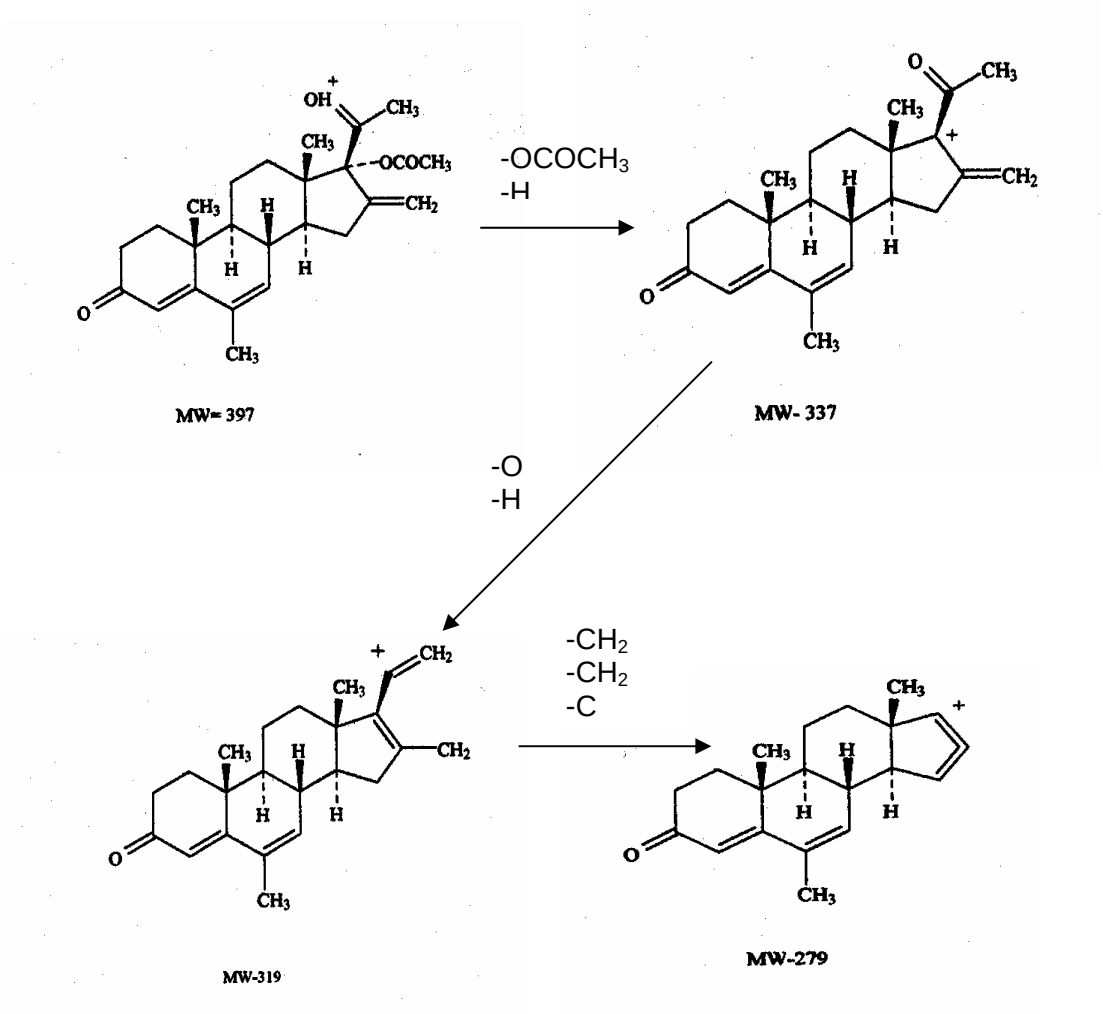
SOP No: **CLG-MGA1.00**		Page 1 of 13
Title: **Confirmation of Melengesterol Acetate by APCI/LC/MS^3**		
Revision: .00	Replaces: NA	Effective: 3-18-03

Contents

A. INTRODUCTION

1. Theory and Structures

This method utilizes the extracts from the determinative procedure (CLG-MGA) for animal fat. The extracts are evaporated, dissolved in the HPLC mobile phase and analyzed by LC/MS/MS/MS (LC/MS^3). Sample and external full scan MS^3 results are compared along with the ion ratio 319/279 for confirmation.



