Topical Dermatologic Corticosteroids: In Vivo Bioequivalence

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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Generic Drugs
Revision 1

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Topical Dermatologic Corticosteroids: In Vivo Bioequivalence Guidance for Industry[1]

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance is intended to assist applicants who submit abbreviated new drug applications (ANDAs) for topical dermatologic corticosteroid products of all potency groups[2], hereinafter referred to as *topical corticosteroids*. This guidance describes recommendations for in vivo studies to demonstrate the bioequivalence of topical corticosteroids.

When finalized, this guidance will replace the guidance for industry *Topical Dermatologic Corticosteroids: In Vivo Bioequivalence* that was issued in June 1995.[3] Revising this guidance will provide clarity for potential ANDA applicants on the appropriate pilot and pivotal studies and other recommendations for pharmacodynamic approach to assess the bioequivalence of topical dermatologic corticosteroids. These recommendations have evolved since the original guidance was issued in 1995.

This guidance provides recommendations for the study design, method qualification, data analysis, and data reporting for the pilot dose-duration vasoconstrictor response study and pivotal vasoconstrictor bioequivalence study used to demonstrate bioequivalence of topical corticosteroids. The guidance also discusses considerations and approaches for estimating key study parameters (e.g., dose corresponding to half the maximal vasoconstrictor response (ED50)) and sample size for the pivotal vasoconstrictor bioequivalence study).

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of

[1] This guidance has been prepared by the Office of Generic Drugs in consultation with the Office of Clinical Pharmacology in the Center for Drug Evaluation and Research at the U.S. Food and Drug Administration. You may submit comments on this guidance at any time. Submit comments to Docket No. FDA-2017-D-6821 (available at https://www.regulations.gov/docket?D=FDA-2017-D-6821). See the instructions in that docket for submitting comments on this and other Level 2 guidances.

[2] The potency of topical corticosteroids is the amount of drug needed to produce a desired therapeutic effect. The vasoconstrictor assay could be used to determine potency.

[3] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The Federal Food, Drug, and Cosmetic Act (FD&C Act) generally requires an ANDA to contain, among other things, information to show that the proposed generic drug product (test product) is bioequivalent to its reference listed drug (RLD).[4] *Bioequivalence* "is the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study."[5]

This guidance describes an in vivo pharmacodynamic approach to demonstrate the bioequivalence of topical corticosteroids. Topical corticosteroids are known to cause vasoconstriction of the dermal vasculature that produces the pharmacodynamic effect of skin blanching. The magnitude of blanching (change in skin color) depends upon the potency of the corticosteroid, and it increases relative to the amount of the corticosteroid permeating into the skin, when study parameters are suitably controlled. Thus, the pharmacodynamic vasoconstrictor response can be a surrogate measure of the rate and extent to which a topical corticosteroid becomes available at the site of action in the skin.

A pilot vasoconstrictor study is routinely performed to define appropriate parameters for a pivotal vasoconstrictor study used to support a demonstration of bioequivalence between a test topical corticosteroid and its reference standard, which ordinarily is the RLD. Therefore, this guidance recommends that ANDA applicants who propose to use an in vivo pharmacodynamic approach to demonstrate bioequivalence between a test topical corticosteroid and its reference standard conduct two in vivo vasoconstrictor studies: (1) a pilot dose-duration vasoconstrictor response study, using the reference standard; and (2) a pivotal vasoconstrictor bioequivalence study, comparing the test topical corticosteroid and reference standard. The proposed methodology, including the study design, model selection, and model optimization for the pilot dose-duration vasoconstrictor response study, and the statistical method for the pivotal vasoconstrictor bioequivalence study are discussed in more detail in subsequent sections of this guidance.

The purpose of the pilot dose duration vasoconstrictor response study (or *pilot vasoconstrictor study* or *pilot study*) is to determine the dose duration-response relationship of the topical corticosteroid to be studied in the pivotal vasoconstrictor bioequivalence study. The results of the pilot vasoconstrictor study provide the dose duration-response information necessary to

[4] See section 505(j)(2)(A), (j)(2)(C), and (j)(4) of the FD&C Act (21 U.S.C. 355(j)(2)(A), (j)(2)(C), and (j)(4)); see also 21 CFR 314.94. Bioequivalence to the RLD may be demonstrated via comparative assessments of the test product to the designated reference standard (RS). See, e.g., § 314.3(b) (21 CFR 314.3(b)) (defining *reference standard*).

[5] § 314.3(b) (defining *bioequivalence*); see also section 505(j)(8)(B) of the FD&C Act (describing when a drug shall be considered to be bioequivalent to a listed drug); see also 21 CFR 320.23(b).

determine the parameters ED_{50}, D_1, and D_2[6] to be used in the prospective applicant's pivotal vasoconstrictor bioequivalence study. Development and validation of a suitably sensitive and discriminating region of a dose duration-response standard curve is essential to estimate ED_{50}, D_1, and D_2 for a vasoconstrictor response. This approach is analogous to using a standard curve to characterize the linearity, range, and limits of quantification for a bioanalytical method for a drug in a biological fluid. The pivotal study should be performed under the same conditions as the pilot study for each topical corticosteroid under investigation.

