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Draft Guidance on Metronidazole

October 2022

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Active Ingredient: Metronidazole

Dosage Form; Route: Gel; vaginal

Recommended Studies: Two options: (1) one in vitro bioequivalence study and other characterization tests or (2) one in vivo bioequivalence study with pharmacokinetic endpoints and one in vivo bioequivalence study with clinical endpoint

I. Option 1: One in vitro bioequivalence study and other characterization tests

To demonstrate bioequivalence for metronidazole vaginal gel, 0.75% using in vitro studies, the following criteria should be met:

1. The test product should contain no difference in inactive ingredients or in other aspects of the formulation relative to the reference standard that may significantly affect the local or systemic availability of the active ingredient. For example, if the test product and reference standard are qualitatively (Q1) and quantitatively (Q2) the same, as defined in the most recent version of the FDA guidance for industry on *ANDA Submissions – Refuse-to-Receive Standards*[a], and the criteria below are also satisfied, the bioequivalence of the test product may be established using a characterization-based bioequivalence approach.

2. The test product and reference standard should have the same physicochemical and structural (Q3) attributes, based upon acceptable comparative Q3 characterization tests with a minimum of three batches of the test product and three batches (as available) of the reference standard. The test product and reference standard batches should ideally represent the product at different ages throughout its shelf life. Refer to the most recent version of the FDA guidance for industry on *Physicochemical and Structural (Q3)*

Characterization of Topical Drug Products Submitted in ANDAs[a] for additional information regarding comparative Q3 characterization tests. The comparison of the test product and reference standard should include characterizations of the following Q3 attributes:

a. Characterization of visual appearance and texture
b. Characterization of phase states and structural organization of matter
    - Microscopic examination with representative high-resolution microscopic images at multiple magnifications
c. Characterization of rheological behavior which may be characterized using a rheometer that is appropriate for monitoring the non-Newtonian flow behavior of semi-solid dosage forms. The following evaluations are recommended:
    - A characterization of shear stress vs. shear rate and viscosity vs. shear rate. At minimum, this should consist of numerical viscosity data at three shear rates (low, medium, and high).
    - A complete flow curve across the range of attainable shear rates, until low or high shear plateaus are identified.
    - Yield stress values should be reported if the material tested exhibits plastic flow behavior.
d. Characterization of pH
e. Characterization of specific gravity
f. Characterization of any other potentially relevant Q3 attributes

3. The test product and reference standard should have an equivalent rate of metronidazole release based upon an acceptable in vitro release test (IVRT) bioequivalence study comparing a minimum of one batch each of the test product and reference standard using an appropriately validated IVRT method.

    Type of study: Bioequivalence study with IVRT endpoint
    Design: Single-dose, two-treatment, parallel, multiple-replicate per treatment group study design using an occluded pseudo-infinite dose, in vitro
    Strength: 0.75%
    Test system: A synthetic membrane in a diffusion cell system
    Analyte to measure: Metronidazole in receptor solution
    Equivalence based on: Metronidazole (IVRT endpoint: drug release rate)
    Additional comments: The IVRT study should be conducted at 37°C based on the route of administration of this drug product. Refer to the most recent version of the FDA guidance for industry on *In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs*[a] for additional information regarding the development, validation, conduct and analysis of acceptable IVRT methods/studies. The batches of test product and reference standard evaluated in the IVRT bioequivalence study should be included among those for which the Q3 attributes are characterized.

II. Option 2: One in vivo bioequivalence study with pharmacokinetic endpoints and one in vivo bioequivalence study with clinical endpoint

1. Type of study: Bioequivalence study with pharmacokinetic endpoints
   Design: Single-dose, two-way crossover, in vivo
   Strength: 0.75% [dose: one applicator full (5 grams containing approximately 37.5 mg of metronidazole in the to-be-marketed or currently marketed applicator provided with the product), administered intravaginally]
   Subjects: Non-pregnant, non-lactating females, general population
   Analyte to measure: Metronidazole in plasma
   Equivalence based on: Metronidazole
   Additional comments: No sexual intercourse or use of spermicides, tampons, douches, diaphragms, or condoms or insertion into the vagina of any drug or non-drug product are permitted within 48 hours of dosing. Exclude subjects with any vulvar or vaginal condition that may affect drug absorption (e.g., vulvovaginitis). Measure applicator weight after filling and after dosing to calculate weight of dose. Subjects should remain supine for at least 4 hours after dosing. The study conditions should be consistent across the study and the bioanalytical method should be sufficiently sensitive to be able to adequately characterize the pharmacokinetic profiles of the test product and reference standard. Refer to the most recent version of the FDA guidance for industry on *Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA*[a] for additional information regarding the analysis of the bioequivalence study with pharmacokinetic endpoints.

