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Draft – Not for Implementation

Draft Guidance on Estrogens, Conjugated

November 2024

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Active Ingredients:	Estrogens, conjugated
Dosage Form:	Cream
Route:	Topical, vaginal
Strength:	0.625 mg/gm
Recommended Studies:	Characterization studies to support active ingredient sameness, and 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

Overview:
This draft guidance provides recommendations for the development of generic drug products for naturally sourced conjugated estrogens vaginal topical cream derived from pregnant mares' urine. First, FDA provides recommendations for tests to support a demonstration of active ingredient sameness. Second, FDA provides recommendations for demonstrating bioequivalence of this drug product.

Recommendations for demonstrating active ingredient sameness:
Conjugated estrogens is an active ingredient obtained from a natural source. It contains a mixture of many steroidal components derived from pregnant mares' urine. The conjugated estrogens united states pharmacopeia (USP) monograph[1] defines 10 individual steroidal components and the acceptance criteria in the labeled content of conjugated estrogens. The identification and quantification method in the USP monograph is a gas-chromatograph (GC) method. A liquid chromatography-mass spectrometry (LC-MS) method was developed[2] and optimized in FDA Laboratories and was used to analyze conjugated estrogens from nine batches of the reference list drug (RLD) product, Premarin topical vaginal cream, manufactured over a two-year period. This LC-MS method can identify the top 48 steroidal components consistently present in the RLD samples, as described below.

The pharmaceutical equivalence of a generic conjugated estrogens obtained from the same natural source as the RLD (i.e., equine urine) can be established based on comparative physicochemical characterizations. The sponsor is advised to use the USP GC method, the proposed FDA LC-MS method, or suitable in-house methods to analyze the RLD and the test drug substance/product batches. The analysis should include both steroidal and non-steroidal components. A minimum of six different batches of the RLD product and the test drug substance/product should be tested respectively as the following: For the RLD batches, three different batches of RLD creams (0.625 mg/gm) manufactured within a single year, based on expiration dates, should be studied to assess intra-year consistency. Similarly, three different batches of RLD creams manufactured within a second year should be studied to assess inter-year consistency. This analysis will require six different batches of RLD creams. The sponsor may study additional batches of RLD creams to assess variations of the RLD product. For test batches, three different batches of bulk drug substance blended from pregnant mares' urine from a single collection year, and one batch of test cream (0.625 mg/gm) manufactured from each of the three drug substance batches (three cream batches total) within that year, should be studied to assess intra-year consistency of test bulk drug substance batches and cream batches. Similarly, three different batches of test drug substance and one batch of test drug product manufactured from each of the drug substance batches from a second collection year should be studied to assess inter-year consistency. This process will yield six different batches of test bulk drug substance and six different batches of test creams.

FDA LC-MS analytical procedure:

It is recommended to use ultra-high performance liquid chromatography and high resolving power mass spectrometry (UHPLC-HRMS) for the chemical characterization of conjugated estrogens.

[1] Conjugated Estrogens monograph, USP 36, official from May 1, 2013
[2] See details in the product-specific guidance on conjugated estrogens tablet: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Conjugated_estrogens_004782_RC12-14.pdf.

1. Materials:

Mass spectrometry (Optima) grade methanol, water, acetonitrile, and ammonium acetate or equivalent.
Waters Acquity BEH C_{18} 1.7 µm, 130 Å, 150 x 2 mm column or equivalent
Estrone-3-sulfate (E1-3S), Equilin-3-sulfate (Eq-3S), Δ8,9-dehydroestrone-3-sulfate (DHES), and Dihydroequiin-3-sulfate-17α (DHEq-3S17a) standards. Identity and purity of standard should be verified with orthogonal methods (e.g., HPLC, NMR and MS data).

Mobile phases:

Mobile Phase A: 10 mM ammonium acetate, acetic acid added to pH 4.75
Mobile Phase B: 9:1 methanol/acetonitrile

2. Qualifying standards preparation:

Estrone-3-sulfate (E1-3S), Equilin-3-sulfate (Eq-3S), Δ8,9-dehydroestrone-3-sulfate (DHES), and Dihydroequiin-3-sulfate-17α (DHEq-3S17a)
Prepare a 0.100 mg/mL solution of Estrone-3-sulfate (E1-3S), Equilin-3-sulfate (Eq-3S), Δ8,9-dehydroestrone-3-sulfate (DHES), and Dihydroequiin-3-sulfate-17α (DHEq-3S17a) each in methanol.