Characteristic MGA fragmentation ions

2. Applicability

This procedure is applicable to bovine fat.

B. EQUIPMENT

Note: Equivalent equipment and instrumentation may be substituted.

1. Apparatus and Instrumentation

a. Thermoquest/Finnigan LCQ with APCI interface.

b. HP/Agilent - 050 HPLC equipped with a quaternary pump.

c. Agilent/Zorbax XDB C-8 4.5 x 75 mm, 5 µm particle size.

d. Xpertek-nylon syringe filters - 13 mm x 0.2 µm pore size.

e. B-D 1 mL plastic syringes.

f. Nitrogen evaporator - Organomation model 112

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted.

1. Reagents

a. Methanol - glass distilled.

b. Water - Ultra-filtered 18 Megaohm

c. Formic acid - Sigma Chemical Catalog number F4636.

2. Mobile Phase Solutions

a. Mobile Phase A - 55/45 methanol/water + 0.1% formic acid.

Combine in a 1L graduated cylinder 550 mL methanol, 450 mL water and 1.0 mL of formic acid.

b. Mobile Phase B - 95/5 methanol/water + 0.1% formic acid.

Combine in a graduated cylinder 950 mL methanol, 50 mL water and 1.0 mL formic acid.

D. STANDARDS

1. Melengestrol acetate, 99.5% pure,

ICN Biomedicals

1263 Chilicothe Road Aurora, OH 44202

E. SAMPLE PREPARATION

1. Analyze the following samples by the MGA Determinative method (CLG-MGA) to provide an adequate confirmatory sample set:
   a. tissue blank
   b. tissue fortified at the level of interest
2. Evaporate the remaining extract from the determinative procedure (CLG-MGA) to dryness at approximately 40°C using the N-EVAP.
3. To the dry extract add 1000 µL of sample diluent (Mobile Phase A).
4. Filter through a 0.2 µm nylon syringe filter into a 1.8 mL autosampler vial.

   Note: Extracts should not be analyzed if the extract filtrate cannot be filtered to a clear solution.
5. Inject 30 µL into the system.

F. ANALYTICAL PROCEDURE

1. Data Acquisition
   a. HPLC Conditions

      The following are examples of HPLC Conditions. The analyst should optimize these parameters for the instrument being used.

      i. Flow rate 400 µL/min.

      ii. Mobile phase gradient profile:

0.00 min.	100% Mobile phase A
5.00 min.	100% Mobile phase B
12.00 min.	100% Mobile phase B
18.00 min	100% Mobile phase A

   b. MS Parameters

      The following are examples of the MSD parameters currently being used. Analyst should optimize these parameters for the instrument being used.

      i. APCI interface Parameters:

         (a) Vap. Temp 470°C

(b) Sheath flow 70
(c) Auxilary flow 0.0
(d) Discharge current 5.0 µA
(e) Capillary temp. 160°C
(f) Capillary voltage 10
(g) Tube lens offset 2.0

ii. Acquisition parameters

(a) Full Microscans 2
(b) Injection time 200

iii. Precursor ions, isolation width, relative collision energy

(a) 397, 2.0, 25.0
(b) 337, 2.0, 32.0

iv. Selected ions in MS^3 for determining the ratio---319, 279.

c. MS Optimization

i. Compare averaged “background spectrum" to the previous run.

ii. Observe the Positive APCI spectrum of background. Inject a pure 5 ng MGA standard by flow injection and observe the first order spectrum. Note the centroid of the precursor ion at 397. Make a second injection of standard in ms/ms using the exact mass of 397 previously noted in the first injection. Note the centroid of the product ion at 337. Make a third injection in ms/ms/ms using the exact mass of 337 as second precursor as noted in the previous injection. A reasonable full spectrum in MS^3 should be obtained.

iii. The 319/279 ratio should be between 0.1-0.2.

