Draft Guidance on Estradiol

December 2025

Active Ingredient:	Estradiol
Dosage Form:	Insert
Route:	Vaginal
Strengths:	0.004 mg, 0.01 mg
Recommended Studies:	Two options: (1) one in vitro bioequivalence study, one in vivo bioequivalence study with pharmacokinetic endpoints, and other characterization tests or (2) one in vivo bioequivalence study with pharmacokinetic endpoints and one comparative clinical endpoint bioequivalence study

I. Option 1: One in vitro bioequivalence study, one in vivo bioequivalence study with pharmacokinetic endpoints, and other characterization tests

To demonstrate bioequivalence for estradiol vaginal insert using a combination of in vitro studies and an in vivo bioequivalence study with pharmacokinetic endpoints, 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) characteristics, based upon acceptable comparative Q3 characterization of a minimum of three batches of the test product and three batches (as available) of the RS. The RS contains two components: a "shell" that encapsulates a "fill" formulation. The comparative Q3 characterization should be conducted with (1) the test product and RS and (2) the fill formulation of the test product and RS. The test product and RS batches should ideally represent the product at different ages throughout its shelf life. The comparison of the test product and RS should include characterizations of the following Q3 attributes:
    a. Characterization of visual appearance, phase states, and texture.
    b. Characterization of mechanical properties of the gelatin shell (i.e., rupture test, and shell and seam line thickness).
    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. Rheological behavior of the test product and RS should be assessed at 37°C following emulsification of the drug product in a biorelevant medium.
        - 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 comparison of the test product fill formulation and RS fill formulation should include characterization 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. Thermal characterization of the fill formulation (e.g., evaluation of the phase states of the fill formulation as a function of temperature, such as crystal habit and melting behavior of any undissolved particles).
    d. Characterization of rheological behavior which may be characterized using a rheometer that is appropriate for monitoring the non-Newtonian flow behavior of the fill formulation. Rheological behavior of the test product and RS should be assessed at 37 °C following emulsification of the fill formulation in a biorelevant medium.
        - 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.

e. Characterization of specific gravity.

3. The test product and RS should have acceptable estradiol release. The study should be conducted using an acceptable in vitro dissolution bioequivalence study comparing a minimum of one batch each of the test product and RS using an appropriately validated and discriminatory in vitro drug dissolution method. The batches of test product and RS evaluated in the in vitro dissolution bioequivalence study should be included among those for which the Q3 attributes are characterized. The study should be conducted at 37°C based on the route of administration of this drug product. The study should be conducted at a physiologically relevant pH that is justified based on appropriate method development and validation studies. The method should be validated across all strengths for which dissolution data will be generated. As part of the method validation, data should be provided to illustrate that the method is sensitive to changes in critical material attributes, critical process parameters, and critical quality attributes of the drug product, as appropriate.

4. The test product and RS should demonstrate bioequivalence based upon an acceptable in vivo pharmacokinetic study with one batch each of the test product and RS.

Type of study: Bioequivalence study with pharmacokinetic endpoints
Design: Single-dose, two-treatment, two-period, crossover, fasting, in vivo
Strength: 0.01 mg (dose: 1x 0.01 mg insert)
Subjects: Healthy postmenopausal women with no contraindication to estrogen therapy
Analyte to measure: Estradiol in plasma
Bioequivalence based on (90% CI): Estradiol in plasma
Additional comments: Measure baseline estradiol levels at -1.0, -0.5, and 0 hours before dosing. The mean of the pre-dose estradiol levels should be used for the baseline adjustment of the post-dose levels. For each subject, baseline concentrations should be determined for each dosing period, and baseline adjustments should be period-specific. If a baseline correction results in a negative plasma concentration value, the value should be set to 0 prior to calculating the baseline-corrected area under the curve (AUC). Pharmacokinetic and statistical analyses should be performed on both uncorrected and corrected data. Determination of bioequivalence should be based on the baseline-corrected data. The bioanalytical method should be sufficiently sensitive to be able to adequately characterize the pharmacokinetic profiles of the test product and RS. 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. The batches of the test product and RS evaluated in the in vivo pharmacokinetic study should be the same as those evaluated in the dissolution bioequivalence study.

