SOP No: **CLG-ANA.02**		Page 1 of 20
Title: **Determination and Confirmation of Diethylstilbestrol (DES) and Zeranol by GC/MS**		
Revision: .02	Replaces: .01	Effective: 11/10/04

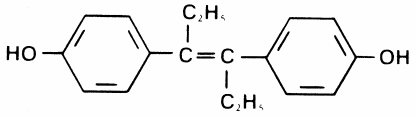
Contents

A. INTRODUCTION

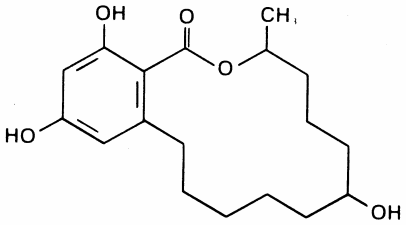
1. Theory

This procedure uses a three-phase solvent extraction of tissue using an aqueous buffer, acetonitrile, dichloromethane, and hexane to effectively remove the majority of the triglycerides and highly non-polar materials prior to loading on the column. This step prevents the overloading of the column, which would reduce recoveries, and simultaneously fractionates compounds into three polarity classes. An extraction cartridge containing a strongly basic anion exchange resin with quaternary amine groups in the chloride form is used to extract the DES and zeranol. The phenolate anion of these compounds has a high affinity for the chloride form of the resin at pH values above 12.0. The acetonitrile extract of the tissue is concentrated, made alkaline, and the analyte is exchanged to the resin. Several solvent washes are performed to remove interfering substances. A 5% aqueous acetic acid wash allows for the elution of a large quantity of interfering anionic materials previously exchanged to the resin. The column is then eluted with a solution of limited solvent strength (25% aqueous methanol) to remove residual acetic acid and additional matrix material prior to the final elution with methanol. The procedure is amendable to automation using the four-solvent Dupont Prep I centrifugal column elution device, which significantly increases sample throughput.

2. Structure



DIETHYLSTILBESTROL
($C_{18}H_{20}O_2$; mol wt 268.34)



ZERANOL
($C_{18}H_{26}O_5$; mol wt 322.41)

3. Applicability

This method is applicable for DES and Zeranol in ovine and bovine liver and muscle at levels ≥ 0.5 ppb DES and 1.0 ppb Zeranol.

B. EQUIPMENT

Note: Equivalent apparatus or instrumentation may be substituted for any of the following.

1. Apparatus

a. Polypropylene centrifuge tubes - 50 mL, screw-cap Falcon.

b. Homogenizer - Tekmar SDT Tissuemizer with 1810 motor and SDT-100EN shaft and generator and TR-10 high torque speed control.

c. Centrifuge - IEC PR-6000, equipped with 12-place angle rotor, and capable of achieving a G force of ≥ 2500.

d. Pasteur disposable pipets.

e. Folded Filter Paper - 125 mm, #2, Whatman.

f. Repipet dispensers - 10 mL, Labindustries, Inc.

g. Low temperature indicator - Precision Scientific, Freas Model 815, or constant temperature water bath (35 - 37 °C).

h. Type AS anion exchange cartridge.

i. Plastic recovery cups.

j. Prep I automated sample processor - Dupont Chemical Co., catalog #60000-908.

k. Reacti-Therm dry block heater with custom-drilled heat blocks, Pierce Chemical Co.

l. Reacti-therm evaporator unit attachment - Pierce Chemical Co.

m. Fisher sample concentrator - Model 190, custom-drilled to accept 20 mL. scintillation vials, catalog #09-549.

n. Vortex mixer.

o. Tapered 1 mL auto sampler vials - 1 CWVC combo-pack and 8 pep-6 polyethylene plug, Chemical Research Supplies.

p. Eppendorf pipets.

q. Disposable pipet - 10 mL.

r. pH meter - Orion Model 601 A.

s. Syringe - 100 µL.

t. Rotorack or Eberbach shaker (optional).

u. Balance - MT5, capable of reading to + 0.001 mg, Mettler-Toledo, Inc, Columbus, OH.

2. Instrumentation

GC/MS - Hewlett-Packard, 5890 GC, 5970A series mass selective detector and 98258 computer.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted for any of the below.