The purpose of the pivotal vasoconstrictor study is to demonstrate bioequivalence of the test product to the RLD using an in vivo approach. Alternatively, an in vitro characterization-based approach to establish the bioequivalence of a topical corticosteroid product may be acceptable when the proposed generic formulation contains no difference in inactive ingredients or in other aspects of the formulation relative to the RLD that may significantly affect the local or systemic availability of the active ingredient(s). Prospective applicants are encouraged to submit a controlled correspondence, if appropriate, or to request a product development meeting for relevant complex products that may be submitted in an ANDA to discuss specific scientific issues or questions (e.g., a proposed study design or issues related to method qualification, dose duration-response, or other aspects of a pilot dose duration-response study before conducting the pivotal vasoconstrictor study), or to discuss an alternative bioequivalence approach (e.g., a characterization-based approach).[7,8] An applicant must submit with their ANDA a complete study report for the bioequivalence study upon which the ANDA relies for approval.[9]

III. PHARMACODYNAMIC VASOCONSTRICTOR STUDIES

A. Vasoconstrictor Method Qualification

The chromameter is the apparatus most commonly used to measure the pharmacodynamic skin blanching response induced following the application of topical corticosteroids. Prior to collecting data for vasoconstrictor studies, the chromameter should be calibrated and qualified for its intended use. In addition, the repeatability and ruggedness[10] of chromameter measurements by different operators should be qualified. These qualifications should be

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[6] ED_{50}: half of the maximal vasoconstrictor response; D_1: the dose duration equal to approximately 0.5 times the population ED_{50}; and D_2: the dose duration equal to approximately 2 times the population ED_{50} for the simple E_{max} model used.

[7] See the guidance for industry *Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA* (November 2020) for more information on product development meetings.

[8] See also the draft guidances for industry *Physicochemical and Structural (Q3) Characterization of Topical Drug Products Submitted in ANDAs* (October 2022), *In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs* (October 2022), and *In Vitro Permeation Test Studies for Topical Drug Products Submitted in ANDAs* (October 2022). When final, these guidances will represent FDA's current thinking on these topics.

[9] 21 CFR 314.94(a)(7).

[10] Repeatability expresses the precision under the same operating conditions over a short interval of time. Ruggedness is the reproducibility of the method under a variety of normal, but variable, test conditions. Variable conditions might include different machines, operators, and reagent lots. Ruggedness provides an estimate of experimental reproducibility with unavoidable error.

completed before the start of a study. If studies have multiple groups, method qualifications should be performed, at a minimum, before the start date of the first group.

1. Chromameter Qualification

Chromameter qualification is conducted with calibrated chromameters to support a demonstration of the ruggedness of the chromameter measurements across multiple chromameter units. Multiple chromameter units can be set up to measure the vasoconstrictor response in both the pilot dose-duration vasoconstrictor response study and the pivotal vasoconstrictor bioequivalence study. All chromameters used in these pilot and pivotal vasoconstrictor studies should be reported with their specific identification numbers and qualified to ensure consistent performance in study data collection. Chromameter qualification should be performed on all chromameters planned to be used in pilot and pivotal vasoconstrictor studies using one operator, one subject, and, with at least four readings each at one designated skin site. Intra-chromameter variability is calculated as the variability within multiple readings at one skin site by one operator using one chromameter. Inter-chromameter variability is calculated as the variability in readings between different chromameters, with the mean value of multiple readings from each chromameter at one skin site by one operator. The chromameter qualification should be repeated with at least four study subjects, using at least four skin sites in each study subject to demonstrate the reproducibility of the chromameter measurements. To determine procedure consistency between and within chromameters, the variability (% coefficient of variation (CV)) for the intra-chromameter and the inter-chromameter measurements should be not more than 15% in each and every subject.

2. Operator Qualification

Operator qualification is conducted to support a demonstration of the ruggedness of the chromameter measurements across multiple operators. The operators who conduct pilot and pivotal vasoconstrictor studies should be reported with their specific identification numbers or names and qualified to ensure that each one is operating the chromameters and measuring the skin response consistently. Operator qualification should be performed by multiple operators using one chromameter on one subject with at least four readings each at one designated skin site. Intra-operator variability is calculated as the variability within multiple readings by one operator using one chromameter at one skin site. Inter-operator variability is calculated as the variability between different operators, with the mean value of multiple readings from each operator, using one chromameter at one skin site of the same subject. The operator qualification should be repeated with at least four study subjects, with at least four skin sites in each study subject, to support a demonstration of method reproducibility. To determine procedure consistency between and within operators, the variability (CV) for the intra-operator and the inter-operator measurements should be not more than 15% in each and every subject.

B. Dose Duration-Response Model

The conditions under which the pivotal vasoconstrictor bioequivalence study is performed should be optimized to assure that the test topical corticosteroid and reference standard are compared in the sensitive (steep) portion of the response curve, where the vasoconstrictor

response would be sensitive and discriminating to differences in the bioavailability of the corticosteroid between the test and reference standard. Development of a dose duration- response relationship for a topical corticosteroid relies on consistent administration of a predetermined dose of the drug product to the skin. Development of a dose duration-response relationship for a topical corticosteroid will identify the sensitive dose duration-response region to support pivotal study design. The time course of the response should be measured until it returns to baseline to ensure that at each dose duration, the maximal pharmacodynamic response is observed.

To identify the sensitive and discriminating region of the dose duration-response curve for the pharmacodynamic skin blanching effect, it is useful to (1) produce conditions that are expected to deliver increasing amounts of a corticosteroid drug into the skin (a practical way to modulate the amount of drug (corticosteroid) delivered into the skin is to dose the fixed amount of topical corticosteroid product on the skin for progressively increasing dose durations), and (2) measure the resulting skin blanching effect caused by dermal vasoconstriction.