Waiver request: The waiver request for 0.004 mg strength of the insert product containing sufficient data may be approved based on (i) acceptable demonstration of bioequivalence of the 0.01 mg strength using the bioequivalence approach outlined within Option 1, (ii) the formulations of the lower and higher strengths of the test product are exactly the same, except for the amount of estradiol and the corresponding change in the amount of the diluent, and have the same manufacturing process, (iii) acceptable comparative Q3 characterization tests using a minimum of three batches of the lower strength of the test product and three batches of the higher strength of the test product; the relationship of the Q3 attributes of the two strengths of the test product should be compared to the relationship of the Q3 attributes of the two strengths of the RS, and (iv) an acceptable dissolution study with a minimum of one batch of each strength of the test product and one batch of each strength of the RS; the data should be generated using a dissolution method that is validated for both the lower and higher strength.

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

To demonstrate bioequivalence for estradiol vaginal insert 0.004 mg and 0.01 mg strengths using a combination of in vivo bioequivalence study with pharmacokinetic endpoints and comparative clinical endpoint bioequivalence study, the following criteria should be met: (i) acceptable in vivo bioequivalence study with pharmacokinetic endpoints on the 0.01 mg strength, (ii) acceptable comparative clinical endpoint bioequivalence study on the 0.004 mg strength, and (iii) the formulations of the lower and higher strengths of the test product are exactly the same, except for the amount of estradiol and the corresponding change in the amount of the diluent, and have the same manufacturing process.

1. Type of study: Bioequivalence study with pharmacokinetic endpoints
   Design: Single-dose, two-treatment, two-period, crossover, fasting, in vivo
   Strength: 0.01 mg (dose: 1 x 0.01 mg insert)
   Subjects: Healthy postmenopausal women with no contraindication to estrogen therapy
   Analyte to measure: Estradiol in plasma
   Bioequivalence based on (90% CI): Estradiol in plasma
   Additional comments: Refer to the "Additional comments" section of the bioequivalence study with pharmacokinetic endpoints described in Option 1.

2. Type of study: Comparative clinical endpoint bioequivalence study
   Design: Randomized, double blind, parallel, placebo-controlled in vivo
   Strength: 0.004 mg
   Subjects: Postmenopausal women with symptoms of vulvar and vaginal atrophy (VVA) and no contraindication to estrogen therapy
   Additional comments: Specific recommendations are provided below.

For applicants intending to develop only estradiol vaginal insert 0.01 mg, FDA recommends submitting questions related to the study design of the comparative clinical endpoint bioequivalence study through an appropriate communication pathway prior to conducting the study. For applicants intending to develop only estradiol vaginal insert 0.004 mg, FDA recommends submitting questions related to the study design of in vivo bioequivalence study with pharmacokinetic endpoints through an appropriate communication pathway prior to conducting the study. Refer to the most recent version of the FDA guidance for industry on *Formal Meetings Between FDA and ANDA Applicants of Complex Products Under GDUFA*[a] for additional information describing the procedures on how to clarify regulatory expectations regarding your individual drug development program.

Additional comments regarding the comparative clinical endpoint bioequivalence study:

1. FDA recommends conducting a comparative clinical endpoint bioequivalence study in the treatment of postmenopausal VVA. Subjects are to be randomized to receive the estradiol vaginal insert, 0.004 mg test product, RS, or placebo. The study treatment is to be administered intravaginally once daily for 14 days. The primary endpoint is the proportion of subjects identified as responders at Day 15.

2. Inclusion criteria (the applicant may add additional criteria):
    a. Non-smoking, postmenopausal female subjects with VVA and no contraindication to estrogen therapy.
        i. "Postmenopausal" is defined as 12 months of spontaneous amenorrhea or 6 months of spontaneous amenorrhea with serum follicle-stimulating hormone levels > 40 mIU/ml or 6 weeks postsurgical bilateral oophorectomy with or without hysterectomy.
    b. ≤ 5% superficial cells on vaginal smear cytology.
    c. Vaginal pH > 5.0.
    d. At least one patient self-assessed moderate to severe symptom of VVA from the following list that is identified by the subject as being most bothersome to her:
        i. Vaginal dryness
        ii. Vaginal and/or vulvar irritation/itching
        iii. Dysuria
        iv. Vaginal pain associated with sexual activity
        v. Vaginal bleeding associated with sexual activity
    e. Baseline systolic blood pressure should be no greater than 140 mm Hg and diastolic blood pressure no greater than 80 mm Hg.
    f. Subjects >40 years have documentation of a negative screening mammogram (obtained at screening or within 9 months of study enrollment) and a normal clinical breast examination prior to enrollment in study.
    g. Subjects with an intact uterus have baseline vaginal ultrasonography demonstrating inactive endometrial lining with endometrial thickness less than 4 mm.