1. Reagents

a. Acetonitrile - Burdick & Jackson, high purity solvent grade.

b. Hexane - Burdick & Jackson, high purity solvent grade.

c. Dichloromethane - Burdick & Jackson, high purity solvent grade.

d. Methanol - Burdick & Jackson, high purity solvent grade.

e. Ethyl acetate - Burdick & Jackson, high purity solvent grade.

f. Isopropyl alcohol - Burdick & Jackson, high purity solvent grade.

g. Water - distilled, deionized.

h. Acetic acid, glacial - USP #9522, Canlab Chemicals.

i. Derivatizing agents - BSTFA, Pierce Chemical Co., #38830; TMSI, Pierce Chemical Co., #88623.

j. Sodium hydroxide - reagent grade pellets - #3722-1, J. T. Baker Chemical Co.

k. 2N Sodium acetate trihydrate - Fisher Certified Reagent, #S209B, Fisher Chemical Co.

l. β-Glucuronidase - Type HP-2S Cat No G7770, Sigma Chemical Corp., St. Louis, MO. Activity approximately 100,000 units/mL.

2. Solutions

a. 2N Sodium hydroxide:

80 g/L in distilled, deionized water.

b. 1:1 Isopropanol:methanol:

100 mL of each, measured separately and mixed.

c. 5% Aqueous acetic acid:

5 mL glacial acetic acid made up to 100 mL with distilled, deionized water.

d. 25% Aqueous methanol:

25 parts methanol; 75 parts distilled, deionized water; measured separately and mixed.

e. 0.04M Sodium acetate trihydrate buffer:

5.44 g sodium acetate dissolved in 1 L distilled, deionized water.

D. STANDARDS

Note: Equivalent standards and solutions may be substituted for any of the following.

1. Source

a. Zearalane - zeranol assay internal standard, Schering-Plough Animal Health, Union, NJ.

b. Zeranol - (alpha-zearalanol) #Z 0292, 5 mg, Sigma-Aldrich Chemical Company, St. Louis, MO. (Five bottles are needed to make up the primary standard as directed below.

c. D_8DES - DES internal standard (diethyl-1,1,1',1 '-d4-stilbestrol-3,3',5,5'-d4), Cambridge Isotope Laboratories, Andover, MA.

d. DES - 99% purity, #21894-4, Aldrich Chemical Co., Milwaukee, WI.

e. Diethylstilbestrol monoglucuronide (DES-MG) - #1 6444-5, Aldrich Chemical Co., Milwaukee, WI. Each ampoule contains the equivalent of 1 mg of free DES.

2. Preparation of Standards

a. DES, D_8DES, zearalane, zeranol.

i. Stock solutions:

Weigh 20.00 mg $\pm$ 0.05 mg each of zearalane and zeranol and 10.00 mg $\pm$ 0.05 mg each DES and D_8DES into separate 100 mL volumetric flasks. Dilute to volume with methanol (concentration 200 µg/mL for zearalane and zeranol and 100 µg/mL for DES and D_8DES).

ii. Working solutions:

Dispense 100 µL of zearalane, zeranol, DES, and D_8DES stock solutions into separate 100 mL volumetric flasks. Dilute to volume with methanol (concentration 0.2 ng/µL for zearalane and zeranol, and 0.1 ng/µL for DES and D_8DES).

b. Conjugated DES.

i. Stock solution (10 μg/mL free DES):

Take 1 ampoule of DES-MG (equivalent to 1 mg free DES) and dissolve the contents with methanol in a 100 mL volumetric flask.

ii. Working solution (0.1 ng/μL free DES):

Dispense 1 mL of stock solution into a 100 mL volumetric flask. Dilute to volume with methanol.

Note: Allow standards to equilibrate to room temperature before use.

3. Storage Conditions: All standards should be kept frozen at < -10 °C if not in use.

4. Shelf Life Stability

a. Stock solutions: One year.

b. Working standards: Up to 6 months.

E. SAMPLE PREPARATION

Sample tissue should be thawed but cold before processing. Cut tissue pieces from various locations, avoiding fat. Place liver tissue in a blender and muscle tissue in a *Robocoupe* ® or grinder. Process tissue just long enough to produce a homogenous blend without warming the tissue. Place samples in a freezer after preparation.