2. Confirmation Criteria

a. Retention time of unknown should be within ± 5% of the recovered standard.

b. The spectrum obtained from a suspect compound should visually match the spectrum obtained from a contemporaneous standard. Since full scan data may include hundreds of significant data points for comparison, strict numerical criteria need not be applied.

c. The ion ratio 319/279 of the unknown should match that of the pure standard within ± 20% relative.

d. Tissue blank has no confirmable target compound.

3. Operational Criteria for Sample Repeat Injection

a. For unknown samples that will not confirm in the initial analyses and the system suitability has not been compromised, repeat the injection. Subsequently, an injection of the pure standard is made.

b. If upon re-injection the sample still fails to confirm, repeat the extraction using the determinative method.

c. If upon re-extraction the sample fails the confirmation, the sample should be reported as non-detected for MGA.

4. Sample Chromatograms and Spectra

Refer to Section K, “Chromatograms and Spectra”.

G. CALCULATIONS (Not Applicable)

H. HAZARD ANALYSIS

1. Method Title — Confirmation of Melengesterol Acetate by APCI-LC-MS^3.

2. Required Protective Equipment — Safety glasses, vinyl or latex gloves, and laboratory coat.

3. Hazards

Reagents	*Hazard*	*Recommended Safe Procedures*
Hexane Methanol Iso-octane	Flammable. Avoid breathing vapors. May cause skin irritation	Keep in well closed containers in a cool place and away from fire. Use it under well ventilated hood.
Formic Acid	Danger. Corrosive. Liquid and mist may cause severe burns to all body tissue. May be fatal if swallowed and harmful if inhaled. Flammable liquid and vapor.	Keep in a tightly closed container. Store in a cool, dry ventilated area away from sources of heat or ignition.

4. Disposal Procedures

Reagents	*Hazard*	*Recommended Safe Procedures*
Organic solvents	See above	Collect waste in tightly sealed container and store away from non-compatibles in a cool, well ventilated, flammable liquid storage area/cabinet for disposal in accordance with local, state, and Federal regulations.
Formic Acid		Dispose of container and unused contents in accordance with federal, local, and state requirements.

I. QUALITY ASSURANCE PLAN

1. Performance Standard
   a. No false positives from blank tissues.
   b. Zero false negatives at tolerance.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
A characteristic MS^3 scan should be obtained using the m/z = 337 precursor ion.	Compare MS^3 scan to a standard scan.

3. Readiness To Perform (FSIS Training Plan)

   a. Phase I. Standards — On three separate days, inject standards at the level of interest and determine the ratios of the ions of interest.

   b. Phase II. On three different days, analyze a blank and a sample spiked at the level of interest and determine the ratios of the ions of interest.

      NOTE: Phases I and II may be performed concurrently.

   c. Phase III. Analyze 6 incurred or fortified tissues at levels $\geq$ 12.5 ppb. One of the 6 unknowns should be blank.

4. Intralaboratory Check Samples

   a. Frequency: One per week or as samples are analyzed.

   b. Records of results are to be maintained by the analyst and reviewed by the supervisor and QAM.

   c. Acceptability criteria

      If unacceptable values are obtained, then:

      i. Stop all official analyses for that analyst.

      ii. Take corrective action.

5. Sample Acceptability and Stability

   The stability of the extracts is to be determined.

6. Sample Set

   a. Standards

   b. Tissue blank

   c. Tissue blank fortified at level of interest with suspect drug

   d. Samples

7. Sensitivity

   a. Lowest reliable confirmation (LRC): To be determined.

   b. Minimum proficiency level (MPL): 12.5 ppb.

