DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

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Joseph G. Valentino, J.D.
Senior Vice President and General Counsel
The United States Pharmacopeial
Convention, Inc.
12601 Twinbrook Parkway
Rockville, MD 20852

REF: 3-00-001-O

Dear Dr. Valentino:

This letter proposes establishment of two new monographs for "Synthetic Conjugated Estrogens, USP" and "Synthetic Conjugated Estrogens Tablets, USP." These monograph proposals are enclosed for your convenience.

The drug substance monograph will provide public standards for various mixtures of sodium steroid esters (for example, *Synthetic Conjugated Estrogens, A*) and the drug product monograph will provide public standards for tablets formulated from such mixtures, (for example, Cenestin® Tablets). The proposed monographs have been redacted in accordance with disclosure limitations imposed by the Freedom of Information Act. We therefore encourage you to obtain the redacted information directly from the NDA sponsor in order to publish the proposals as complete monographs.

We hope these comments will be helpful to the USP and the Committee of Revision in their deliberations. Please feel free to contact Larry Ouderkirk on my staff if you have any questions. If you have extensive questions, we will be happy to arrange for your representatives to meet with the CDER scientists responsible for the draft monographs. Please use the reference number provided above on any ensuing correspondence.

As a final note, we would like to advise you that due to public interest in this matter, we will be placing a copy of this letter and the accompanying proposed monograph on the following CDER Internet website:

http://www.fda.gov/cder/regulatory/initiatives/cestrogens/

Sincerely,

Yana Ruth Mille

Yana Ruth Mille
Chief
Compendial Operations Staff, HFD-354
Office of Pharmaceutical Science
Center for Drug Evaluation & Research

"SYNTHETIC CONJUGATED ESTROGENS, USP" – PROPOSED MONOGRAPH

Synthetic Conjugated Estrogens is a mixture of the sodium salts of the sulfate esters of a number of estrogenic substances. It is a dispersion of these estrogenic substances in a suitable diluent. Synthetic Conjugated Estrogens contains, as the sodium sulfate esters, estrone, equilin, and 17α-dihydroequilin in such proportions that the total of sodium estrone sulfate and sodium equilin sulfate is not less than 79.5% and not more than 88.0% of the labeled content of Synthetic Conjugated Estrogens, and the total of the sodium sulfate esters of estrone, equilin and 17α-dihydroequilin is not less than 90.0 % and not more than 110.0 % of the labeled content of Synthetic Conjugated Estrogens.

Packaging and Storage -- Preserve in well-closed containers.

Labeling – The name should include the appropriate letter modifier based on the composition of the Synthetic Conjugated Estrogens. Label it to state the content of Synthetic Conjugated Estrogens on a weight-to-weight basis.

USP Reference Standards <11> -- USP Estrone RS. USP Equilin RS. USP 17α-Dihydroequilin RS. USP Estradiol RS.

Synthetic Conjugated Estrogens, A

Synthetic Conjugated Estrogens, A, contains, as the sodium sulfate esters, expressed in terms of the labeled content of Synthetic Conjugated Estrogens, not less than and not more than of estrone, not less than and not more than of equilin, not less than and not more than of 17 α-dihydroequilin, not less than and not more than of 17 β-dihydroequilin, not less than and not more than of 17 α-estradiol, not less than and not more than of 17 β-estradiol, not less than and not more than of equilenin, not less than and not more than of 17 α-dihydroequilenin, and not less than and not more than of 17 β-dihydroequilenin. The ratio of sodium equilin sulfate to sodium estrone sulfate is not less than and not more than .

Identification – The following results are obtained with respect to the *Assay preparation* treated as directed for *Procedure* in the *Assay*.

A: The chromatogram exhibits peaks for estrone and equilin at relative retention times corresponding to those exhibited in the chromatogram of the *Standard preparation*.

B: The chromatogram of Synthetic Conjugated Estrogens, A exhibits additional peaks or shoulders, corresponding to 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin at retention times of about respectively, relative to that of 17α-dihydroequilin.

C: The chromatogram of Synthetic Conjugated Estrogens, A exhibits additional peaks corresponding to 17β-estradiol, 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin at retention times of about respectively, relative to that of 3-O-methylestrone.

Limit of estrone, equilin, and 17α-dihydroequilin (free steroids) --
Internal standard solution, pH 5.2 acetate buffer, Stock solution, and System suitability solution -- Proceed as directed in the *Assay*.

Free steroids standard solution -- Proceed as directed for *Standard preparation* in the *Assay*.

