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Draft Guidance on Nitroglycerin

November 2024

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Active Ingredient:	Nitroglycerin
Dosage Form:	Ointment
Route:	Intra-anal
Strength:	0.4%
Recommended Studies:	Two options: (1) one in vitro bioequivalence study and other characterization tests or (2) one in vivo bioequivalence study with clinical endpoint

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

To demonstrate bioequivalence for nitroglycerin intra-anal ointment, 0.4% 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 (RS) that may significantly affect the local or systemic availability of the active ingredient. For example, if the test product and RS 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 RS 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 RS. The test product and RS 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 RS 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
        - Analysis of globule size distribution
    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.
        - The linear viscoelastic response (storage and loss modulus vs. frequency) should be measured and reported. Any non-linear viscosity behavior over a range of shear rates should also be investigated, measured, and reported.
    d. Characterization of oleaginous components
    e. Characterization of specific gravity
    f. Characterization of any other potentially relevant Q3 attributes

3. The test product and RS should have an equivalent rate of nitroglycerin release based upon an acceptable in vitro release test (IVRT) bioequivalence study comparing a minimum of one batch each of the test product and RS 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: 0.4%
    Test system: A synthetic membrane in a diffusion cell system
    Analyte to measure: Nitroglycerin in receptor solution
    Bioequivalence based on: Nitroglycerin (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 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 RS 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 clinical endpoint

1. Type of study: Bioequivalence study with clinical endpoint
   Design: Randomized, double-blind, parallel, three-arm, placebo-controlled in vivo
   Strength: 0.4%
   Subjects: Male and non-pregnant, non-lactating female adults with chronic anal fissure
   Additional comments: Specific recommendations are provided below.

Additional comments regarding the bioequivalence study with clinical endpoint:

1. FDA recommends conducting a bioequivalence study with a clinical endpoint in the treatment of moderate to severe pain associated with chronic anal fissure. Subjects should be randomized to receive a test product of nitroglycerin ointment, the RS, or placebo. One inch of study drug is to be administered intra-anally every 12 hours for up to 3 weeks. The primary endpoint is the change in pain intensity measured in mm on the Visual Analog Scale (VAS). All subjects should take two 325 mg acetaminophen tablets (650 mg) approximately 30 minutes before application of the study product because they may need to use a medication for the management of pain (e.g., headache) associated or not associated with the use of nitroglycerin.

2. Inclusion criteria (the applicant may add additional criteria):
   a. Male and non-pregnant, non-lactating female adults aged 18 to 75 years
   b. Have a single, chronic, posterior midline anal fissure defined as having anal pain for the 6 weeks prior to Screening and showing the presence of at least 1 of the following: sentinel skin tag, hypertrophied anal papillae, exposed internal anal sphincter or fibrotic fissure margins or fibrotic anal sphincter
   c. 24-hour average pain VAS of at least 50 mm at Day 0 visit

3. Exclusion criteria (the applicant may add additional criteria):
   a. Have more than one anal fissure, an anal fistula, or an anal abscess
   b. Have inflammatory bowel disease
   c. Have fibrotic anal stenosis
   d. Have an anal fissure secondary to an underlying condition (e.g., human immunodeficiency virus, tuberculosis, syphilis)
   e. Had any anal surgery
   f. Had previous or current pelvic radiation treatment
   g. Have severe and chronic illness which may have put the patient at risk when participating in the study or may have influenced the results of the study
   h. Have acute or chronic renal and/or hepatic impairment

i. Have clinically significant, abnormal laboratory results, electrocardiogram, and vital signs
j. Pregnant, lactating, or planning a pregnancy
k. Subjects are allergic to nitroglycerin, other nitrates and nitrites, any components of the test product and RS or acetaminophen
l. Have hypotension or uncorrected hypovolemia, increased intracranial pressure (e.g., head trauma or cerebral hemorrhage) or inadequate cerebral circulation, cardiomyopathies, congestive heart failure, acute myocardial infarction, or poor cardiac function for other reasons, severe anemia, or closed-angle glaucoma
m. Taking nitroglycerin or any other nitric oxide (NO) donors (e.g., arginine), phosphodiesterase type 5 (PDE5) inhibitors (e.g., sildenafil, vardenafil and tadalafil), potassium channel blockers (e.g., nicorandil) or calcium channel blockers (e.g., nifedipine)
n. Use of non-prescription or prescription drugs for the treatment of anal fissure during the study (except for fiber supplements and stool softeners)
o. Use of non-steroidal anti-inflammatory drugs (NSAIDs) including cyclooxygenase 2 inhibitors (e.g., ibuprofen, naproxen, ketoprofen), and aspirin (except daily low-dose aspirin [up to 162 mg per day] for cardiovascular prophylaxis) or any other analgesic for the treatment of headache or any other condition
p. Use of acetaminophen (other than that provided for use in the study) and any other acetaminophen-containing product for the treatment of headache or any other condition
q. Have a history of or ongoing migraine and chronic headaches or any other chronic pain