3. Sample preparation:

Approximately 1 g of cream in 40 mL of 10% methanol in water is shaken for 30 minutes at 52° C. 1.5 mL of the extract is centrifuged at 15000 RPM for 10 minutes. The bottom layer is transferred into a syringe and filtered through a 0.2 µm FTEP filter.

4. Instrumentation:

An ultra-high performance liquid chromatography high resolution mass spectrometer consisting of an ultra-high performance binary pump, vacuum degasser, autosampler, a thermostatted column compartment, an electrospray source, and high-resolution mass spectrometer.

In order to distinguish between a monoisotopic mass of one species and the A+2 isotopic peak of another species, 2 m/z units below, a mass spectrometer with resolving power of 50,000 or more is preferred.

UHPLC conditions:

Column: Waters Acquity BEH C_{18} 1.7 µm, 150 x 2 mm or equivalent
Flow: 0.2 mL/min
Total run time: 50 minutes
Injection volume: 1 µL, loop size 5-20 µL
Column oven temperature: 40° C

Gradient Program:

Time (min.)	%B
0	5
2	5
5	35
30	45
35	95
40	95
40.5	5
50	5

Mass spectrometry conditions:
Ionization method: Electrospray Ionization (ESI)
Source conditions:
Scan type: MS, negative ion mode
Scan range: 115-520 m/z

5. System suitability:
The elution order for the synthetic steroid standards should be DHES, Eq-3S, DHEq-3S17a, E1-3S. Masses to monitor are $C_{18}H_{19}O_5S$, m/z 347.0959 for DHES and Eq-3S, and $C_{18}H_{21}O_5S$, m/z 349.1115 for E1-3S. The retention time of E1-3S should be between 22-26 minutes. The resolution (R) of the close pair, DHES, Eq-3S, DHEq-3S17a, and E1-3S should be at least 1.2 using Equation (1):

$$R = 2(t_{r2}-t_{r1})/1.70(W_{1,h/2}+W_{2,h/2}) \quad (1)$$

where t_r is the retention time and $W_{h/2}$ is the width at the half height for compounds 1 and 2. %RSD of E1-3S should be NMT 2.0%. Number of plates of E1-3S should be NLT 15000. The tailing of E1-3S should be NMT 2.0.

6. Sample analysis and calculation:
Extracted ion chromatograms (EIC) for all masses as in Table 1[3], with a mass tolerance of ± 10 ppm, should be created, and all peak areas should be recorded. Match the peak list with the 48 peaks in Table 2 using the mass and relative retention time (RRt). Relative retention time is calculated based on Equation (2):

$$RRt = R_t/R_{t0} \quad (2)$$

where R_t is the compound retention time, and R_{t0} is the retention time of E1-S which is the most abundant compound in the conjugated estrogens mixture at m/z 349.1115. If the retention time is longer than 31 minutes, calculate retention time using Equation (3):

[3] Masses (m/z) identified in Table 1 are from analysis of RLD product. Steroidal and non-steroidal components are listed separated to improve clarity.

$$RRt = 1.558 \times R_t/R_{t1} \quad (3)$$

where R_{t1} is the retention time of peak m/z 399.2211. This peak was chosen to calculate the RRt of late eluters because it is the most abundant late eluting peak and easy to recognize (R_{t1} is approximately R_{t0} x 1.558).

Divide the peak area of each component in the EIC list by the sum all the peak areas of the 48 components to obtain relative peak area of each component.

7. Data reporting:

For each peak detected, the RRt and exact mass should be reported. Sponsors should also match each peak with the appropriate peak in Table 3 (10 components identified in the Conjugated Estrogen USP monograph) based on the exact mass and RRt. In addition, each peak area should be reported in unit of area % relative to the sum of all 48 components as in Table 2. To aid in assignment, peaks are listed in decreasing order of relative peak area abundance.

Above method is also suitable to analyze non-steroidal components (Table 1). Amount and content of non-steroidal components may vary between RLD and test batches.