J. WORKSHEET

An example of a worksheet, on the following page, can be removed from this book for photocopying.

MELENGESTROL ACETATE CONFIRMATION FORM

EQUIPMENT USED	ID NUMBER
Instrument Used (LMS #):	
Method File Name:	
External Standard:	
Mobile Phase "A":	
Mobile Phase "B":	
Injection Volume (uL):	
Micropipettor Used (MCP#):	
N-Evap Used (WAT#):	

Analyst: _____
Date Started: _____
Date Completed: _____

Set Number : _____

Reviewed by: _____
(Initials and Date)

N-Evap Temperature (40C) _____

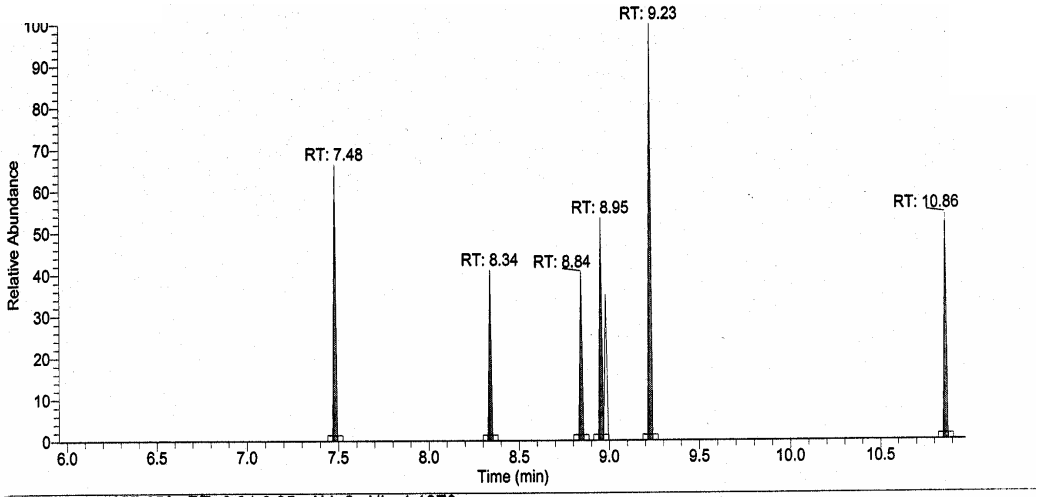
Confirmation Sample Analysis Data

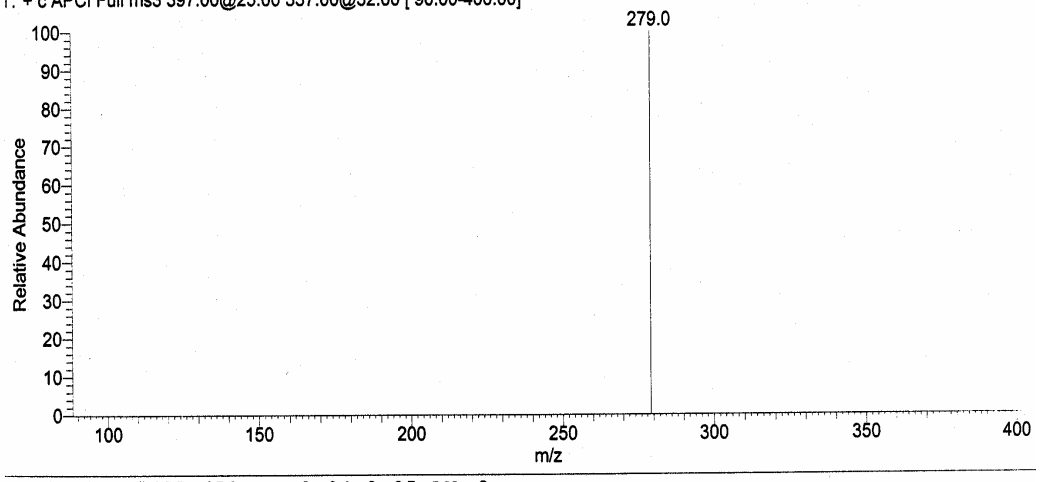
Sample Number	RMS Lab. Number	R-504 Lab. Number	Tissue Code	MGA Ret. Time (min)	MGA RT Vs. Rec. Std. (± 5%)	MGA Ion Ratio m/z (319/279)	MGA Ion Ratio Vs. Ext. Std. ± 30%	Reasonable Mass Spectra match? Y or N	Melengestrol Acetate Result + or -
1	Ext. Std.								
2	Recovery								
3	Blank								
4									
5									
6									
7									
8									
9									
10									
11									
12									

