



**EXHIBIT 17**

**ESTIMATED STATE MEDIAN INCOME  
FOR 4-PERSON FAMILIES, BY STATE,  
FISCAL YEAR 2001<sup>1</sup>—Continued**

States	Estimated state median income 4-person families <sup>2</sup>	60 percent of estimated state median income 4-person families
Wyoming .....	50,989	30,593

<sup>1</sup>In accordance with 45 CFR 96.85, each State's estimated median income for a 4-person family is multiplied by the following percentages to adjust for family size: 52% for one person, 68% for two persons, 84% for three persons, 100% for four persons, 116% for five persons, and 132% for six persons. For family sizes greater than six persons, add 3% for each additional family member and multiply the new percentage by the State's estimated median income for a 4-person family.

<sup>2</sup>Prepared by the Bureau of the Census from the March 1999 Current Population Survey, 1990 Decennial Census of Population and Housing, and 1998 per capita personal income estimates, by state, from the Bureau of Economic Analysis.

Note—FY 2001 covers the period of October 1, 2000 through September 30, 2001. The estimated median income for 4-person families living in the United States is \$56,061 for FY 2001. The estimates are effective for the Low Income Home Energy Assistance Program (LIHEAP) at any time between the date of this publication and October 1, 2000, or by the beginning of a LIHEAP grantee's fiscal year, whichever is later.

[FR Doc. 00-5679 Filed 3-8-00; 8:45 am]

BILLING CODE 4184-01-P

**DEPARTMENT OF HEALTH AND  
HUMAN SERVICES**

**Food and Drug Administration**

[Docket No. 00D-0835]

**Draft Guidance for Industry on  
Conjugated Estrogens, USP: LC-MS  
Method for Both Qualitative Chemical  
Characterization and Documentation of  
Qualitative Pharmaceutical  
Equivalence; Availability**

**AGENCY:** Food and Drug Administration, HHS.

**ACTION:** Notice.

**SUMMARY:** The Food and Drug Administration (FDA) is announcing the availability of a draft guidance for industry entitled "Conjugated Estrogens, USP: LC-MS Method for Both Qualitative Chemical Characterization and Documentation of Qualitative Pharmaceutical Equivalence." This draft guidance is intended to provide recommendations to applicants who wish to submit a new drug application or abbreviated new drug application for

a natural source conjugated estrogens solid oral dosage form. This guidance provides a description of the liquid chromatography-mass spectrometry (LC-MS) method that can be used to address both qualitative chemical characterization and qualitative pharmaceutical equivalence (PE).

**DATES:** Submit written comments on the draft guidance by June 8, 2000. General comments on agency guidance documents are welcome at any time.

**ADDRESSES:** Copies of this draft guidance for industry are available on the Internet at <http://www.fda.gov/cder/guidance/index.htm>. Submit written requests for single copies of the draft guidance to the Drug Information Branch (HFD-210), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Send one self-addressed adhesive label to assist that office in processing your requests. Submit written comments on the draft guidance to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852.

**FOR FURTHER INFORMATION CONTACT:** Wallace P. Adams, Center for Drug Evaluation and Research (HFD-350), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-594-5651.

**SUPPLEMENTARY INFORMATION:** FDA is announcing the availability of a draft guidance for industry entitled "Conjugated Estrogens, USP: LC-MS Method for Both Qualitative Chemical Characterization and Documentation of Qualitative Pharmaceutical Equivalence." Chemical characterization and PE of natural source conjugated estrogens involve both qualitative and quantitative aspects. Qualitative aspects of both chemical characterization and PE involve detection and measurement of certain of the components in conjugated estrogens. The recommended methodology, LC-MS, is applicable to both the drug substance and/or solid oral dosage forms. This draft guidance provides a description of the LC-MS method developed by the Division of Testing and Applied Analytical Development/Office of Pharmaceutical Science/Center for Drug Evaluation and Research for both the qualitative chemical characterization and documentation of qualitative PE of natural source conjugated estrogens. Interpretation of the data for PE is beyond the scope of this guidance and will be addressed in a separate document. Quantitative aspects of chemical characterization and PE use

the gas chromatography (GC) (flame-ionization detector) and high-pressure liquid chromatography (HPLC) (ultraviolet detector) assays described in a draft proposed Conjugated Estrogens, USP, monograph (<http://www.fda.gov/cder/drug/monographs/default.htm>), and they are not the subject of this guidance.

This Level 1 draft guidance is being issued consistent with FDA's good guidance practices (62 FR 8961, February 27, 1997). The draft guidance represents the agency's current thinking on this LC-MS method for both qualitative chemical characterization and documentation of qualitative pharmaceutical equivalence of conjugated estrogens, USP. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes, regulations, or both.