Table 1. List of masses (m/z) to generate extracted ion mass chromatograms (EIC).

m/z Masses for EIC			
Non-Steroidal components		Steroidal Components	
121.0295	231.0333	345.0802	377.1064
137.0244	239.0853	347.0959	379.1221
150.0561	241.0176	349.1115	381.1377
153.0193	243.0333	351.1272	395.1898
178.0510	245.0489	353.1428	397.2054
187.0071	297.1132	355.1585	399.2211
192.0666		363.0897	413.2003
201.0227		365.1064	415.2160
212.0023		367.1585	431.1136
213.0227		369.1741	449.0361
215.0384		371.1898	

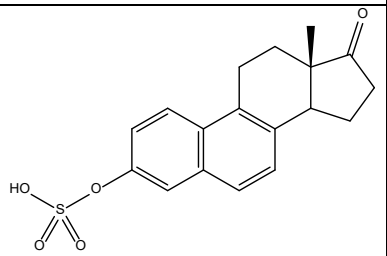
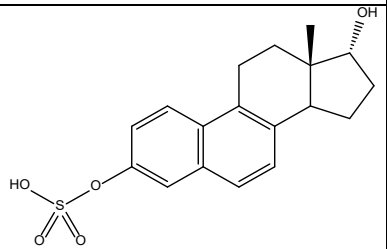
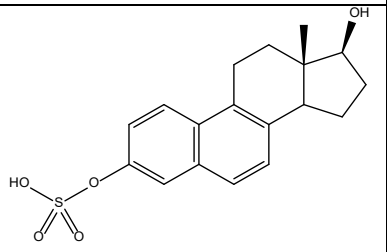
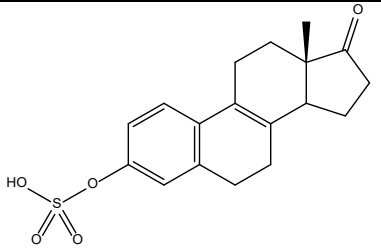
Table 2. List of 48 steroidal components identified in conjugated estrogens cream.

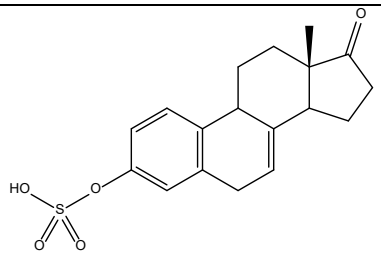
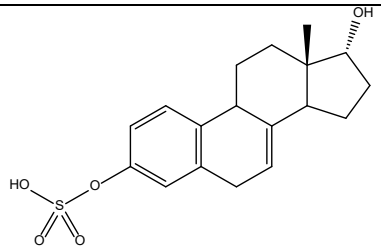
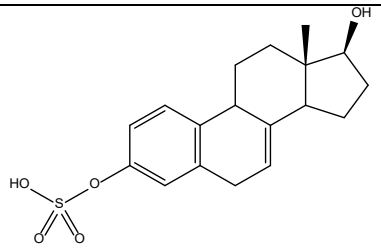
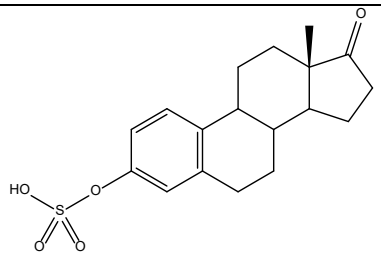
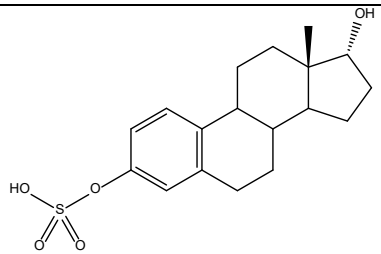
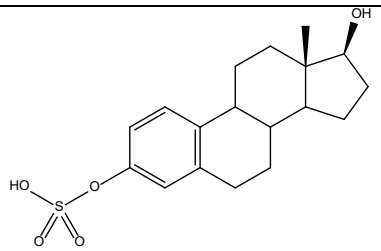
Peak #	Name[4]	RRt[5]	Mass m/z	Composition
1	349@1.00-E1-3S*	1.000	349.1115	$C_{18}H_{22}O_5S$
2	347@0.94-Eq-3S*	0.937	347.0959	$C_{18}H_{20}O_5S$
3	399@1.56	1.558	399.2211	$C_{21}H_{36}O_5S$
4	349@0.98-DHEq3S17a*	0.981	349.1115	$C_{18}H_{22}O_5S$
5	397@1.55	1.553	397.2054	$C_{21}H_{34}O_5S$
6	395@1.55	1.552	395.1898	$C_{21}H_{32}O_5S$
7	351@1.16-E2-3S17a*	1.155	351.1272	$C_{18}H_{24}O_5S$
8	347@0.92-DHES*	0.919	347.0959	$C_{18}H_{20}O_5S$
9	351@1.14	1.140	351.1272	$C_{18}H_{24}O_5S$
10	353@1.33	1.328	353.1428	$C_{18}H_{26}O_5S$
11	413@1.18	1.177	413.2003	$C_{21}H_{34}O_6S$
12	369@1.50	1.497	369.1741	$C_{19}H_{30}O_5S$
13	345@0.85-EqnS*	0.853	345.0802	$C_{18}H_{18}O_5S$
14	399@1.57	1.567	399.2211	$C_{21}H_{36}O_5S$
15	349@0.88-DHEq3S17b*	0.876	349.1115	$C_{18}H_{22}O_5S$
16	353@1.01	1.007	353.1428	$C_{18}H_{26}O_5S$
17	365@0.92	0.921	365.1064	$C_{18}H_{22}O_6S$
18	397@1.57	1.568	397.2054	$C_{21}H_{34}O_5S$
19	379@1.02	1.017	379.1221	$C_{19}H_{24}O_6S$
20	347@0.87-DEqn3S17a*	0.870	347.0959	$C_{18}H_{20}O_5S$
21	397@1.57	1.572	397.2054	$C_{21}H_{34}O_5S$
22	349@0.90	0.895	349.1115	$C_{18}H_{22}O_5S$
23	395@1.54	1.538	395.1898	$C_{21}H_{32}O_5S$
24	353@1.22	1.216	353.1428	$C_{18}H_{26}O_5S$
25	351@0.95-E2-3S17b*	0.952	351.1272	$C_{18}H_{24}O_5S$
26	371@1.24	1.240	371.1898	$C_{19}H_{32}O_5S$
27	369@0.93	0.931	369.1741	$C_{19}H_{30}O_5S$
28	413@1.54	1.539	413.2003	$C_{21}H_{34}O_6S$
29	415@1.13	1.131	415.216	$C_{21}H_{36}O_6S$
30	349@0.82	0.823	349.1115	$C_{18}H_{22}O_5S$
31	355@1.09	1.087	355.1585	$C_{18}H_{28}O_5S$
32	395@1.56	1.564	395.1898	$C_{21}H_{32}O_5S$
33	397@1.54	1.542	397.2054	$C_{21}H_{34}O_5S$
34	399@1.53	1.534	399.2211	$C_{21}H_{36}O_5S$
35	431@1.00	1.000	431.1136	$C_{25}H_{20}O_7$
36	347@0.75-DEqn3S17b*	0.746	347.0959	$C_{18}H_{20}O_5S$
37	397@1.55	1.546	397.2054	$C_{21}H_{34}O_5S$
38	363@0.85	0.854	363.0897	$C_{18}H_{20}O_6S$
39	449@1.00	1.000	449.0361	$C_{19}H_{14}O_{13}$
40	377@0.93	0.932	377.1064	$C_{19}H_{22}O_6S$