F. ANALYTICAL PROCEDURE

Note: All tissues should be kept frozen at all times until assayed.

1. Sample Extraction

a. Weigh 5.0 g + 0.1 g blended liver or ground, homogenized muscle into 50 mL polypropylene centrifuge tube.

b. Fortify each sample and control sample with 50 µL (concentration 0.2 ng/µL) of zearalane and 50 µL (concentration 0.1 ng/µL) of D_8DES internal standard (equivalent to 2.0 and 1.0 ppb) respectively.

c. Prepare controls as follows:

i. For screening sets

Weigh 2 tissue blanks and prepare duplicate recovery samples by adding zeranol (concentration 0.2 ng/µL), and DES-MG (concentration 0.1 ng/µL) as follows:

DES (ppb)	Zeranol (ppb)	*µL of DES fortification solution added*	*µL of Zeranol fortification solution added*
0.50	1.00	25.0	25.0

ii. For quantitative sets, weigh 4 tissue blanks and add zeranol (concentration 0.2 ng/µL), and DES-MG (concentration 0.1 ng/µL) as follows:

Control Concentration (ppb)		*µL of DES fortification solution added*	*µL of Zeranol fortification solution added*
DES	**Zeranol**		
0.00	0.00	0.0	0.0
0.50	1.00	25.0	25.0
1.00	2.00	50.0	50.0
2.00	4.00	100.0	100.0

d. With repipet, add 11 mL of 0.04 M sodium acetate buffer.

e. Homogenize for 1 minute, using Tissuemizer.

f. Adjust pH to 4.25 - 4.75 with glacial acetic acid. Add 5 drops of glacial acetic acid with a Pasteur pipet, vortex mix, and check pH (5 - 8 drops are usually required).

g. Add 100 µL ß-glucuronidase to each sample (approximately 10,000 units). Cap tube and vortex mix thoroughly for about 10 seconds.

h. Incubate overnight (16 -18 hours) at 37 °C or place overnight in a constant temperature water bath set at 35 - 37 °C.

i. After incubation, add 16.0 mL acetonitrile to each tube, using a repipet, and shake moderately by hand or in a Rotorack or Eberbach shaker for about 5

minutes.

j. Centrifuge samples at a minimum G force of 2450g for 5 minutes.

Note: g force = $(1 .118 \times 10^{-5})$(radius of rotation in cm)$(RPM)^2$.

radius of rotation = distance from center shaft to the furthest inside tip of the tube.

k. Filter the supernatant through fluted filter paper into a 50 mL screw-cap polypropylene centrifuge tube.

Note: If a small amount of floating debris is transferred with the supernatant, it will not interfere with the test.

l. Add 2 mL dichloromethane and 8 mL hexane to the supernatant.

m. Shake moderately by hand for 1 minute (or use Rotorack or Eberbach shaker).

n. Centrifuge at a minimum G force of 1060 for 2 minutes.

o. Transfer middle layer (acetonitrile) to a clean 20 mL scintillation vial, using a 10 mL disposable pipet, without removing any of the other layers.

p. With repipet, add another 4 mL of acetonitrile to the remaining two layers in the 50 mL centrifuge tube.

q. Shake moderately by hand for 1 minute (or use Rotorack or Eberbach shaker).

r. Centrifuge at a minimum G force of 1060 for 2 minutes.

s. Repeat step o above, recovering as much of the middle acetonitrile layer as possible using a 10 mL disposable pipet, without removing any of the other layers.

Stop point: Samples may be stored for up to 1 hour at room temperature at this point.

t. Gently evaporate the combined acetonitrile layers to dryness with filtered house or compressed air at 60 ± 5 °C, using the Fisher Model 190 sample concentrator, or a gentle stream of nitrogen, using the custom-drilled heating block.

Note: All acetonitrile must be removed. This takes 45 - 60 minutes.

u. With repipet, add 2 mL 1:1 isopropanol:methanol and dissolve residue. Let set 5 minutes and vortex until dissolved.

v. With disposable pipet, add 1.5 mL 2N sodium hydroxide solution and vortex mix or swirl for 5 -10 seconds.

