Guidance for Industry

Conjugated Estrogens, USP— LC-MS Method for Both Qualitative Chemical Characterization and Documentation of Qualitative Pharmaceutical Equivalence

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
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LC-MS Method for Both Qualitative Chemical Characterization
and
Documentation of Qualitative Pharmaceutical Equivalence

I. INTRODUCTION

This guidance is intended to provide recommendations to applicants who wish to submit a new drug application (NDA) or abbreviated new drug application (ANDA) for a natural source conjugated estrogens solid oral dosage form. This guidance provides a description of the liquid chromatography-mass spectrometry (LC-MS) method, which can be used to address both qualitative chemical characterization and qualitative pharmaceutical equivalence (PE).

Chemical characterization and PE of natural source conjugated estrogens involve both qualitative and quantitative aspects. Qualitative aspects of both chemical characterization and qualitative PE involve detection and measurement of the components in conjugated estrogens at or above 0.1 area % of the sum of the three quantitatively major estrogens: estrone sulfate, equilin sulfate, and 17"-dihydroequilin sulfate ("sum of three"). The recommended LC-MS method is applicable to both the drug substance and/or solid oral dosage forms.

This guidance provides a description of the LC-MS method developed by the Division of Testing and Applied Analytical Development/Office of Pharmaceutical Sciences/Center for Drug Evaluation and Research for both the qualitative characterization and documentation of qualitative PE of natural source conjugated estrogens. Interpretation of the data for PE purposes is beyond the scope of this guidance and will be addressed in a separate document. Quantitative aspects of chemical characterization and PE use the GC (flame-ionization detector) and HPLC (ultraviolet detector) assays (described in the draft proposed Conjugated Estrogens, USP, monograph)[2] and are not the subject of this guidance.

[1] This guidance has been prepared by the Natural Source and Synthetic Conjugated Estrogens Working Group of the Complex Drug Substances Coordinating Committee in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration. This guidance represents the Agency's current thinking on a LC-MS method for both qualitative chemical characterization and documentation of qualitative pharmaceutical equivalence for conjugated estrogens drug substance and solid oral dosage forms. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. Alternative approaches may be used if such approaches satisfy the requirements of the applicable statutes, regulations, or both.

[2] Draft Conjugated Estrogens, USP, monograph proposed by FDA to USP. The draft monograph is available on the CDER internet website at http://www.fda.gov/cder/drug/monographs/default.htm.

II. PROCEDURE[3, 4]

Estrogen standard solutions:

Prepare separate aqueous solutions of sodium estrone-3-sulfate, piperazine equilin-3-sulfate, and sodium 17"-dihydroequilin-3-sulfate, each at approximately 0.05 mg/mL. Analyze these solutions separately using the gradient LC-MS method to define the three most abundant estrogen sulfates in Conjugated Estrogens, USP, by comparing the retention times (RTs) to the three most abundant components in Conjugated Estrogens, USP, reference standard tablets or to the pioneer Conjugated Estrogens Tablets, USP. While multiple peaks should be detected at 349 atomic mass units (AMU), estrone sulfate and 17"-dihydroequilin sulfate should be the two largest peaks, with estrone sulfate larger than 17"-dihydroequilin sulfate. Similarly, multiple peaks should be detected at 347 AMU, with equilin sulfate being the largest peak. RTs should increase in the order 17"-dihydroequilin sulfate, equilin sulfate, and estrone sulfate.

USP reference standard tablets and test preparation:

Follow USP 24, *Conjugated Estrogens Tablets* under *Assay* to obtain the sample powder, using Conjugated Estrogens, USP, reference standard tablets, or test preparation consisting of the pioneer Conjugated Estrogens Tablets, USP, or test tablets. For assay of the bulk drug, use the powdered bulk drug substance or other suitable sample. Accurately weigh a portion of the sample powder equivalent to about 0.25 mg Conjugated Estrogens into a screwcap vial. Add 2.00 mL water and vigorously shake to yield a concentration equivalent to about 0.125 mg conjugated estrogens/mL. Alternately mix the water-powder mixture with a Vortex stirrer and treat with an ultrasonic bath until a uniform fine suspension is obtained. Filter the suspension through a 0.2 µm surfactant-free cellulose acetate 25 mm membrane syringe filter (e.g., Nalgene Catalog No. 190-2520, Nalge Company).

Buffer, 1.0 M Ammonium Acetate, pH 6.0: Dissolve approximately 7.7 g ammonium acetate (ACS reagent grade) in 90 mL water, and adjust to pH 6.0 with glacial acetic acid. Transfer the solution to a 100 mL volumetric flask and dilute to volume with water.

Mobile Phase A: 12% acetonitrile-10 mM Buffer – In a 500-mL volumetric flask, add 400 mL water, 5.0 mL 1.0 M Buffer, mix, add 60 mL acetonitrile, mix, dilute to volume with water, mix. Filter the mobile phase through a polyvinylidene difluoride membrane filter, 0.22 µm (e.g., Durapore, filter type GV, Catalog No. GVWP 04700, Millipore Corporation).