3. Exclusion criteria (the applicant may add additional criteria):
    a. Male subject
    b. Premenopausal, perimenopausal, pregnant or lactating subject
    c. Undiagnosed abnormal genital bleeding
    d. Known, suspected, or history of breast cancer
    e. Known or suspected estrogen-dependent neoplasia
    f. History of endometrial cancer or risk factors for endometrial cancer
    g. Subject with tobacco use or body weight >90 kg
    h. Active deep venous thrombosis, pulmonary embolism, or a history of these conditions
    i. High risk of venous thrombosis or arterial thrombosis
    j. Active arterial thromboembolic disease (e.g., stroke and myocardial infarction), or a history of these conditions
    k. Anaphylactic reaction or angioedema with the RS
    l. Liver impairment or disease
    m. Protein C, protein S, or antithrombin deficiency, or other thrombophilic disorders
    n. History of cholestatic jaundice, hypertension, coronary heart disease or other serious heart problems, diabetes, hypercholesterolemia, hypercalcemia, hypoparathyroidism, hypertriglyceridemia, systemic lupus erythematosus, renal impairment, residual endometriosis post-hysterectomy, asthma, epilepsy, migraine, porphyria, hepatic hemangiomas
    o. History of narcotic abuse, drug abuse or alcoholism
    p. Within 6 months prior to dosing, estrogen pellet therapy or progestin injectable drug therapy
    q. Within 3 months prior to dosing, progestin implants and estrogen alone injectable drug therapy
    r. Within 8 weeks prior to dosing, oral estrogen and/or oral or intrauterine progestin therapy
    s. Within 4 weeks prior to dosing, transdermal estrogen alone or transdermal estrogen/progestin products
    t. Within 1 week prior to dosing, vaginal hormonal products (rings, creams, gels)
    u. Within 4 to 6 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization
    v. Taking thyroid hormone replacement therapy
    w. Taking inducers of CYP3A4 such as St. John's wort, anticonvulsants, phenylbutazone, rifampin, rifabutin, nevirapine and efavirenz
    x. Taking inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, ritonavir, nelfinavir and grapefruit juice

4. A listing of the prescription and over-the-counter drug products that are contraindicated during the study should be provided, such as:
    a. Antihypertensives and pressor agents
    b. Estrogens, other than study medication

5. The recommended primary endpoint of the study is the proportion of subjects in the per protocol (PP) population that are identified as responders at Day 15. A responder is defined as a subject with:
    a. At least a 25% reduction from baseline in the sum of % basal/parabasal + % intermediate cells on vaginal cytology; and
    b. Vaginal pH < 5.0 with a change from baseline vaginal pH of at least 0.5; and
    c. Improvement in severity of the most bothersome symptom of VVA

6. 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
    m. Modified intent-to-treat (mITT) population flag (yes/no)
    n. Reason for exclusion from mITT population
    o. 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 intermediate epithelial cells on vaginal cell cytology (i.e., % intermediate)
    x. Study Day 15 intermediate epithelial cells on vaginal cell cytology (i.e., % intermediate)
    y. Baseline basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    z. Study Day 15 basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    aa. Baseline vaginal pH
    bb. Study Day 15 vaginal pH
    cc. Baseline score of most bothersome symptom of VVA identified at baseline
    dd. Study Day 15 score of most bothersome symptom of VVA identified at baseline
    ee. Final designation as responder/non-responder
    ff. Compliance rate (%)

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

7. 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. 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. Baseline intermediate epithelial cells on vaginal cell cytology (i.e., % intermediate)
    o. Study Day 15 intermediate epithelial cells on vaginal cell cytology (i.e., % intermediate)
    p. Baseline basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    q. Study Day 15 basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    r. Baseline vaginal pH
    s. Study Day 15 vaginal pH
    t. Baseline score of most bothersome symptom of VVA identified at baseline
    u. Study Day 15 score of most bothersome symptom of VVA identified at baseline
    v. Final designation as responder/non-responder
    w. Additional treatment required during the visit (yes/no)
    x. Adverse event reported during the visit (yes/no)
    y. Concomitant medication during the visit (yes/no)

8. 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 comparative clinical endpoint bioequivalence studies.

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

Document History: Recommended December 2025

Unique Agency Identifier: PSG_208564

[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.