Note: Column cleanup must proceed at this point.

w. Take apart TYPE AS cartridges; decant liquid above the ion exchange bed. Place the cartridges on the inner ring of the rotor. Place the plastic waste cups and plastic sample recovery cups on the outside ring of the rotor.

x. Load sample solutions from step v above onto the cartridge bed. Rinse the vials

with 1.0 mL of isopropanol:methanol and also add this to the cartridges.

y. Close the lid of the Prep I and select Program 7. Advance the program to step 2 with the STEP ADVANCE switch and start the processor with the STEP ACTIVATE/HOLD switch. Allow the unit to run for 4 minutes and then stop it with the STEP ACTIVATE/HOLD switch. Open the lid and empty the waste cups and return them to the outer rotor.

z. With repipet, add 4 mL methanol to each cartridge and repeat step y above.

aa. Add the following solvents to solvent reservoirs 1, 2, 3, and 4 respectively.

i. Wash solvent 1: Place 18 mL of distilled deionized water in reservoir 1. This is equivalent to 1.4 mL per cartridge.

ii. Wash solvent 2: Place 33 mL of 5% aqueous acetic acid in reservoir 2. This is equivalent to 2.75 mL per cartridge.

iii. Wash solvent 3: Place 22 mL of 25% aqueous methanol in reservoir 3. This is equivalent to 1.75 mL per cartridge.

iv. Elution solvent: Place 33 mL ± 3 mL of 100% methanol in reservoir 4. This is equivalent to 2.75 mL ± 0.25 mL per cartridge.

Note: The volume of methanol elution solvent may require adjustment (± 3 mL) such that the final volume in step cc below is < 0.5 mL. The specific volume required for each Prep I unit in each laboratory can be predetermined by the analyst.

bb. Select Program 7 and advance to step 3 with the STEP ADVANCE switch. Set the evaporation temperature to 55 °C (approximately setting 5). Start the program with the START switch.

cc. After the program is finished, remove the plastic recovery cups and quantitatively transfer methanol eluate (0.1 - 0.5 mL) to a tapered 1 mL auto sampler vial using small MeOH rinses. Evaporate just to dryness under low nitrogen flow in a custom-drilled heat block set at 60 ± 5 °C. Note: If volume of eluate + rinses exceeds amount that can be safely concentrated without sample loss, use multiple partial transfer/evaporation cycles to effect concentration. The sample is ready for GC/MS analysis.

Note: Extracts must be stored in a freezer and should be analyzed as soon as possible but, if necessary, may be stored frozen up to a week without significant

loss.

2. Instrumental Settings

a. GC parameters

Note: Optimize instrumental conditions to obtain maximum sensitivity and adequate baseline resolution of peaks of interest, especially zeranol. The following table represents one optimization possibility.

i.	GC	Hewlett Packard 5890
ii.	Column	Crosslinked 100% methylsilicone HP special purpose 25.0 m, 0.2 mm i.d., 0.33 micron film thickness (16 m, 0.20 mm i.d., 0.5 micron film thickness has been used successfully)
iii.	Injector	Heated splitless, 260 °C minimum
Iv.	Linear carrier gas flow	30 cm/sec, helium
v.	Source Temperature	250 °C
vi.	Interface Temperature	250 °C
vii.	Temperature Program	Hold at 130 °C for 1 minute. Ramp at 20° /minute to 230 °C. Ramp at 5 °C/minute to 295 °C. Ramp at 5 °C/minute to 310 °C. Hold for 10 minutes.

b. MS parameters.

i.	Mass spectrometer	Hewlett Packard 5970A-MSD
ii.	Ionization mode	Electron impact
iii.	Electron energy	70 eV
iv.	Calibration	Autotune
v.	Electron Multiplier Voltage	Approximately 1300 V

3. General Operation

a. Reconstitute the dry residue with 10 µL of ethyl acetate and derivatize the samples with BSTFA plus 2% TMSI catalyst using an on-column derivatization technique.

b. Draw 1.0 - 2.0 µL of sample into the syringe, followed by a 1 µL air space

and 2 - 3 µL of derivatization agent. (Wipe syringe needle with Kimwipe soaked with ethyl acetate before inserting into the derivatization agent).

c. Inject the entire contents (3 - 5 µL total) into the heated injection part of the GC operating in the splitless mode.