Mobile Phase B: 60% acetonitrile-10 mM Buffer – In a 500-mL volumetric flask, add 180 mL water, 5.0 mL 1.0 M Buffer, mix, add 300 mL acetonitrile, mix, dilute to volume with water, mix. Filter the mobile phase as described for Mobile Phase A.

[3] Equivalent procedures that provide comparable data are acceptable.

[4] Use of water purified to about 18 megohm.cm resistivity (e.g., water prepared using the Milli-Q Water System, Millipore Corporation) is recommended for all described procedures.

Gradient Program

Time (min)	% A	%B	Comments
0	100	0	Initial conditions
47	20	80	Linear gradient
48	0	100	Linear gradient
54	0	100	Washout time

Instrumentation:

Liquid Chromatograph-Mass Spectrometer consisting of a binary pump, a vacuum degasser, an autosampler, a thermostatted column compartment, and an atmospheric pressure ionization-electrospray detector.[5]

High performance liquid chromatography (HPLC) conditions and procedure:

Column	L1 packing,[6] USP 24/NF 19, <621>
Initial system equilibration:	Prior to assay of samples, make one injection of the conjugated estrogens sample solution and run the gradient program. Do not use data from this run.
Between-run equilibration:	Equilibrate with the initial mobile phase for 20 min
Run Time:	74 min: 54 min (gradient program) plus 20 min (equilibration)
Flow Rate:	0.35 mL/min
Injection volume:	12 µL
Column Temperature:	25°C

Mass spectrometer (MS) conditions and procedure:

API-Electrospray Ionization, Negative Ion Mode

Gain:	2.0
Fragmentor Voltage:	100 volts

[5] The instrumentation used by the Division of Testing and Applied Analytical Development was a Hewlett Packard Liquid Chromatograph-Mass Spectrometer [1100 HPLC-Mass Selective Detector (MSD)] consisting of a binary pump (model G1312A), a vacuum degasser (model G1322A), an autosampler (model G1329A), a thermostatted column compartment (model G1316A), and an LC-MSD atmospheric pressure ionization (API)-electrospray detector (model G1946A). Equivalent instrumentation that provides comparable data is acceptable.

[6] YMC ODS-AM S3 120A, 3.0 x 150 mm, 3 µm spherical particle size column (Waters Associates), or equivalent column that provides comparable data.

Selected Ion Monitoring Mode: The Agency analyzed nine AMUs in each run. When performing their analyses, applicants should select specific AMUs for each of several runs, each run differing only in the AMUs scanned, except for AMUs 347 and 349, which should be included in each run. The number of AMUs scanned within each run affects the sensitivity, with decreasing sensitivity as the number of AMUs increases. Therefore, an attempt should be made to include about the same number of AMUs in each run.

During the data analysis of each run, each AMU should be extracted from the total ion chromatogram and the extracted ion chromatogram (EIC) should be integrated. The relative retention time (RRT) of a specific peak within a given EIC should be calculated by dividing the retention time (RT) of that peak by the RT of estrone sulfate in the AMU 349 EIC recorded during the same run. In determining the area % for a particular peak, the areas of estrone sulfate and 17"-dihydroequilin sulfate measured at 349 AMU and the area of equilin sulfate measured at 347 AMU should be added. This sum should then be divided into the area of the particular peak recorded during the same run.

Data collection time: From 3 min to 48 min post-injection

Spray Chamber:

Drying Gas Flow:	10 L/min
Drying Gas Temperature:	350°C
Nebulizer Pressure:	45 PSI
Capillary Voltage:	3500 volts

AMUs of negative ions $ 232 containing peaks consistently present during FDA analysis at $ 0.1 area % (relative to the *sum of three*) for Conjugated Estrogens, USP (Premarin, Wyeth-Ayerst)*

239	243	245	265	267	269
283	287				
303	345	347	349	351	353
355	361	363	365	367	369
371	373	375	377	379	381
385	387	389	395	397	399
401	407	411	413	415	429
445	447	449	451	461	465
467	476	479	481	487	494
495	496				
503	511	520	521		

*Most AMUs gave multiple peaks

Based on the Agency's experience, approximately 56 AMUs should be observed (see above table), excluding isotopes, for which the chromatograms exhibit approximately 230 to 260 peaks at $ 0.1 area %. Also, approximately 21 of these AMUs should be observed for which the chromatograms exhibit approximately 40 peaks at $ 1.0 area %. It is anticipated that additional analyses may reveal fewer peaks consistently present at $ 0.1 area % and $ 1.0 area %.

III. QUALITATIVE DATA REPORTING

For either qualitative chemical characterization or qualitative PE, the applicant should report RRTs of each peak relative to the estrone sulfate peak. In addition, each peak should be quantitated and reported in units of area % relative to the sum of the areas of the estrone sulfate, equilin sulfate, and 17"-dihydroequilin sulfate peaks (*sum of three*).

FDA is developing a draft guidance in which the Agency will make detailed recommendations on how to interpret the qualitative LC-MS data and acceptance criteria for documentation of qualitative PE.