Test solution -- Proceed as directed for *Assay preparation* in the *Assay* with the following exceptions: do not add the sulfatase enzyme preparation, and transfer 6.0 mL of the filtrate instead of 3.0 mL in the preparation of the test specimen. Prepare a reagent blank in the same manner.

Chromatographic system -- Proceed as directed in the *Assay* with the additional requirement that the relative standard deviation for the ratio of the peak response of estrone to that of the internal standard in the *Free steroids standard solution* is not greater than on the basis of not less than two replicate injections.

Procedure -- Separately inject equal volumes (about 1 µL) of the *Free steroids standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak responses. Calculate the ratio, R_U, of the combined peak areas of estrone, equilin, and 17α-dihydroequilin relative to the area of the internal standard in the *Test solution*, correcting for any reagent blank peaks. The ratio, R_U/R_S, where R_S is the peak response ratio of estrone to that of the internal standard obtained from the *Free steroids standard solution*, is not more than .

Chromatographic purity –

Internal standard solution, Stock solution, pH 5.2 acetate buffer, System suitability solution, Standard preparation, and *Chromatographic system* – Proceed as directed in the *Assay*.

Test preparation – Prepare as directed for *Assay preparation* in the *Assay*.

Procedure – Proceed as directed in the *Assay*. Calculate the content of any individual unknown impurity by dividing the peak response of the unknown peak by the sum of the all the peak responses minus the internal standard. The requirements are met if no individual impurity is more than and the sum of the individual impurities is not more than .

Organic volatile impurities, *Method V* <467>: meets the requirements.
Solvent -- Use dimethyl sulfoxide as the solvent.

Heavy metals, *Method II* <231>: meets the requirements.

Assay --

Internal standard solution- Prepare a solution of 3-O-methylestrone in alcohol containing about 150 µg/mL.

Stock solution- Using accurately weighed quantities of USP Estrone RS, USP Equilin RS, and USP 17α-Dihydroequilin RS, prepare, by quantitative and stepwise dilution, a solution in alcohol having known concentrations of about 160, 70, and 50 µg per mL, respectively.

pH 5.2 acetate buffer- Mix 79 mL of sodium acetate TS with 21 mL of 1 *N* acetic acid, dilute with water to 500 mL, and mix. Adjust to a pH of 5.2 ± 0.1 by the addition of 1 *N* acetic acid, or sodium acetate TS, if necessary.

System suitability solution- Dissolve a quantity of USP Estradiol RS (17β-estradiol) in alcohol to obtain a solution containing about 2 µg per mL. Pipet 1.0 mL of this solution, 1.0 mL of *Stock solution* and 1.0 mL of *Internal standard solution* into a centrifuge tube fitted with a tight screw-cap or stopper. Proceed as directed for *Standard preparation* beginning with "Evaporate the mixture."

Standard preparation- Pipet 1.0 mL of the *Stock solution* and 1.0 mL of the *Internal standard* into a suitable centrifuge tube fitted with a suitable polytef-lined screw-cap or stopper. Evaporate the mixture to dryness with the aid of a stream of nitrogen, maintaining the temperature below 50°. To the residue add 15 µL of pyridine and 65 µL of bis(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly using a Teflon-lined cap, mix thoroughly on a vortex mixer, and let stand for 15 minutes. Add 0.5 mL toluene and mix.

Assay preparation -- Transfer an accurately weighed quantity of Synthetic Conjugated Estrogens, A equivalent to about 0.5 g of synthetic conjugated estrogens to a 100 mL volumetric flask. Dilute to volume with water and mix. Pipet 10.0 mL of this solution to a 50 mL volumetric flask, and dilute to volume with water and mix. Pipet 2.0 mL aliquots into each of two separate 50-mL centrifuge tubes, fitted with polytef-lined screw-caps, containing 15 mL of *pH 5.2 acetate buffer* and 1 g of barium chloride. Cap the tubes tightly, and shake for 30 minutes. If necessary, adjust the solution with 1 *N* acetic acid or sodium acetate to a pH of 5.0 ± 0.5. Use one as an *Assay Preparation* and reserve the other for use in the *Free Steroids Limit Test*. Add a suitable sulfatase enzyme preparation equivalent to 2500 Units, and place in a water bath maintained at 50° for 20 minutes, shaking occasionally. Add 15.0 mL of methylene chloride to the warm mixture, again cap the tube, and shake by mechanical means for 15 minutes.