[4] Components are named using nominal mass (m/z) and relative retention time (RRt). * denotes the known steroidal components in the Conjugated Estrogens USP monograph. See Table 3 for details.
[5] RRt is the average value from multiple runs that calculated using formula (2) or (3) depending on Rt.

41	367@1.41	1.413	367.1585	$C_{19}H_{28}O_5S$
42	381@1.15	1.147	381.1377	$C_{19}H_{26}O_6S$
43	351@0.81	0.814	351.1272	$C_{18}H_{24}O_5S$
44	351@1.08	1.078	351.1272	$C_{18}H_{24}O_5S$
45	369@1.51	1.510	369.1741	$C_{19}H_{30}O_5S$
46	413@1.51	1.507	413.2003	$C_{21}H_{34}O_6S$
47	367@1.14	1.141	367.1585	$C_{19}H_{28}O_5S$
48	413@0.93	0.925	413.2003	$C_{21}H_{34}O_6S$

Table 3. List of steroidal components in the conjugated estrogens USP monograph.

Peak # from Table 2	Name	Shortened name	Structure	$[M-H]^-$ m/z
13	Equilenin-3-sulfate	EqnS		345.0802
20	Dihydroequilenin-17α-3-sulfate	DEqn3S17a		347.0959
36	Dihydroequilenin-17β-3-sulfate	DEqn3S17b		347.0959
8	$\Delta^{8,9}$-dehydrostrone-3-sulfate	DHES		347.0959

Peak # from Table 2	Name	Shortened name	Structure	$[M-H]^-$ m/z
2	Equilin-3-sulfate	Eq-3S		347.0959
4	Dihydroequilin-17α-3-sulfate	DHEq3S17a		349.1115
15	Dihydroequilin-17β-3-sulfate	DHEq3S17b		349.1115
1	Estrone-3-sulfate	E1-3S		349.1115
7	Dihydroestrone-17α-3-sulfate	E2-3S17a		351.1272
25	Dihydroestrone-17β-3-sulfate	E2-3S17b		351.1272

Sameness of active ingredient
The applicant should report all steroidal components consistently present at a level ≥0.10% (relative percentage of peak area, as described in Session **I 7** (Data Reporting) identified using the FDA LC-MS method or a suitable and validated in-house method.