Note: 4 - 5 µL of ethyl acetate solvent should be injected after every positive sample to prevent carry over interferences.

4 Sample Chromatogram

See Section K for chromatograms.

5. Retention times

		Approximate retention times (min) with above conditions:
a.	cis DES	10.8
b.	trans DES	11.9
c.	Zearalane	14.9
d.	Zeranol	17.5

6. Ions Monitored

Analyte		**Ions (Dwells = 100 milliseconds)**
a.	Zearalane	435
b.	Zeranol	538, 523, 433, 379
c.	D_8DES	420
d.	DES	412, 397, 383

G. CALCULATIONS

1. Procedure

a. Screening calculations

i. Report samples as positive if they meet the confirmation criteria specified in G. 2. a - d.

ii. Report samples as negative if they fail to meet the confirmation criteria specified in G. 2. a - d.

b. Quantitative calculations

Read tangential area counts or peak height for the selected ions from the integration report and tabulate.

i. Obtain calibration curve values by ratioing the ion area of the analyte in question to the appropriate internal standard, as below:

$$\text{zeranol} = \frac{\text{Area counts or peak height for ions 538 + 523 + 433}}{\text{Area counts or peak height for ion 435}}$$

$$\text{DES} = \frac{\text{Area counts or peak height for ion 412, cis + trans}}{\text{Area counts of peak height for ion 420, cis + trans}}$$

ii. Using linear regression, construct a standard curve of DES or zeranol ion ratios vs. concentration of DES or zeranol.

The equation: $y = mx + b$.

where: y = ion ratio

x = conc. in ppb (µg/kg)

m = slope

b = y intercept

The correlation coefficient should be > 0.995.

iii. From the ion ratios, using the regression slope and intercept, calculate the concentration for each sample.

2. Criteria for Confirmation (Screening and Quantitative analyses)

Note: The set must include a tissue blank and three fortifications to bracket the estimated level of the suspect positive.

a. All monitored ions must be present for all isomers.

b. Blank tissue must show no interferences.

c. Ion ratios must agree within 30% of those of the fortified standard.

Compounds	**Ion Ratios**
Zeranol	523/538 433/538 379/538
DES (cis + trans)	383/412 397/412

d. Retention time must agree within 0.3 min of that for the fortification extract.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Safety glasses, plastic gloves, labcoat.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Acetonitrile, hexane, dichloromethane, ethyl acetate	These solvents are corrosive and flammable. Vapors can cause severe skin, eye, and respiratory irritation.	Work should be performed in an efficient fume hood away from heat-generating devices.
Sodium hydroxide	Caustic; may cause irreversible skin and eye damage.	Wear the required protective equipment. Avoid spillage or spattering.

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Acids and Bases	See Above	Neutralize (pH 5-8) and flush into waste sink with large amounts of water if waste meets Federal/State/Local regulations.
Organic Solvent Mixtures	See Above	Segregate chlorinated from nonchlorinated solvents until disposal by contract or in-house specialist.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

   a. For screening sets:

      i. No false negatives at 0.5 ppb for DES and 1.0 ppb for Zeranol.

      ii. No false positives for the negative control.

   b. For quantitative sets:

Analyte	*Analytical Range*	*Acceptable Recovery*	*Acceptable Repeatability % CV*
DES	$\geq$ 0.5 ppb	80 - 130	$\leq$ 20
Zeranol	$\geq$ 1.0 ppb	78 - 120	$\leq$ 20

The Measurement Uncertainty and Method Detection Limit should be recalculated yearly or whenever a change that affects method accuracy, precision, or sensitivity occurs.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
a. Sample weight	5.0 g ± 0.1 g
b. Hydrolysis pH	4.5 ± 0.25
c. ß-Glucuronidase	100 µL
d. Incubation	35 - 37 °C
e. Evaporation to dryness	Remove all acetonitrile
f. Sodium Hydroxide	1.5 mL of 2N

3. Readiness To Perform (FSIS Training Plan)

a. Familiarization

i. Phase I: Standards. External standard curve is not applicable for this method. Proceed to Phase II.

ii. Phase II: Analyst's self-fortified samples. Duplicate recoveries at the fortified levels as listed below for three consecutive sets including:

(a) 1 set of liver fortified with DES and zeranol.