Centrifuge for 10 minutes at approximately 3000 rpm or until the lower layer is clear. Transfer as much of the organic phase as possible, and dry by filtering rapidly through a filter consisting of a pledget of dry glass wool and about 5 g of anhydrous sodium sulfate in a small funnel. Protect from loss by evaporation. Transfer 3.0 mL of the solution to a suitable centrifuge tube fitted with a tight screw-cap or stopper. Add 1.0 mL of *Internal standard solution* and evaporate the mixture to dryness with the aid of a stream of nitrogen in a heating block maintaining the temperature below 50°. To the dry residue add 15 µL of dried pyridine and 65 µL of is (trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. Immediately cover the tube tightly using a Teflon-lined cap, mix thoroughly on a vortex mixer, and let stand for 15 minutes. Add 0.5 mL toluene and mix.

Chromatographic system (see Chromatography <621>)- The gas chromatograph is equipped with a flame-ionization detector held at 260°, a 0.25-mm x 15-m fused silica capillary column bonded with a 0.25 µm layer of phase G19, and a split injection system. The column is held at 220° and the injection port at 260°. The carrier gas is hydrogen flowing at the rate of 2 mL per minute, and the split flow rate is 40 to 60 mL per minute. Inject about 1 µL of the *System suitability solution* into the gas chromatograph. Adjust the operating conditions as necessary to maintain the elution time of the 3-O-methylestrone peak at between 17 and 25 minutes. The relative retention times are about for 17β-estradiol, 17α- dihydroequilin, estrone, equilin, and 3-O-methylestrone, respectively. The tailing factor for the estrone peak is not more than ; the resolution, R, between estrone and equilin is not less than ; and the relative standard deviation of the 17α-dihydroequilin, estrone, and equilin peak area ratios, calculated individually, is not greater than for not fewer than four injections of the *Standard preparation*.

Procedure- Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Separately calculate the quantities, in mg, of estrone, equilin, 17α-dihydroequilin, 17α-estradiol, 17β-estradiol, 17β-dihydroequilin, 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin as their sodium sulfate salts in the portion of Synthetic Conjugated Estrogens, A taken by the formula:

$$0.005(1.381C_S)(R_U/R_S),$$

in which 1.381 is the factor converting free estrogen to the conjugate sodium salt, C_S is the concentration, in mg per g, of USP Estrone RS or USP Equilin RS in the *Stock solution* to the weight of the sample, and R_U and R_S are the ratios of the peak response of the appropriate analyte to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively. For 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin, use the peak response for 17α-dihydroequilin

in the R_S ratio. For estrone, 17α-dihydroequilenin, 17β-dihydroequilenin, 17β-estradiol, and equilenin, use the peak response for estrone in the R_S ratio. For equilin, use the peak response for equilin in the R_S ratio.

"SYNTHETIC CONJUGATED ESTROGENS TABLETS, USP"
PROPOSED MONOGRAPH

Synthetic Conjugated Estrogens Tablets contain, as the sodium sulfate esters, estrone, equilin and 17α-dihydroequilin in such proportions that the total of sodium estrone sulfate and sodium equilin sulfate is not less than 79.5 % and not more than 88.0 % of the labeled content of Synthetic Conjugated Estrogens, and the total of estrone, equilin, and 17α-dihydroequilin is not less than 90.0 % and not more than 110.0 % of the labeled content of Synthetic Conjugated Estrogens.

Packaging and storage -- Preserve in well-closed containers.

Labeling – The name should include the appropriate letter modifier based on the composition of the Synthetic Conjugated Estrogens and the labeling indicates the tablet strength.

USP Reference Standards <11> -- USP Estrone RS. USP Equilin RS. USP 17α-Dihydroequilin RS. USP Estradiol RS.

Synthetic Conjugated Estrogens Tablets, A
The tablets contain other conjugated estrogen components as defined in the Synthetic Conjugated Estrogens, A monograph.

Identification – Tablets respond to the *Identification* test under Synthetic Conjugated Estrogens, A.

Drug release <724> -- Proceed as directed for *Extended-release Articles -- General Drug Release Standard* under *Drug Release*.
Medium: water; 900 mL.
Apparatus 1: 50 rpm.
Mobile phase -- Prepare a filtered and degassed mixture of 0.025 *M* monobasic potassium phosphate, pH 3.0 and acetonitrile (78:22). Make adjustments if necessary (see *System Suitability* under *Chromatography* <621>).

Standard solution – Prepare a stock standard of 40 µg per mL estropipate and 18 µg per mL sodium equilin sulfate. Use an amount of methanol equal to approximately 2% of the volumetric flask to dissolve the materials. Dilute to volume with purified water. From the stock standard solution, prepare a working standard of 0.4 µg per mL estropipate and 0.18 µg per mL equilin sulfate diluted with purified water.