4. The primary endpoint is the change in 24-hour average pain intensity measured in mm on the VAS from baseline (Day 0) to average of Days 14 to 18 of treatment.

5. The subjects should take acetaminophen (twice per day with 12 hours interval; approximately 30 minutes before application of the study product) from Day 0 prior to the use of the study product and during the study. Baseline VAS score should be recorded following the administration of acetaminophen at Day 0 to adjust the effect of acetaminophen on the management of pain associated with chronic anal fissure.

6. The protocol should include a list of the prescription and over-the-counter drug products, procedures, and activities that are prohibited during the study, such as any new therapy for treatment of pain (e.g., oral, topical, or parenteral NSAIDs, aspirin or narcotic pain medication), NO donors (e.g., arginine), PDE5 inhibitors (e.g., sildenafil, vardenafil and tadalafil), potassium channel blockers (e.g., nicorandil) or calcium channel blockers (e.g., nifedipine).

7. Patients are advised or allowed to adopt conservative care consisting of fiber supplements, adequate fluid intake, and sitz baths. If patients are on a stable dose of stool softener prior to the study, they can continue on that dose for the duration of the

study. Patients need to record their daily number of sitz baths. Patients should not take a sitz bath until at least one hour after treatment application to allow absorption of the ointment.

8. The application method of the study product should be instructed to subjects by un-blinded study staff. Subjects should also be informed of the possible side effects (e.g., headaches and dizziness) following the use of nitroglycerin ointment and the medication of acetaminophen prior to the application of the study product.

9. Applicants should 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. Actual treatment (character)
    l. Safety population flag (yes/no)
    m. Reason for exclusion from safety population
    n. Modified Intent-to-Treat (mITT) population flag (yes/no)
    o. Reason for exclusion from mITT population
    p. Per-Protocol (PP) population flag (yes/no)
    q. Reason for exclusion from PP population
    r. Randomized population flag (yes/no)
    s. Date/time of first application to treatment
    t. Date/time of last application to treatment
    u. End of study date
    v. End of study status
    w. Subject required additional treatment due to unsatisfactory treatment response (yes/no)
    x. VAS (in mm) on Day 0 (baseline)
    y. VAS (in mm) on Days 7
    z. VAS (in mm) on Days 14
    aa. VAS (in mm) on Days 15
    bb. VAS (in mm) on Days 16
    cc. VAS (in mm) on Days 17
    dd. VAS (in mm) on Days 18
    ee. Compliance rate (%) of the study product
    ff. Compliance rate (%) of acetaminophen
    gg. Subject missed the pre-specified number of scheduled doses for more than pre- specified number of consecutive days (yes/no)

hh. Subject missed the pre-specified number of scheduled acetaminophen doses for more than pre-specified number of consecutive days (yes/no)
ii. Concomitant medication (yes/no)
jj. Adverse event(s) reported (yes/no)

10. Applicants should provide basic data structure dataset (BDS) 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. mITT population flag (yes/no)
    i. 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. Evaluator: identity of evaluator
    o. VAS (in mm) (e.g., for Day 0 (baseline), Day 7, 14, 15, 16, 17, and 18)
    p. Concomitant medication reported during the study (yes/no)
    q. Additional treatment required during the study (yes/no)
    r. Adverse event reported during the study (yes/no)
    s. Laboratory testing during the study (yes/no)

11. Refer to the most recent version of the 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.

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

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[a] For the most recent version of a guidance, check the FDA guidance website 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 website at https://www.accessdata.fda.gov/scripts/cder/psg/index.cfm.