.8
	Median	-17.17	-23.92	-20.995	-19.315	-23.52	-29.88	-24.22	-34.605
	Max	26.59	-9.3	1.03	-0.24	-10.37	-11.3	-14.04	-9.02

Note: $AUEC_{0\text{-}24}$: Area under the effect curve; N: Sample; SD: Standard Deviation; CV%: Coefficient of Variation; Min: Minimum; Max: Maximum.

Table 4: Pilot phase analysis outcome-Per protocol set (N=12).

ED_{50} (hours)	D_1 (hours)	D_2 (hours)
0.52	0.26	1.04

Note: Determination of ED_{50}, D_1 and D_2 following the human skin blanching test after application of the reference product clobetasol propionate 0.05% cream at the 8 predefined dose-durations; ED_{50}: Dose duration (D) at which effect is half-maximal; D_1: Dose duration corresponding to approximately half ED_{50}; D_2: Dose duration corresponding to approximately twice ED_{50}; N: Sample.

Table 5: Pivotal part $AUEC_{0-24}$ of test at ED_{50} and reference at D_1, ED_{50} and D_2 dose durations.

Dose duration	Test product: clobetasol propionate 0.05% gel $AUEC_{0-24}$, ED_{50} [1]	Reference product: clobetasol propionate 0.05% cream $AUEC_{0-24}$, ED_{50} [1] Clobesol ® $AUEC_{0-24}$ (Mean ± SD)
D_1 (15 min)	-	-24.738 ± 11.921
ED_{50} (30 min)	-27.807 ± 12.520	-29.280 ± 12.521
D_2	-	-39.041 ± 17.324

Note: [1] Calculated as the arithmetic mean of 4 replicates of $AUEC_{0-24}$ at the dose duration ED_{50}; N=40 detectors; $AUEC_{0-24}$: Area under the effect curve; ED_{50} : Dose duration (D) at which effect is half-maximal; N: Sample; SD: Standard Deviation; CV%: Coefficient of Variation; Min: Minimum; Max: Maximum.

Table 6: Pivotal part: Bioequivalence results- pharmacodynamic set (N=40).

Comparison	Point estimate	G	K	90% CI
Test versus reference	1.68	0.01	0.32	88.59, 101.73

Note: $AUEC_{0-24}$ calculated as the arithmetic mean of 4 replicates at the dose duration ED_{50} ; ED_{50} : Dose duration (D) at which effect is half-maximal; N: Sample; G and K: Calculated using the Locke's method; CI: Confidence interval calculated using the Locke's method.

Table 7: Number and percentage of subjects with adverse events by study part and preferred term-Safety set.

MedDRA description		Pilot part-N=12	Pivotal part-N=90
SOC	PT	n (%)	n (%)
Nervous system disorders	Headache	0	8 (8.9)
Respiratory, thoracic and mediastinal disorders	Rhinitis allergic	0	2 (2.2)
	Cough	0	1 (1.1)
	Oropharyngeal pain	0	1 (1.1)
Infections and infestations	Cystitis	0	2 (2.2)
Reproductive system and breast disorders	Dysmenorrhea	0	1 (1.1)

DISCUSSION

The present study designed accurately in compliance with the relevant FDA guideline demonstrated that the test gel and the reference cream are bioequivalent. The two products were tested in the pivotal part, using the experimental conditions determined in the pilot part (ED_{50}=30 min, D_1=15 min and D_2=60 min). In 40 Caucasians (27 women and 13 men), the 90% confidence interval of the ratio test/reference corresponding to 88.6%-101.7% fell within the pre-established acceptance interval 80%-125%.

The tolerability of both formulations was excellent according to the safety results.

The FDA guideline discourages the application of visual methods of measurement of the blanching effect preferring the use of a chromameter, as used in the present study. The chromameter offers reliable and repeatable results provided that certain precautions are taken to avoid to bias the measurements [13,15-17]. It is important to avoid skin compression and align the chromameter correctly in order to ensure data repeatability [13,16,17]. Furthermore, the reliability of the measurements was also ensured in the present study by carefully avoiding, nevi, veins, moles and hair-covered areas or uneven skin tone due to varying vascularity since the presence of such variations on the skin surface, scarring, etc., can influence the data reliability [18].

The ED_{50} of the reference cream (0.52 h) was of the same order of magnitude as the ED_{50} estimate found for a clobetasol propionate 0.05% formulation in Chinese skin (cream: 0.42 h and ointment: 0.40 h) and in Caucasians with an unspecified clobetasol propionate 0.05% cream (36 min) [19,20].

In the pivotal part of the present study, the number of detectors was in accordance with the number requested by the FDA guidance (40-60 detectors) in order to perform the bioequivalence test. The enrolment of 90 responders was necessary to have at least 40 detectors (44%) [9,21,22].

CONCLUSION

Gallicano argued that the FDA guidance sample size is not based on any justified calculation considering that the number of necessary subjects may vary with the within-subject variability in the blanching response. He hypothesised that successful bioequivalence studies depend on the clinic's ability to recruit a fair-skinned population of subjects that show high and consistent skin blanching to even low potency topical steroids in order to minimise the variability in the vasoconstrictor response.

In conclusion, under the study conditions and in compliance with the FDA guidance, the test clobetasol propionate 0.05% gel was found to be bioequivalent to the reference cream.

STATEMENT OF ETHICS

The documentation of the study was reviewed by the competent Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki and the general principles of ICH Harmonised Guidelines for GCP and in compliance with local regulations. Study subjects did not undergo any study procedure before signing the written informed consent form.

DISCLOSURE STATEMENT

All the authors had potential conflicts of interest. In detail, the relationships of C.Q-R., F.M., and A.F.D.D. with the Sponsor were regulated by financial agreements. P.B. and M.P. are employees of Esapharma S.p.A.

FUNDING DETAILS

Esapharma S.p.A., Italy gave the financial support to the project. The relationships between the Sponsor, Esapharma S.p.A., and CPCAD, and CROSS Research S.A. were regulated by financial agreements.

AUTHOR CONTRIBUTIONS

The Sponsor reviewed and approved the study design and reviewed and approved the clinical study report. Esapharma S.p.A. reviewed and approved the manuscript for publication.

C.Q-R. wrote the study protocol was responsible for the clinical activities and collected the data, F.M. performed the pharmacodynamic and safety analysis, P.B. and M.P. reviewed and approved the design of the study and the draft manuscript and A.F.D.D. drafted the manuscript. All authors read and approved the manuscript.

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