Test solution -- Filter a portion of the solution under test.

[NOTE -- It is recommended that the filters selected be tested for binding affinity.]

Chromatographic system -- The liquid chromatograph is equipped with a 210-nm detector and a 4.6-mm X 7.5-cm column that contains 3-µm packing L1. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard solution*, and record the responses as directed under *Procedure*: the relative standard deviation for the estrone sulfate and equilin sulfate peaks is not more than 2.0%, and the resolution between equilin sulfate and estrone sulfate is not less than 1.2. [NOTE – If estrone is present it may be retained on the column for a period longer than 50 minutes and interfere in later chromatographic runs.]

Procedure -- Separately inject equal volumes (between 20 and 200 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the responses for the estrone sulfate and equilin sulfate peaks. The relative retention times are about 0.9 for equilin sulfate and 1.0 for estrone sulfate, the estrone sulfate peak being the last major peak in the chromatogram. Calculate the percentage of estrone sodium sulfate released by the formula:

$$100(r_U/r_S),$$

in which r_U and r_S are the peak responses obtained from the *Test solution* and the *Standard solution*, respectively.

Times and tolerances -- The percentages of sodium estrone sulfate and sodium equilin sulfate dissolved at the times specified conform to *Acceptance Table 1*.

Time (hours)	Amount dissolved
	between
	between
	not less than

Uniformity of dosage units – Assay 10 individual Tablets as directed in the *Assay*, and calculate the average content of Synthetic Conjugated Estrogens, as the average of the total contents of sodium estrone sulfate, sodium equilin sulfate, and sodium 17 alpha-dihydroequilin, in the 10 Tablets. The requirements are met if the content of each of the Tablets is not less than 85.0 % and not more than 115.0 % of the average content of Synthetic Conjugated Estrogens. If the content of not more than 2 Tablets falls outside the range of 85.0 % to 115.0 % of the average content but not outside the range of 75.0 % to 125.0 %, assay an additional 20 Tablets. The requirements are met if the content of not more than 2 of the 30 Tablets falls outside the limits of 85.0 % and 115.0 % of the average, and no unit is outside the range of 75.0 % to 125.0 % of the average content.

Chromatographic purity – Proceed as directed in the *Impurities* under Synthetic Conjugated Estrogens, A. The requirements are met if no individual impurity is more than and the sum of the individual impurities is not more than .

Assay --

Internal standard solution, Stock solution, pH 5.2 acetate buffer, System suitability solution, Standard preparation, and *Chromatographic system* -- Proceed as directed in the *Assay* under *Synthetic Conjugated Estrogens, A.*

Assay preparation -- Weigh and finely powder not less than 20 of the Tablets. Transfer an accurately weighed quantity of the powder, equivalent to about 2 mg of total synthetic conjugated estrogens, to a 50-mL centrifuge tube fitted with a polytef-lined screw-cap and containing 15 mL of *pH 5.2 acetate buffer* and 1 g of barium chloride. Proceed as directed in the *Assay preparation* under *Synthetic Conjugated Estrogens, A,* beginning with ``Cap the tube tightly."

Procedure- Separately inject equal volumes (about 1 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Separately calculate the quantities, in mg, of estrone, equilin, 17α-dihydroequilin, 17α-estradiol, 17β-estradiol, 17β-dihydroequilin, 17α-dihydroequilenin, 17β-dihydroequilenin, and equilenin as their sodium sulfate salts in the portion of Synthetic Conjugated Estrogens, A taken by the formula:

$$0.005(1.381C_S)(R_U/R_S),$$

in which 1.381 is the factor converting free estrogen to the conjugate sodium salt, C_S is the concentration, in mg per g, of *USP Estrone RS* or *USP Equilin RS* in the *Stock solution* to the weight of the sample, and R_U and R_S are the ratios of the peak response of the appropriate analyte to that of the internal standard obtained from the *Assay preparation* and the *Standard preparation*, respectively. For 17α-estradiol, 17α-dihydroequilin, and 17β-dihydroequilin, use the peak response for 17α-dihydroequilin in the R_S ratio. For estrone, 17α-dihydroequilenin, 17β-dihydroequilenin, 17β-estradiol, and equilenin, use the peak response for estrone in the R_S ratio. For equilin, use the peak response for equilin in the R_S ratio. The limits for these components are specified in the Synthetic Conjugated Estrogens monograph.