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Draft – Not for Implementation

Draft Guidance on Estradiol

December 2025

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

Active Ingredient:	Estradiol
Dosage Form:	Tablet
Route:	Vaginal
Strengths:	10 mcg, 25 mcg
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 tablet, 25 mcg 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 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 (dimensions) with high resolution photographs
    b. Characterization of particle size distribution, crystal habit, and polymorphic form of estradiol in the drug product
    c. Characterization of disintegration time

3. The test product and RS should have acceptable dissolution of estradiol. 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. 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: 25 mcg (dose: 1x 25 mcg tablet) [**NOTE**: if approval is sought only for 10 mcg, dose with 1x10 mcg tablet]
    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 10 mcg strength of the tablet product containing sufficient data may be approved based on (i) acceptable demonstration of bioequivalence of the 25 mcg 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 strengths.

To only develop an estradiol vaginal tablet, 10 mcg test product using the bioequivalence approach outlined in Option 1, all studies outlined within the characterization-based bioequivalence approach (including the in vivo pharmacokinetic study) should be performed using the 10 mcg strength of the test product and the RS.

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

To demonstrate bioequivalence for estradiol vaginal tablet, 10 mcg and 25 mcg 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 25 mcg strength, (ii) acceptable comparative clinical endpoint bioequivalence study on the 10 mcg 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: 25 mcg (dose: 1x 25 mcg tablet) [**NOTE**: if approval is sought only for 10 mcg, dose with 1x10 mcg tablet]
   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: 10 mcg (dose: 1x10 mcg once daily for 14 days) [**NOTE**: if approval is sought only for 25 mcg, dose with 1x25 mcg once daily for 14 days]
Subjects: Postmenopausal women with symptoms of vulvar and vaginal atrophy (VVA) and no contraindication to estrogen therapy.
Additional comments: Specific recommendations are provided below.

To develop a single strength of an estradiol vaginal tablet test product using the bioequivalence approach outlined in Option 2, an in vivo bioequivalence study with pharmacokinetic endpoints and a comparative clinical endpoint bioequivalence study should be performed using the single strength of the test product and RS.

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 tablet, 10 mcg 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 on study Day 15 (i.e., one day after the administration of the fourteenth dose of study treatment).

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 subject 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 be no greater than 140 mm Hg and diastolic blood pressure be 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. Estrogen, progesterone or testosterone drug product, other than study product.
    b. Vaginal drug products other than study product (e.g., vaginal antifungals).

5. The recommended primary endpoint of the study is the proportion of subjects in the per protocol (PP) population that are identified as responders on Day 15 (i.e., one day after the administration of the fourteenth dose of study treatment). 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 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 Week 12 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 Week 12 basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    r. Baseline vaginal pH
    s. Study Week 12 vaginal pH
    t. Baseline score of most bothersome symptom of VVA identified at baseline
    u. Study Week 12 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)

7. Provide the Subject-Level Analysis Dataset (ADSL), one record per subject, using the following headers, 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 Week 12 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 Week 12 basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    aa. Baseline vaginal pH
    bb. Study Week 12 vaginal pH
    cc. Baseline score of most bothersome symptom of VVA identified at baseline
    dd. Study Week 12 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)

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.

Additional information:

Device:
The reference listed drug (RLD) is presented as a vaginal tablet that is inset into an applicator. The device constituent part is the applicator.

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

- Single-use, single-dose applicator with inset tablet
- Applicator length and diameter
- Number of doses

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 RLD, 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. For additional information, refer to the most recent version of the FDA guidance for industry on *Comparative Analyses and Related Comparative Use Human Factors Studies for a Drug-Device Combination Product Submitted in an ANDA*.[a]

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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.