Although various models are available to express a relationship between drug dose and pharmacodynamic effect, the Agency recommends use of the E_{max} model below to describe the dose duration-response of topical corticosteroids, which describes the measure of effect (E) in terms of a baseline effect (E_0), a maximal effect (E_{max}) and a dose duration (D) at ED_{50}:

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

Alternative models can be used, with justifications and appropriate model selection procedures, if a prospective applicant finds the above E_{max} model is not appropriate (see Appendix IV). Prospective applicants should justify their selected E_{max} model and are encouraged to use the pharmacodynamic vasoconstrictor study data to support the dose duration selection from a dose duration-response model for population estimation. In the population dose duration-response model, both fixed effect and/or random effect for E_{max} and ED_{50} can be considered. The type of model parameter distribution assumption (normal or log-normal) for E_{max} and ED_{50} parameters within the population analysis should be specified. Prospective applicants should describe their model optimization procedures and provide the rationale for ED_{50} selection in the pre-ANDA meeting request or ANDA submission. Some aspects of model optimization that are recommended to be included are provided below:

- E_{max} model selection
- Estimation methods comparison
- Model parameter selection
- Error models selection
- Initial estimates procedure[11]

The in vivo vasoconstrictor response (detected as skin blanching) generally approaches a maximum when the dermal vasculature is not able to vasoconstrict further. At relatively high

[11] For detailed modeling procedures, refer to the guidance for industry *Population Pharmacokinetics* (February 2022).

strengths for highly potent topical corticosteroids, there may be a diminishing change in the vasoconstrictor response to increases in dose duration (flattening the response curve at the upper end). Conversely, at relatively low strengths for low potency topical corticosteroids, it may be challenging to elicit a vasoconstrictor response despite increases in dose duration (flattening the response curve at the lower end). Therefore, a prospective applicant should design the pilot vasoconstrictor study to cover a full dose duration-response curve appropriately according to the potency of topical corticosteroids, and hence improve the dose duration-response model.

C. Study Design

1. Pilot Study

- This dose duration-response study should be based on the reference standard only, with randomization of dose-duration skin sites.

- Untreated control sites on each arm should be used to enable correction of active drug skin sites for color changes during the study unrelated to drug exposure. Because the vehicle corresponding to the reference standard is not generally available, untreated control sites refer to untreated areas of skin, not to areas of skin to which vehicle has been applied.

- Dose durations (e.g., from 0.25 to 6.0 hours) should be designed properly to explore the dose duration-response relationship and to determine the appropriate dose duration for the pivotal study. Pharmacodynamic responses are measured in terms of area under the effect curve (AUEC) by readings of a chromameter at the end of each dose duration after the removal of residual topical corticosteroid.

- Dose duration-response data should be modeled using a nonlinear mixed effect modeling method to determine the population ED_{50} value, which will serve as the approximate dose duration for the pivotal vasoconstrictor study.

- A minimum of twelve subjects is recommended.

2. Pivotal Study

- This pharmacodynamic bioequivalence study uses replicates of single dose duration of test topical corticosteroid and reference standard based on the population ED_{50} identified in the pilot study. Also, the replicates of each of the dose durations (D_1 and D_2) of the reference standard should be included in the pivotal study.

- For a bioequivalence analysis, selection of an individual subject is based upon an acceptable ratio of mean reference AUEC at D_2 over mean reference AUEC at D_1 for each subject. The minimum value of the ratio should be 1.25 and both mean AUEC values at D_1 and D_2 are negative,[12] if simple E_{max} model is proposed. However, other

[12] Refer to section J.1.(b) for AUEC calculation

values for the ratio can be used with justification depending on the selected dose duration-response model. The individual subject who meets this dose duration-response criterion (under conditions when both mean D_1 and D_2 values are negative) is defined as a detector (i.e., evaluable subject).

- It is the applicant's responsibility to design an adequately powered pivotal bioequivalence study. It is recommended that applicants enroll a sufficient number of subjects to yield a number of detectors sufficient to power the study. When determining the sample size of enrolled subjects, dropouts and estimated required number of detectors should be taken into consideration. Based on observations from studies submitted in ANDAs, forty or more detectors are generally used for the pivotal study. The sample size determination for the pivotal study should be prespecified in the protocol and justified. Sufficient subjects should be recruited, randomized with respect to dose duration skin site, and dosed at the beginning of the study to ensure that the desired number of detectors will be available for analysis. All detectors should be included in the analysis.

D. Subject Inclusion Criteria

- Males and non-pregnant, non-lactating females, general population.

- Subjects demonstrating adequate vasoconstrictor response to the reference standard. (Refer to section F for subject screening for response).

- Willing to shower using the same soap/cleansers throughout the study (Screening Visit through study completion).

- Willing to follow study restrictions. (Refer to section I.1.(c)-(f)).

E. Subject Exclusion Criteria

- Clinically significant hypertension or circulatory disease.

- Smoking within one week of study.

- Caffeine intake greater than 500 mg per day prior to or during the study. Coffee, tea, and energy drinks should all be considered as important caffeine sources.

- Clinically significant history of alcoholism or drug abuse.

- Use of topical dermatologic drug therapy (either as therapy or participation in the clinical study) on ventral forearms within one month prior to the study.

- Adverse reactions to topical or systemic corticosteroids.

- Any current or past medical condition, including active dermatitis or any other dermatologic condition, which might significantly affect the pharmacodynamic response to the administered drug.

- Would require shaving ventral forearms to ensure consistent dosing on the skin surface.
- Use of any vasoactive (constrictor or dilator) medication (prescription or over-the-counter) that could modulate blood flow. Examples of such drugs include nitroglycerin, antihypertensives, antihistamines, nonsteroidal anti-inflammatory drugs, aspirin, and over-the-counter cough/cold products containing antihistamines and/or either phenylpropanolamine or phentolamine.

- Any obvious difference in skin color between arms.