1. Identification test for steroidal components
All test drug substance batches should contain the 48 steroidal components identified by the FDA LC-MS method (Table 2). All components should be identified by RRt and exact mass.

2. USP quantification test for 10 steroidal components
The components identified in the conjugated estrogens USP monograph should be present in all test drug substance batches within the acceptance criteria specified in the USP monograph. Data obtained using the USP GC method or an appropriately validated in-house method is acceptable.

3. Control of major non-USP steroidal components
All steroidal non-USP components consistently present at a level ≥1.0% (LC-MS method, relative percentage of peak area, as described in Session **I 7** (Data Reporting) in the RLD should be present in the test drug substance batches at a level comparable to the RLD batches, otherwise should be justified or qualified.

4. Control of additional steroidal components in the test drug substance batches
Any steroidal components that present at a level ≥1.0% (LC-MS method, relative percentage of peak area, as described in Session **I 7** (Data Reporting) in any test drug substance batches, but not consistently present at a level ≥1.0% in the RLD batches, should be justified or qualified.

5. Total steroidal components content test
The ratio of the sum of 10 USP steroidal components (LC-MS peak area) to the sum of the 48 steroidal components identified by the FDA LC-MS method (Table 2) should be calculated as the following:

Ratio = Σ 10 USP steroidal components / Σ 48 steroidal components (Table 2)

The ratios obtained from the test drug substance batches should be at a level comparable to the ratios from the RLD batches, otherwise should be justified.

In addition, any additional steroidal peaks (other than 48 listed in Table 2) observed at the level >LOQ should be reported and the sum of all steroidal peaks (including the 48 peaks in the ID test) with levels >LOQ should be reported.

6. Non-steroidal components in the test drug substance batches

Non-steroidal components in test drug substance batches should be analyzed in comparison to the RLD batches. Although same profile of non-steroidal components is not expected, appropriate acceptance criteria should be set so that the levels of common non-steroidal components in the test drug substance batches are not higher than that in the RLD batches. For new non-steroidal components in the test drug substance batches not found in the RLD batches, follow the Guidance for Industry *ANDAs: Impurities in Drug Substances*[a] to set acceptance criteria.

Recommendations for demonstrating bioequivalence:

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

To demonstrate bioequivalence for conjugated estrogens topical vaginal cream, 100 mg 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 (T) 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 T 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 T product may be established using a characterization-based bioequivalence approach.

2. The T 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 T product and three batches (as available) of the RS. The T 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 T 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 pH
e. Characterization of specific gravity
f. Characterization of any other potentially relevant Q3 attributes

3. The T product and RS should have an equivalent rate of drug release based upon an acceptable in vitro release test (IVRT) bioequivalence study comparing a minimum of one batch each of the T 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: 0.625 mg/gm
Test system: A synthetic membrane in a diffusion cell system
Analytes to measure: Estrone sulfate in receptor solution
Bioequivalence based on: Estrone sulfate (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 T product and RS evaluated in the IVRT bioequivalence study should be included among those for which the Q3 attributes are characterized.

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

Type of study: Bioequivalence study with pharmacokinetic endpoints
Design: Single-dose, two-treatment, two-period crossover in vivo
Strength: 0.625 mg/gm (dose: 2 gm of the cream product)
Subjects: Healthy postmenopausal women with no contraindication to estrogen therapy
Analytes to measure: Baseline-adjusted unconjugated estrone, baseline-adjusted total estrone, unconjugated equilin, and total equilin in plasma.
Bioequivalence based on: Baseline-adjusted unconjugated estrone, baseline-adjusted total estrone, unconjugated equilin, and total equilin computed from blood sampling through 72 hours.