Interested persons may submit to the Dockets Management Branch (address above) written comments on the draft guidance. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. The draft guidance and received comments are available for public examination in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

Dated: March 1, 2000.

**Margaret M. Dotzel,**

*Acting Associate Commissioner for Policy.*

[FR Doc. 00-5751 Filed 3-6-00; 2:58 pm]

BILLING CODE 4160-01-F

**DEPARTMENT OF HEALTH AND  
HUMAN SERVICES**

**Health Care Financing Administration**

[Document Identifier: HCFA-R-205/  
Supplement]

**Agency Information Collection  
Activities: Proposed Collection;  
Comment Request**

**AGENCY:** Health Care Financing Administration, HHS.

In compliance with the requirement of section 3506(c)(2)(A) of the Paperwork Reduction Act of 1995, the Health Care Financing Administration (HCFA), Department of Health and Human Services, is publishing the following summary of proposed collections for public comment. Interested persons are invited to send

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# Guidance for Industry

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

### *DRAFT GUIDANCE*

*This guidance document is being distributed for comment purposes only.*

Comments and suggestions regarding this draft document should be submitted within 90 days of publication of the *Federal Register* notice announcing the availability of the draft guidance. Submit comments to Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20857. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions on the content of the draft document contact Wallace P. Adams, 301-594-5651.

U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
March 2000

BP

# **Guidance for Industry**

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

*Additional copies are available from:*

*Office of Training and Communications  
Division of Communications Management  
Drug Information Branch, HFD-210  
5600 Fishers Lane  
Rockville, MD 20857  
(Tel) 301-827-4573*

*(Internet) <http://www.fda.gov/cder/guidance/index.htm>*

**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research (CDER)  
March 2000**

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**Guidance for Industry<sup>1</sup>**  
**Conjugated Estrogens, USP—**  
**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 $\alpha$ -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)<sup>2</sup> and are not the subject of this guidance.

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<sup>1</sup> 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.

<sup>2</sup> 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>.

33 **II. PROCEDURE<sup>3, 4</sup>**

34

35 Estrogen standard solutions:

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

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49

50 USP reference standard tablets and test preparation:

51

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

62

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

66

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

71

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

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<sup>3</sup> Equivalent procedures that provide comparable data are acceptable.

<sup>4</sup> 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.

75

Gradient Program

76

77

Time (min)

% A

%B

Comments

78

79

0

100

0

Initial conditions

80

47

20

80

Linear gradient

81

48

0

100

Linear gradient

82

54

0

100

Washout time

83

84

Instrumentation:

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86

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.<sup>5</sup>

87

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High performance liquid chromatography (HPLC) conditions and procedure:

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92

Column

L1 packing,<sup>6</sup> USP 24/NF 19, <621>

93

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.

94

95

96

Between-run equilibration:

Equilibrate with the initial mobile phase for 20 min

97

Run Time:

74 min: 54 min (gradient program) plus 20 min

98

(equilibration)

99

Flow Rate:

0.35 mL/min

100

Injection volume:

12 µL

101

Column Temperature:

25°C

102

103

Mass spectrometer (MS) conditions and procedure:

104

105

API-Electrospray Ionization, Negative Ion Mode

106

Gain:

2.0

107

Fragmentor Voltage:

100 volts

<sup>5</sup> 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.

<sup>6</sup> 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.

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

114  
 115 During the data analysis of each run, each AMU should be extracted from the total ion  
 116 chromatogram and the extracted ion chromatogram (EIC) should be integrated. The  
 117 relative retention time (RRT) of a specific peak within a given EIC should be calculated  
 118 by dividing the retention time (RT) of that peak by the RT of estrone sulfate in the AMU  
 119 349 EIC recorded during the same run. In determining the area % for a particular peak,  
 120 the areas of estrone sulfate and 17 $\alpha$ -dihydroequilin sulfate measured at 349 AMU and the  
 121 area of equilin sulfate measured at 347 AMU should be added. This sum should then be  
 122 divided into the area of the particular peak recorded during the same run.

123  
 124 Data collection time: From 3 min to 48 min post-injection

125  
 126  
 127 Spray Chamber:

128  
 129 Drying Gas Flow: 10 L/min  
 130 Drying Gas Temperature: 350°C  
 131 Nebulizer Pressure: 45 PSI  
 132 Capillary Voltage: 3500 volts  
 133  
 134

AMUs of negative ions  $\geq$  232 containing peaks consistently present during FDA analysis at  
 $\geq$  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

135

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

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### 143 III. QUALITATIVE DATA REPORTING

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

149

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

153