F. Subject Screening for Response

In this guidance, a *responder* is defined as a subject who shows the skin blanching vasoconstriction response to a single-dose duration of the corresponding reference standard under the same occlusive or non-occlusive conditions used in the pilot and pivotal vasoconstrictor studies. Quantification of skin blanching in the pilot and pivotal vasoconstrictor studies by a chromameter is considered to be the most satisfactory response measurement. However, *responder* status may be based on visual readings with the discrete multiple unit scale (0 - 3 or 0 – 4). A dose duration of 4 hours or 6 hours is suggested, with skin blanching assessment 2 hours following drug product removal. A *responder* shows a visual reading of at least one unit.

Inclusion of *nonresponders* reduces the ability of a study to detect true differences between the test topical corticosteroid and reference standard, should they exist. Therefore, for both the pilot dose duration-response study and the pivotal bioequivalence study, only *responders* should be included for the enrollment.

To conserve skin sites on the ventral forearm for use in the dose duration-response study or bioequivalence study, *responder* status may be based on studies conducted at sites other than the forearm (e.g., upper arm).

Criteria for identification of responders, including dose duration, magnitude of response, and skin site tested, should be included in the study report.

G. Occlusion Versus Nonocclusion

When use of occlusion is allowed in the label of the specific reference standard, the pilot dose duration-response vasoconstrictor study and pivotal vasoconstrictor (bioequivalence) study may be conducted using a non-absorbent occlusive film. Occlusion may be appropriate only for the lower potency products in the vasoconstrictor study. Caution is recommended, as observations from pilot studies data suggest that the ED_{50} (the dose duration to be used in the pivotal study)

decreases with increasing topical corticosteroid product potency.[13] Evaluation of dose duration-response requires dose duration data at some time (i.e., D_1) less than the ED_{50}. Very short dose durations are difficult to conduct experimentally and tend to produce high variability in response. If occlusion is used for the pilot vasoconstrictor study, it should also be used for the pivotal vasoconstrictor study.

H. Methods of Application and Removal

Staggered application with synchronized removal (i.e., the topical corticosteroid is applied to skin sites at different times, and removed at the same time) could be utilized in the pilot and pivotal vasoconstrictor studies (see Appendix I).

I. Study Day Activities and Restrictions

1. Pilot Study

a) Subjects should begin the study sessions at approximately the same time (within one hour) each study day.

b) Verification by history of adequate washout of excluded drugs that could modulate blood flow (constrictor or dilator).

c) No exercise with either arm, and no strenuous exercise overall, for duration of study session.

d) No bathing or showering during the periods of drug application and assessment of skin blanching.

e) No use of creams, emollients, or similar products on forearms for 24 hours prior to, and throughout, the study.

f) The forearms should be free of any dirt or particulate matter that would interfere with proper drug application or the assessment of a pharmacodynamic response. Cleansing of the skin is not encouraged because of the possible effects on drug uptake and the pharmacodynamic response to the drug product. If necessary, cleansing should be performed not less than 2 hours before drug product application. If cleansing is performed, this should be noted in the study report.

g) Whether the study is conducted using occlusion or under non-occlusive conditions, the use of a protective, non-occlusive guard is recommended to prevent smearing or removal of the topical corticosteroid from the skin site. Care should be taken to avoid contact between the guard and the topical corticosteroid to prevent inadvertent contamination of untreated control sites or other test sites.

[13]Singh GJP, W P Adams, Lesko LJ, Shah VP, et al. Development of in vivo bioequivalence methodology for dermatologic corticosteroids based on pharmacodynamic modeling; Clin Pharmacol Ther 1999 Oct, 66(4): 346-57.

h) Skin sites should be no closer than 3–4 cm to the antecubital fossa or to the wrist.

i) The reference standard should be applied to skin sites of identical surface area on the ventral forearms. Suggested dose durations for the pilot study are 0.25, 0.5, 0.75, 1, 1.5, 2, 4 and 6 hours, but may vary depending on the topical corticosteroid under investigation.

j) Eight dose durations, i.e., active drug sites, should be equally divided between the two arms.

k) Amount of drug product, skin site size, and spacing between sites should be determined prior to the initiation of the study. For example, investigators may use doses of 5-12 microliters (μL) of formulation per centimeter $(cm)^2$ of skin surface area, and 1.6 cm diameter sites. Sites may be spaced as close as 2.5 cm center-to-center and may be in a straight line or staggered pattern, depending on skin surface suitability (e.g., vascularity, nevi, etc.) and arm length. If vasoconstrictor effects of two adjacent test sites overlap and the investigator cannot discern between the vasoconstrictor effect at each test site, the subject should be excluded from the data analysis.

l) Application to each subject of eight dose durations (in duplicate; see Appendix II) and four untreated control sites should be randomly assigned among the 20 sites, maintaining two untreated control sites, eight dosed sites on each arm (ten sites per arm), and duplicate measurements for each duration.

m) Prior to measurement of the pharmacodynamic skin blanching (vasoconstrictor) response at the end of the application period, remaining topical corticosteroid should be gently removed from the skin. This may be accomplished by either of the methods below:

- Three consecutive swabbings with dry cotton swabs.

- Washing all skin sites with mild skin cleanser and water, blotting the sites dry with a nonabrasive towel, and allowing to air-dry for at least 5 minutes prior to evaluation. Cleanse arm surfaces with a minimum amount of mild liquid skin cleanser, for example one drop of a liquid cleanser worked to a lather in wetted hands, followed by rinsing. If after 5 minutes the subject has any visible cutaneous effects related to washing, a longer waiting period may be necessary. This method is suitable for the staggered application with synchronized removal method.

n) Assessment of baseline skin color and skin blanching at each site.
Examples of assessment time periods for staggered application with synchronized removal are:

- For all dose durations and untreated control sites, baseline readings within 1 hour prior to drug application of the longest dose duration, and at 0, 2, 4, 6, 8, 10, 12, 20, and 24 hours or longer until the response returns to baseline after drug product removal (see Appendix I). Dose duration will depend upon the topical corticosteroid being studied.