(b) 1 set of liver fortified with DES.

(c) 1 set of muscle fortified with DES and zeranol.

DES (ppb)	Zeranol (ppb)
0	0
0.5	1.0
1.0	2.0
2.0	4.0

iii. Phase III: Check samples for analyst accreditation.

(a) 13 liver samples, including:

1) 5 samples fortified with DES (between 0 and 2.0 ppb).

2) 8 samples fortified with DES and zeranol.

(b) 8 muscle samples fortified with DES and zeranol.

(c) Report analytical findings to the Quality Assurance Manager (QAM)

Notification from QAM is required to commence official analysis.

b. Acceptability criteria.

i. Refer to section I.1.a. above for screening sets.

ii. Refer to section I.1.b. above for quantitative sets.

2. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One per week by each analyst as samples analyzed.

ii. Records to be maintained for:

(a) All replicate findings.

(b) Appropriate charts.

(c) Coefficient of correlation of all internal standard curves.

b. Acceptability criteria.

Refer to Section I.1 above.

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrices: Liver or muscle.

b. Sample receipt size: Varied; enough to obtain matrix required for all quantitative and confirmation tests and reserve sample.

c. Condition upon receipt: Frozen.

d. Sample storage:

i. Time: ≤ 6 months.

ii. Condition: Frozen.

6. Sample Set

a. 12 units as determined by DuPont Prep I apparatus.

b. Screen set will include:

i. 2 fortifications at action level,

i. a blank, and

ii. up to 9 samples.

c. Quantitation set will include:

i. 4-point fortification curve (as described in section F.1 .f.),

ii. an analyst recovery and

iii. up to 7 samples.

7. Sensitivity

Minimum proficiency level (MPL): 0.5 ppb DES, 1.0 ppb Zeranol

J. WORKSHEET

The worksheet on the following page, DES and Zeranol, can be removed for photocopying.

Anabolic Steroids Worksheet

Weighed (Init/AC/Date): _____
Extracted (Init./AC/Date): _____
MS Analysis (Init./AC/Date): _____
Reviewed By (Init./AC/Date): _____
Method: CLG-ANA.XX
Set Number: _____
Species: _____

Sample FRZ-_____ to Holding FRZ-_____
Holding FRZ-_____ to FRZ-_____

Incubator: _____
Initial Incubation Time (PM) _____
Final Incubation Time (AM) _____
Total Incubation Time _____

Equipment ID:
Balance: _____
pH meter: _____
Micropipetor(s): _____
Centrifuge: _____
Concentrator: _____
Concentrator Temp: _____
Auto-Prep I: _____
Reacti-Vap: _____
Reacti-Vap Temp: _____
GC/MS: _____
GC/MS Method File: _____

Incubation Temp., (35-37 C, PM) _____
Incubation Temp., (35-37 C, AM) _____

Reagent ID:
Zeranol Standard : _____
Zeralane Standard : _____
DES - MG Standard : _____
DgDES Standard : _____
0.04 M NaOAc: _____
Acetic acid, glacial: _____
100 uL B - Glucuronidase: _____
Acetonitrile: _____
Hexane: _____
Methylene Chloride: _____
1:1 IPA/MeOH: _____
2N NaOH: _____
Water: _____
5% HOAc: _____
25% MeOH/H_2O: _____
Type AS cartridge: _____
Methanol: _____
Ethyl Acetate: _____
2% TMSI in BSTFA: _____

Note: Check and print out instrument parameters before collecting data.