REMARKS:

K. Chromatograms and Spectra

1. Figure 1 Blank Fat

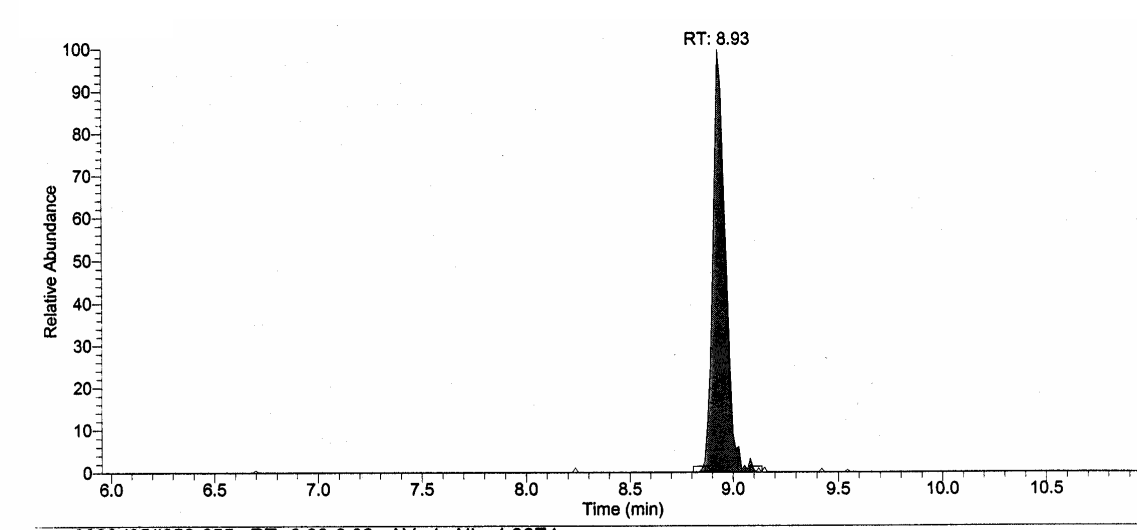




mga0920d04#657-658 RT: 8.94-8.95 AV: 2
T: + c APCI Full ms3 397.00@25.00 337.00@32.00 [90.00-400.00]

m/z	Intensity	Relative
279.03	1162.0	100.00

2. Figure 2 12.5 ppb Fat Recovery



mga0920d05#652-656 RT: 8.88-8.93 AV: 5
T: + c APCI Full ms3 397.00@25.00 337.00@32.00 [90.00-400.00]

m/z	Intensity	Relative
261.10	14203.0	22.07
279.05	64358.0	100.00
294.11	9885.0	15.36
295.09	6736.6	10.47
319.11	9177.0	14.26

Approved by:	**Date Approved:**
Tom Mallinson	2-7-03
Terry Dutko	2-5-03
Jess Rajan	2-6-03
Charles Pixley	2-5-03
Phyllis Sparling	2-18-03

Approval signatures on file.