- Time zero (0) is defined as within 15 minutes after drug product removal.

2. *Pivotal Study*

a) Follow the recommendation listed in the section III.I.1 above where applicable. To remove potential operator bias, the analyst (e.g., chromameter operator) should be blinded to the product treatment assignments.

b) Application of dose durations to skin sites on the ventral forearms of each subject should be randomly assigned, maintaining the recommendations described below. Sites may be occluded or nonoccluded, based on the considerations of section III.G above and the study design used in the pilot study. Untreated control skin sites should also be included. Dose durations and control sites on each arm should include:

R: the reference standard at the dose duration corresponding approximately to ED_{50}, as determined with the reference standard in the pilot study (e.g., two sites per arm)

T: the test topical corticosteroid at the same dose duration corresponding approximately to ED_{50} as for the reference standard (e.g., two sites per arm)

D_1: the shorter dose duration reference standard calibrator (e.g., two sites per arm)

D_2: the longer dose duration reference standard calibrator (e.g., two sites per arm);and

UNT: the untreated control (e.g., two sites per arm)

The total number of treated sites is 16 (i.e., eight sites per arm). The eight treatments and two UNTs each arm should be randomized, as noted above. Application patterns on each arm should be complementary, i.e., D_2 is complementary to D_1, R is complementary to T, and UNT is complementary to UNT. As examples, where T is assigned a specific skin site location on one arm, R should be assigned to the corresponding skin site on the other arm. Where UNT is assigned a specific skin site location on one arm, UNT should be assigned to the corresponding skin site on the other arm.

A representative application sequence for a particular subject might be:

ANTECUBITAL FOSSA

Left Arm	Right Arm
D1	D2
T	R
UNT	UNT
R	T
D1	D2
UNT	UNT
T	R
D2	D1
R	T
D2	D1

WRIST

The specific pattern of skin sites, i.e., medial (ulnar) to lateral (radial), and superior to inferior, should be described in the study report/study protocol.

c) The staggered application with synchronized removal method consistent with the methodology used in the pilot study should be used for D_1, D_2, and ED_{50} dose durations.

d) Refer to section III.I.1(n) Assessment of baseline skin color and skin blanching at each site.

J. Data Analyses and Pharmacodynamic Modeling

1. AUEC Calculation for the Pilot and Pivotal Studies

a) Adjust (by subtraction) the chromameter raw data of each skin blanching response versus time profile (both active drug sites and untreated control sites) for the baseline value at that site. Correct each baseline-adjusted active drug site for the mean of the two baseline-adjusted untreated control sites on the same arm.

b) Using the trapezoidal rule, compute the AUEC for each baseline-adjusted, untreated control site -corrected dose duration (see Appendix III):

$AUEC_{(0\text{-}t)}$ for the staggered application with synchronized removal method
0: within 15 minutes after drug removal
t: at least 24 hours after drug removal

2. Pharmacodynamic Modeling for the Pilot Study

a) Fitting dose duration-response data by averaging across subjects at each dose duration is not recommended. Rather, the data should be fitted by using all observations of all individual subjects simultaneously using nonlinear mixed effects modeling. The modeling software should provide population estimation for ED_{50} and E_{max} parameters for the data from at least 12 subjects.

493
494 b) Determine the ED_{50} (the dose duration corresponding to half-maximal response).
495
496 c) Determine D_1 and D_2 corresponding to approximately one-half ED_{50} and two
497 times ED_{50} (for simple E_{max} model used), respectively, for use in the pivotal
498 study.[14] These values bracket ED_{50}, correspond to approximately 33% and 67%
499 respectively of the maximal response, and represent the sensitive portion of the
500 dose duration-response curve.
501
502 *3. Data Analysis for the Pivotal Study*
503
504 a) Only the data of *detectors* should be included in the data analysis. The dose
505 duration-response criterion to define detector is:
506
507 $$\frac{AUEC\ at\ D_2}{AUEC\ at\ D_1} \geq 1.25$$
508
509 AUEC at D_2=average of AUECs at D_2 from both left arm and right arm
510 AUEC at D_1=average of AUECs at D_1 from both left arm and right arm
511
512 b) The bioequivalence comparison should be based on AUEC values computed
513 according to Appendix III at the dose duration corresponding approximately to ED_{50}
514 (treatments T and R).
515 i. The statistical analysis requires the use of untransformed data because
516 AUEC values of treatments T and R, calculated from baseline-adjusted,
517 untreated control site-corrected data, are generally negative, although
518 sometimes positive. The presence of both positive and negative data
519 prevents the use of conventional statistical transformations. Locke's
520 method[15] provides an exact confidence interval from untransformed data.
521
522 ii. Using data from the detectors, the 90% confidence interval should be
523 calculated for the ratio of the average AUEC (e.g., $AUEC_{0\text{-}24hr}$) response
524 due to the test product (average of four replicates) to the average AUEC
525 (e.g., $AUEC_{0\text{-}24hr}$) response due to the reference product (average of four
526 replicates) should be calculated using Locke's method. The formulae and a
527 worked example based on the data are given in Appendix V.
528
529 The 90% confidence interval for the test to reference AUEC ratio should
530 be within the 80.00-125.00% interval.
531

[14] The estimated ED_{50} value may be rounded by up to 15 minutes to obtain the ED_{50} value used in the pivotal study. For potent corticosteroids with short ED_{50} values, these recommendations may require adjustment. If so, FDA may be consulted via a controlled correspondence or, for relevant complex products, via a pre-ANDA meeting.