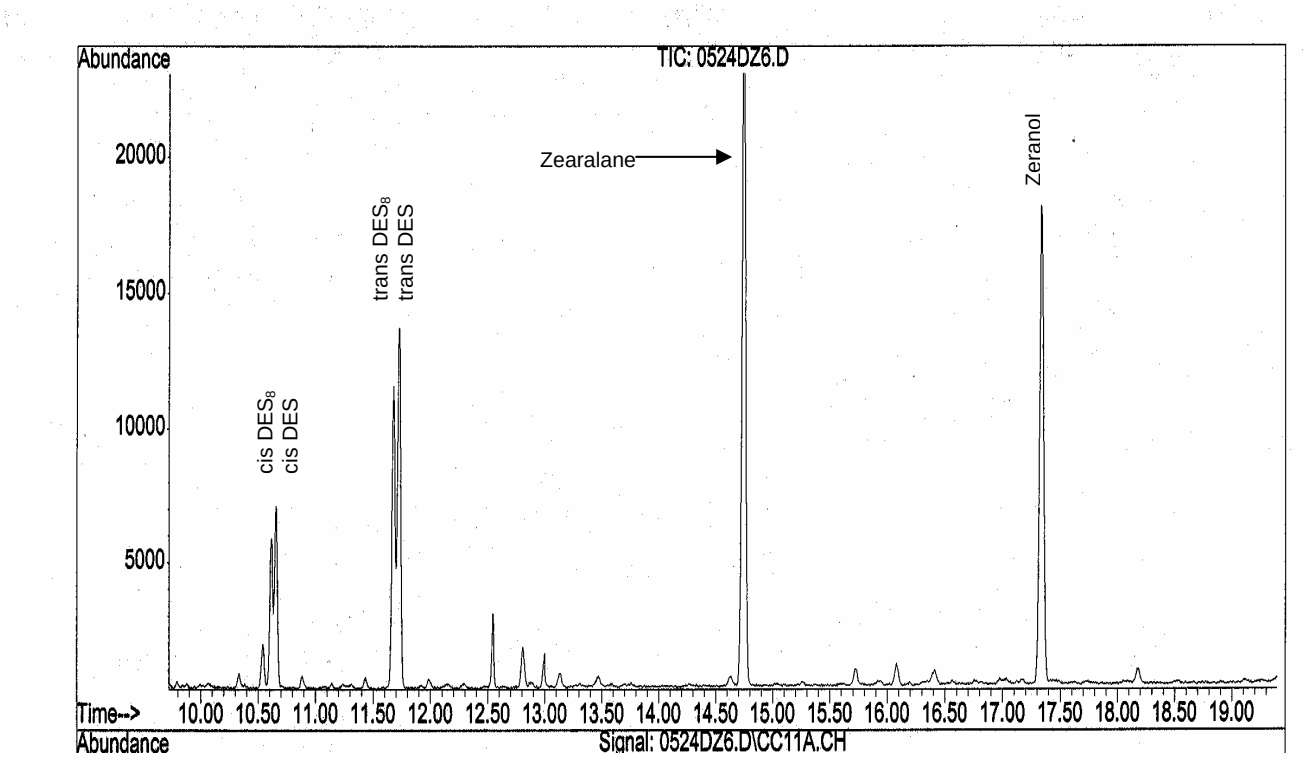
Sample Analysis Data

Sample No.	R501	R510	Tissue Type	Sample Wt. (5.00 ± 0.10 g)	Sample Temp. C	Sample pH (4.50 ± 0.25)	DES (m/z 412) Ret. Time (w/in ± 0.3 min. of Fort. Extract(s))	Zeranol (m/z 538) Ret. Time (w/in ± 0.3 min. of Fort. Extract(s))	DES (ppb)	Zeranol (ppb)
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										

REMARKS:

K. APPENDIX

1. Chromatogram



1 ppb Standard of DES, Zearalane, and Zeranol.

2. References

a. Determination of Diethylstilbestrol and Zeranol in Bovine Liver, Extraction, Screening, Quantitation, and Confirmation. Prepared by Covey, Silvestre, and Henion, Cornell University, Ithaca, NY for USDA, October 15, 1985.

b. Quantitation of Zeranol and Diethylstilbestrol in Beef Liver, Kidney, and Muscle by GC-MS. Agriculture Canada, Animal Pathology Laboratory, Saskatoon, Saskatchewan, HOR-DP0l.TXT-September 1986.

c. Method Extension Study on Ovine Tissue for Taleranol, DES, and Zeranol. September, 1988.

Approved by:	**Date:**
Tom Mallinson	11-3-04
Terry Dutko	10-19-04
Kathy Holland	10-19-04
Jess Rajan	10-21-04
Charles Pixley	10-19-04
Phyllis Sparling	10-19-04

Approval signatures on file.