2. Type of study: Bioequivalence study with clinical endpoint
   Design: Randomized, double blind, parallel, placebo controlled, in vivo
   Strength: 0.75%
   Subjects: Non-pregnant, non-lactating females with bacterial vaginosis
   Additional comments: Specific recommendations are provided below.

Additional comments related to the bioequivalence study with clinical endpoint:

1. FDA recommends a bioequivalence study with clinical endpoint in the treatment of non-pregnant female subjects with a confirmed clinical diagnosis of bacterial vaginosis (BV). Subjects are to be randomized to receive the test metronidazole vaginal gel, 0.75%, the reference standard, or placebo as one applicator full (approximately 5 grams containing approximately 37.5 mg of metronidazole in the to-be-marketed or currently marketed applicator provided with the product) administered intravaginally once daily at bedtime for 5 days.

2. Inclusion Criteria (the sponsor may add additional criteria):
   a. Non-pregnant, non-lactating females age ≥ 18 years.
   b. Diagnosis of BV, defined as the presence of all of the following criteria:
      - Clinical diagnosis of BV (e.g., off-white or gray, thin, homogenous vaginal discharge associated with minimal or absent pruritus inflammation

- Saline wet mount of vaginal discharge demonstrating the proportion of clue cells to be ≥ 20% of the total epithelial cells.
- Vaginal pH > 4.5, using pH paper that measures from 4.0-6.0.
- Positive "whiff test" (after addition of a drop of 10% potassium hydroxide (KOH) to vaginal discharge).
- Gram stain Nugent score ≥ 7 on first day of dosing (study Day 1).

c. Any subject with childbearing potential has a negative urine pregnancy test on the first day of dosing (study Day 1) using a pregnancy test with a sensitivity of at least 25 mIU/mL hCG.
d. Willing to refrain from using any intra-vaginal product or device other than the study treatment (e.g., other vaginal drugs, spermicide, tampon, douche, diaphragm, condom, or other objects) on study days when study treatment is administered, for 48 hours prior to the first dose of study product, and for 48 hours prior to Test of Cure visit.
e. Agrees to abstain from sexual intercourse on study days when study treatment is administered.
f. Willing to refrain from alcohol ingestion on study Days 1-6.

3. Exclusion Criteria (the sponsor may add additional criteria):
   a. Pregnant, lactating, or planning to become pregnant during the study period.
   b. Menstruating at the baseline visit (when evaluation for BV is performed) or anticipate onset of menses during study drug administration.
   c. Primary or secondary immunodeficiency.
   d. Severe liver disease.
   e. Central nervous system disease.
   f. Evidence of any vulvovaginitis other than BV (e.g., candidiasis, *Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae, Herpes simplex,* or human papilloma virus).
   g. Subject with another vaginal or vulvar condition, which would confound the interpretation of clinical response.
   h. Subject will be under treatment during the study period for cervical intraepithelial neoplasia or cervical carcinoma.
   i. History of hypersensitivity or allergy to metronidazole, parabens, other nitroimidazole derivatives or other ingredients of the formulation.
   j. Use of any of the following medications within 2 weeks of the baseline visit:
      - Systemic steroids (oral or injectable).
      - Disulfiram.
      - Lithium.
      - Topical or systemic antibiotics.
      - Topical or systemic antifungal.
      - Topical or systemic antiparasitic.
   k. Use of intra-vaginal product or device (e.g., other vaginally administered drugs, spermicide, tampon, douche, diaphragm, condom, other objects) within 48 hours of study Day 1 dosing.
   l. Current use of anticoagulation therapy or cimetidine.

4. At the baseline visit, documentation of the participant's medical history should include menopausal status. For postmenopausal women, document the month and year of the last menses. For premenopausal women, documented information should include: the first day of the last menstrual period, regularity of menses, use of contraception, past episodes of BV, and sexual history (e.g., sex of intimate partners and history of sexually transmitted infections).

5. The protocol should include a list of prescription and over-the-counter drug products that are prohibited during the study, such as:
    a. Any anticoagulation therapy.
    b. Systemic corticosteroid or immunosuppressive drugs.
    c. Systemic or topical antibiotics, other than study product.
    d. Cimetidine.
    e. Lithium.
    f. Any product inserted into the vagina during treatment (e.g., on study Days 1-5) and for 48 hours prior to Test of Cure visit.
    g. Subjects should be instructed not to engage in vaginal intercourse during treatment (e.g., on study Days 1-5). Subjects should be cautioned about drinking alcohol during treatment.

6. The primary endpoint of the study is the responder rate, defined as both a clinical cure (resolution of the abnormal vaginal discharge, a negative whiff (amine) test for any amine "fishy" odor (KOH Test), and the presence of clue cells at less than 20% of the total epithelial cells on microscopic examination of the saline wet mount)[1], AND a bacteriological cure (Nugent score <4), evaluated at the Test of Cure visit (study Day 22-30).

7. Subjects who used any BV therapy, other than study product, during the study or had a Nugent score ≥4 at the Test of Cure visit should be considered therapeutic failures.