[15] Locke CS. An exact confidence interval from untransformed data for the ratio of two formulation means. J Pharmacokinet Biopharm 1984;12:649-55.

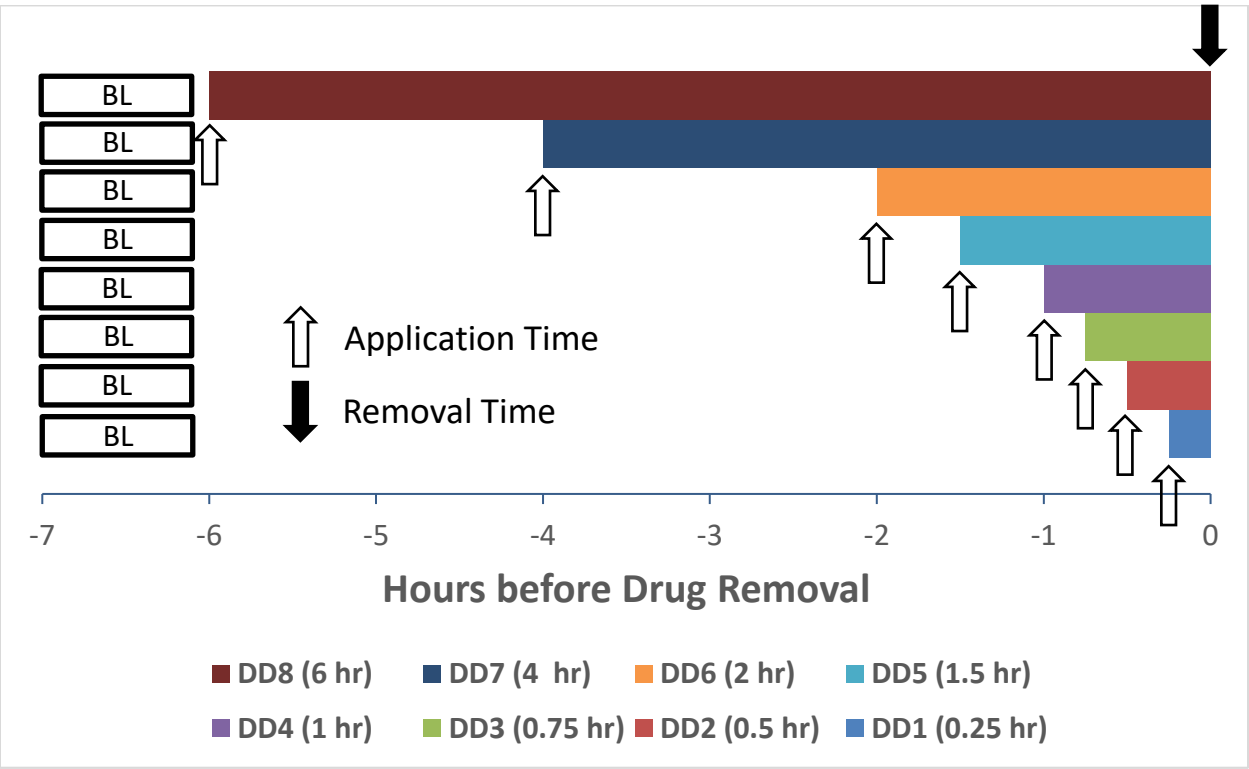
4. Formatted Data Submission

The study data for the pilot and pivotal studies should be submitted, as recommended by the Agency, in the following format: https://www.fda.gov/media/87599/download. Chromameter raw data; baseline-adjusted data; baseline-adjusted, untreated control site-corrected data; and AUEC data should be arranged in separate files.

All study data, including the data of *nondetectors*, should be submitted. An explanation (e.g., *nondetector*, overlap of vasoconstrictor effect due to an adjacent site, etc.) should accompany any data not used in the vasoconstrictor study evaluation. The randomization code, indicating the specific skin sites to which each dose duration and control site was assigned, should be submitted with the study report.

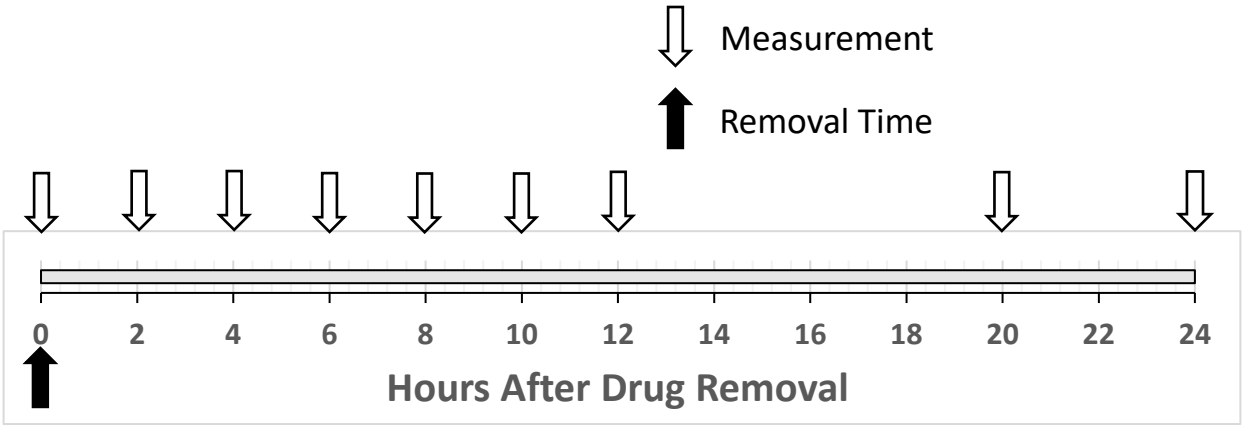
APPENDIX I: SCHEMATIC FOR STAGGERED APPLICATION WITH SYNCHRONIZED REMOVAL FOR PILOT STUDY PROTOCOLS