Additional comments: Baseline (pre-dose) levels of unconjugated and total estrone (sum of unconjugated estrone, estrone sulfate and estrone glucuronide) in plasma determined at -1.0, -0.5 hrs, and pre-dose (time zero) should be averaged to obtain a single baseline value for each of unconjugated estrone and total estrone. Total equilin is the sum of unconjugated equilin, equilin sulfate and equilin glucuronide. Equilin is not endogenous in the human, thus baseline plasma levels are zero. Pharmacokinetic and statistical analyses should be performed on both uncorrected and corrected data. Determination of bioequivalence should be based on the baseline-corrected data. 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 T product and RS evaluated in the in vivo pharmacokinetic study should be the same as those evaluated in the IVRT bioequivalence study.

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

1. Type of study: Bioequivalence study with pharmacokinetic endpoints
   Design: Single-dose, two-treatment, two-period crossover in vivo
   Strength: 0.625 mg/gm (dose: 2 gm of the cream product)
   Subjects: Healthy postmenopausal women with no contraindication to estrogen therapy
   Analytes to measure: Baseline-adjusted unconjugated estrone, baseline-adjusted total estrone, unconjugated equilin, and total equilin in plasma.
   Bioequivalence based on: Baseline-adjusted unconjugated estrone, baseline-adjusted total estrone, unconjugated equilin, and total equilin computed from blood sampling through 72 hours.
   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-group, placebo-controlled, in vivo
   Strength: 0.625 mg/gm
   Subjects: Postmenopausal women with symptoms of vulvar and vaginal atrophy and no contraindication to estrogen therapy
   Additional comments: Specific recommendations are provided below.

Additional comments regarding the in vivo bioequivalence studies with clinical endpoint:

1. FDA recommends conducting a comparative clinical endpoint bioequivalence study in the treatment of postmenopausal vulvar and vaginal atrophy (VVA) comparing the T product versus the RS and vehicle control, each administered as 0.5 g once daily for 21 days. The primary endpoint is the proportion of subjects identified as responders on Day 22.

2. Inclusion criteria (the sponsor 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 should be no greater than 140 mmHg and diastolic blood pressure no greater than 80 mmHg (subjects with well-controlled hypertension on a stable treatment regimen are acceptable)
    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 sponsor may add additional criteria):
    a. Male subject
    b. Premenopausal, perimenopausal, pregnant or lactating
    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. Tobacco use
    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 or myocardial infarction), or a history of these conditions
    k. History of hypersensitivity to any component of the RS or T product
    l. Liver impairment or disease
    m. Protein C, protein S, or antithrombin deficiency, or other thrombophilic disorders
    n. Active drug abuse or alcoholism
    o. Any other disease or condition that would result in unacceptable risk to the subject based upon the opinion of the investigator

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 prior to dosing, surgery of the type associated with an increased risk of thromboembolism, or requiring periods of prolonged immobilization
v. Taking inducers of CYP3A4 such as St. John's wort, anticonvulsants, phenylbutazone, rifampin, rifabutin, nevirapine and efavirenz
w. 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 prohibited during the study should be provided and include estrogen products other than study treatment.

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 22. 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

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 22 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 22 basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
aa. Baseline vaginal pH
bb. Study Day 22 vaginal pH
cc. Final designation as responder/non-responder
dd. Compliance rate (%)
ee. Subject missed the pre-specified number of scheduled doses for more than pre-specified number of consecutive days (yes/no)
ff. Adverse event reported (yes/no)
gg. 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. Baseline intermediate epithelial cells on vaginal cell cytology (i.e., % intermediate)
    n. Study Day 22 intermediate epithelial cells on vaginal cell cytology (i.e., % intermediate)
    o. Baseline basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    p. Study Day 22 basal/parabasal epithelial cells on vaginal cell cytology (i.e., % basal)
    q. Baseline vaginal pH
    r. Study Day 22 vaginal pH

s. Final designation as responder/non-responder
t. Additional treatment required during the visit (yes/no)
u. Adverse event reported during the visit (yes/no)
v. 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 the comparative clinical endpoint bioequivalence study.

9. Refer to the Study Data Standards Resources website https://www.fda.gov/industry/fda-data-standards-advisory-board/study-data-standards-resources.

Additional information:

Device:
The RLD product is presented in a tube co-packaged with 1 or 2 vaginal applicators. The vaginal applicators are the device constituent parts.

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 T device including:

- Number of co-packaged applicators
- Compatibility of the applicator with the container opening
- Dose markings on the applicator plunger rod
- Applicator can be disassembled and washed with soap and water

User interface assessment:
An abbreviated new drug application 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 the product-specific guidance, refer to the FDA product specific guidance website at: https://www.accessdata.fda.gov/scripts/cder/psg/index.cfm