8. Provide the Subject-Level Analysis Dataset (ADSL), one record per subject, using the following headings, if applicable:
    a. Study identifier
    b. Unique subject identifier
    c. Subject identifier for the study
    d. Study site identifier (if applicable)
    e. Age
    f. Age units (years)
    g. Sex
    h. Race
    i. Name of planned treatment
    j. Name of actual treatment
    k. Safety population flag (yes/no)
    l. Reason for exclusion from safety population

[1] Note that the inclusion criteria use all four Amsel criteria but only three are used to support the primary study endpoint.

m. Modified Intent-to-Treat (mITT) population flag (yes/no)
n. Reason for exclusion from mITT population
o. Per-Protocol (PP) population flag (yes/no)
p. Reason for exclusion from PP population
q. Randomized population flag (yes/no)
r. Date/time of first exposure to treatment
s. Date/time of last exposure to treatment
t. End of study date
u. End of study status
v. Subject required additional treatment due to unsatisfactory treatment response (yes/no)
w. Baseline vaginal discharge consistent with clinical diagnosis BV (yes/no)
x. Baseline clue cells on wet mount (≥20%, <20%, or none)
y. Baseline vaginal pH
z. Baseline KOH "whiff test" (positive/negative)
aa. Baseline Nugent score
bb. Chlamydia trachomatis (positive/negative)
cc. Neisseria gonorrhoeae test, (positive/negative)
dd. Urine pregnancy test (positive/negative)
ee. Clinical cure (Day 22-30) (yes/no)
ff. Normal physiological vaginal discharge (Day 22-30) (yes/no)
gg. KOH "whiff test" (Day 22-30) (positive/negative)
hh. Clue cells on wet mount (Day 22-30) (≥20%, <20%, or none)
ii. Bacteriological cure (Day 22-30) (yes/no)
jj. Nugent score (Day 22-30) (0, 1, 2, 3, or 4)
kk. Treatment success (Day 22-30) (responder) (yes/no)
ll. Compliance rate (%)
mm. Subject missed the pre-specified number of scheduled doses for more than pre-specified number of consecutive days (yes/no)
nn. Adverse event reported (yes/no)
oo. Concomitant medication (yes/no)

9. Provide the basic data structure (BDS) dataset with records per subject, per visit, per analysis timepoint, using the following headings, if applicable:
    a. Study identifier
    b. Unique subject identifier
    c. Subject identifier for the study
    d. Study site identifier (if applicable)
    e. Name of planned treatment
    f. Name of actual treatment
    g. Safety population flag (yes/no)
    h. Modified ITT population flag (yes/no)
    i. Per-Protocol (PP) population flag (yes/no)
    j. Analysis date
    k. Analysis visit
    l. Study visit within the designated window (yes/no)

m. Analysis timepoint (e.g., hour 0, hour 2) (if applicable)
n. Abnormal vaginal discharge (yes/no)
o. Clinical cure (yes/no)
p. Normal physiological vaginal discharge (yes/no)
q. KOH "whiff test" (positive/negative)
r. Clue cells on wet mount (≥20%, <20%, or none)
s. Bacteriological cure (yes/no)
t. Nugent score (0, 1, 2, 3, or 4)
u. Treatment success (responder) (yes/no)
v. Additional treatment required during the visit (yes/no)
w. Adverse event reported during the visit (yes/no)
x. Use of any vaginal products other than study product (yes/no)
y. Concomitant medication during the visit (yes/no)

10. Refer to the most recent version of the FDA product-specific guidance on *Adapalene; Benzoyl Peroxide Topical Gel* (NDA 207917)[b] for a recommended approach to statistical analysis and study design for bioequivalence studies with clinical endpoint.

11. Refer to the study data standards resources, https://www.fda.gov/industry/fda-resources-data-standards/study-data-standards-resources.

Additional information:

Device:
The reference listed drug (RLD) product is presented in a tube copackaged with five vaginal applicators that can attach to the opening of the tube. The vaginal applicators are the device constituents used to measure and administer the drug dose.

FDA recommends that prospective applicants examine the size and shape, external critical design attributes, and external operating principles of the RLD device when designing the Test (T) device including:

- End of vaginal applicator can attach to opening of drug container to fill the applicator with the correct dose
- Vaginal applicators can be disassembled and washed with soap and water

User interface assessment:
An ANDA for this product should include complete comparative analyses so FDA can determine whether any differences in design for the user interface of the proposed generic product, as compared to the Reference (R) device, are acceptable. and whether the product can be expected to have the same clinical effect and safety profile as the RLD when administered to patients under the conditions specified in the labeling.

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Unique Agency Identifier: PSG_020208

[a] 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.
[b] For the most recent version of a product-specific guidance, check the FDA product-specific guidance web page at https://www.accessdata.fda.gov/scripts/cder/psg/index.cfm.