Figure A1: Example of Baseline (BL) Measurement, Drug Application and Drug Removal



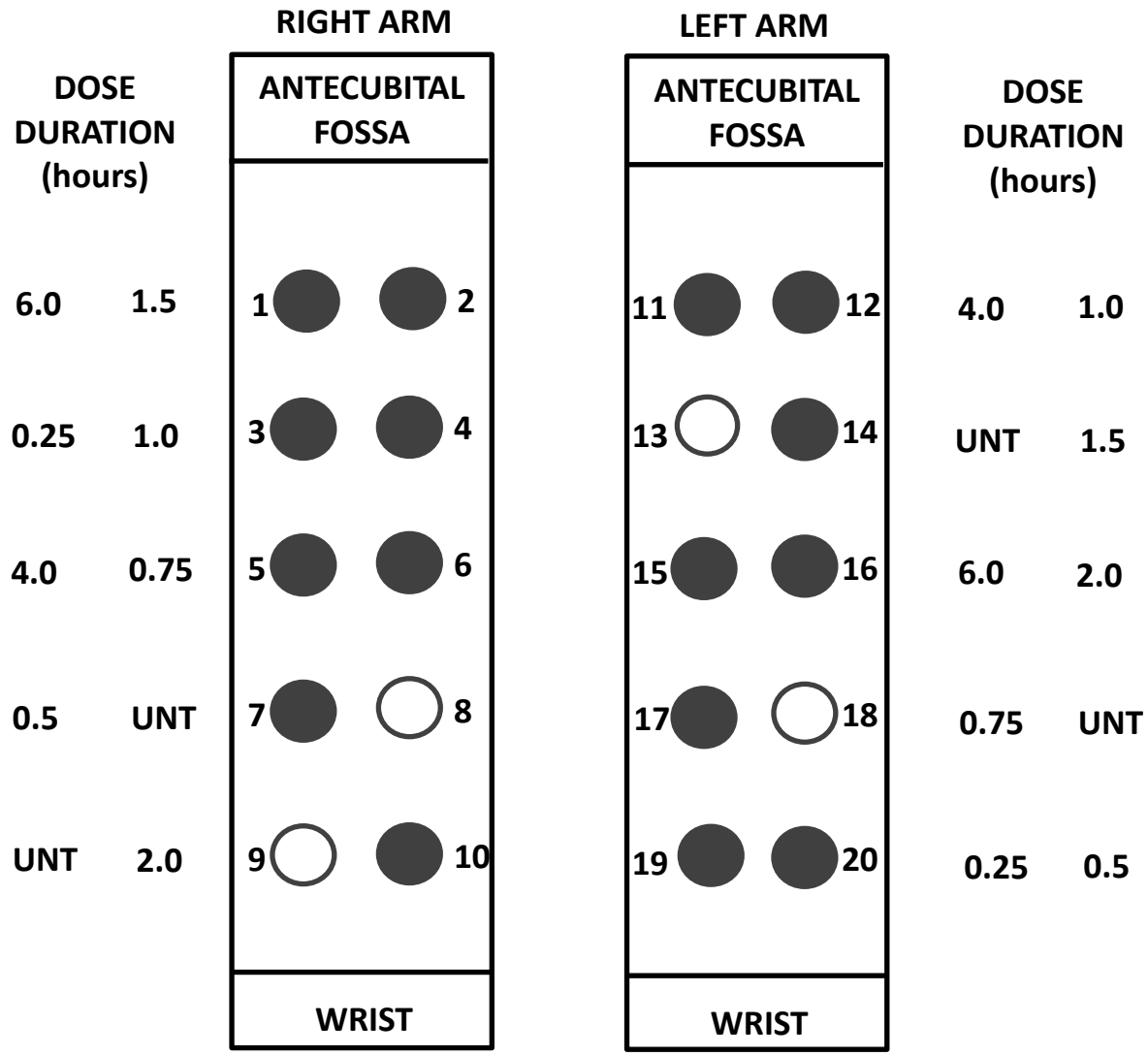
DD: Dose Duration

Figure A2: Skin Blanching Measurements



Note: Time zero (0) is defined as the within 15 minutes after drug product removal.

APPENDIX II: EXAMPLE FOR SKIN BLANCHING STUDY DESIGN FOR PILOT DOSE-DURATION RESPONSE STUDY



Light circle: untreated site; Dark circle: treated site with different dose-duration

Dose duration of 0.25 to 6.0 hours represent times for exposure of skin to the reference standard.

APPENDIX III: CALCULATION OF AUEC

Step 1. Calculate baseline-adjusted, untreated control site-corrected a-scale data ($C_{i,j}$) for each corresponding treated site:

$$C_{i,j} = A_{i,j} - A_{0,j} - A_{i,0}$$

where i is i th measurement after drug removal (hours): e.g., from 0 hr to t (at least 24 hr);
j is the j th dose duration: from dose duration DD_1 to last dose duration DD_n;
$A_{i,j}$ is the raw a-scale data site reading for each corresponding treated site for j th dose duration at time i after drug removal;
$A_{0,j}$ is baseline (pre-dose) reading within one hour prior to drug application of the longest dose duration;
and $A_{i,0}$ is mean of untreated control site reading at time i after drug removal of the same arm.

Step 2. AUEC calculation from the baseline-adjusted and untreated control site-corrected a-scale data ($C_{i,j}$) for the test topical corticosteroid and reference standard for all subjects.

$$AUEC_{t0}^{t_{last}} = \sum_{i=1}^{n} \frac{C_i + C_{i+1}}{2} * \Delta t_i$$

where t_0 denotes the time of first measured pharmacodynamic response, e.g., 0.25 hr after drug removal;
$\Delta t_i = t_{i+1} - t_i$ and t_{last} denotes the time of the last measured pharmacodynamic response

APPENDIX IV: E_{MAX} MODELS

A population modeling approach should be used to develop a simple E_{max} model as shown below, because the E_{max} model needs to account for between-subject variability. Naïve pools (all subjects pooled as one) are no longer recommended by FDA.

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

E is the response (baseline-adjusted, untreated control site-corrected AUEC) at the dose duration of application (D), E_{max} is the maximal response, and ED_{50} is the duration at which half-maximal response occurs.

Alternative sigmoidal models can be used with justifications and appropriate model selection procedures if the above E_{max} model cannot fit dose duration-response response data well. Potential alternative models are provided below:

Sigmoid E_{max} model which incorporates a Hill coefficient γ:

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

Note: D_1 and D_2 should be adjusted using the following equations: $D_1 = (f_1)^{\frac{1}{\gamma}} \times ED_{50}, f_1 \approx \frac{1}{2}$; $D_2 = (f_2)^{\frac{1}{\gamma}} \times ED_{50}, f_2 \approx 2$.

Other alternative models may be acceptable with sufficient justification. For detailed information about population modeling, model verification/validation and E_{max} models, refer to the following guidance and publications:

- Guidance for industry *Population Pharmacokinetics* (February 2022)
- Guidance for industry *Exposure-Response Relationships – Study Design, Data Analysis, and Regulatory Applications* (April 2003)
- Deniz Ozdin, Naveen Sharma, Jorge Lujan-Zilbermann, Philippe Colucci, Isadore Kanfer, Murray P Ducharme, Revisiting FDA's 1995 Guidance on Bioequivalence Establishment of Topical Dermatologic Corticosteroids: New Research Based Recommendations, J PharmSci. 2018;21(1):413-28.
- RN Upton and DR Mould. Basic Concepts in Population Modeling, Simulation, and Model-Based Drug Development: Part 3—Introduction to Pharmacodynamic Modeling Methods. CPT Pharmacometrics Syst Pharmacol. 2014 Jan; 3(1): e88.

APPENDIX V: LOCKE METHOD FOR BIOEQUIVALENCE ASSESSMENT AND A WORKED EXAMPLE

The calculation of the 90% confidence interval for the pivotal bioequivalence data set of Table 1 (Mean AUEC Values of Subjects in the Pivotal Study) is given below. The data used to calculate the confidence interval are the average baseline-adjusted and untreated control site-corrected AUEC values of 'detectors'.

The calculation of the confidence interval is facilitated by the calculation of the following intermediate quantities:

$$\overline{X}_T = \frac{1}{n}\sum_{i=1}^{n} X_{T_i}$$

$$\overline{X}_R = \frac{1}{n}\sum_{i=1}^{n} X_{R_i}$$

$$\hat{\sigma}_{TT} = \frac{\sum_{i=1}^{n}\left(X_{T_i} - \overline{X}_T\right)^2}{n-1}$$

$$\hat{\sigma}_{RR} = \frac{\sum_{i=1}^{n}\left(X_{R_i} - \overline{X}_R\right)^2}{n-1}$$

$$\hat{\sigma}_{TR} = \frac{\sum_{i=1}^{n}\left(X_{T_i} - \overline{X}_T\right)\left(X_{R_i} - \overline{X}_R\right)}{n-1}$$

where n is the number of evaluable subjects,

And define t as the 95th percentile of the t-distribution for n-1 degrees of freedom, then define:

$$G = \frac{t^2\,\hat{\sigma}_{RR}}{n\,\overline{X}_R^2}$$

$G < 1$ is required to have a proper confidence interval. If $G \geq 1$, the study does not meet the in vivo bioequivalence requirements.

Under the assumption that $G < 1$, calculate:

$$K = \left(\frac{\overline{X}_T}{\overline{X}_R}\right)^2 + \frac{\hat{\sigma}_{TT}}{\hat{\sigma}_{RR}}(1-G) + \frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\left(G\frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}} - 2\frac{\overline{X}_T}{\overline{X}_R}\right)$$

The confidence interval limits may now be calculated:

$$\frac{\left(\frac{\bar{X}_T}{\bar{X}_R} - G\frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\right) \mp \frac{t}{\bar{X}_R}\sqrt{\frac{\hat{\sigma}_{RR}}{n}K}}{1 - G}$$

Table 1. Mean AUEC Values of Subjects in the Pivotal Study

Subject	AUEC(0-t) Test Product (Average)	AUEC(0-t) Reference Product (Average)
2	-48.52	-22.20
3	-38.99	-18.65
4	-7.62	-22.42
7	0.98	-10.96
9	-32.05	-37.40
11	-26.18	-26.73
12	-11.62	-12.56

For the example, these are $\bar{X}_T$ = -23.43, $\bar{X}_R$ = -21.56, $\hat{\sigma}_{TT}$ = 323.13, $\hat{\sigma}_{RR}$ = 80.10, and $\hat{\sigma}_{TR}$ = 78.83.

In the example, for n = 7, t (6 degrees of freedom) is 1.9432. G = 0.0930 <1, then K = 2.791.

Based on the data of evaluable subjects, the 90% confidence interval limits are 53.6% and 165.9%, which are not within the acceptable limits of 80.00- 125.00%. .