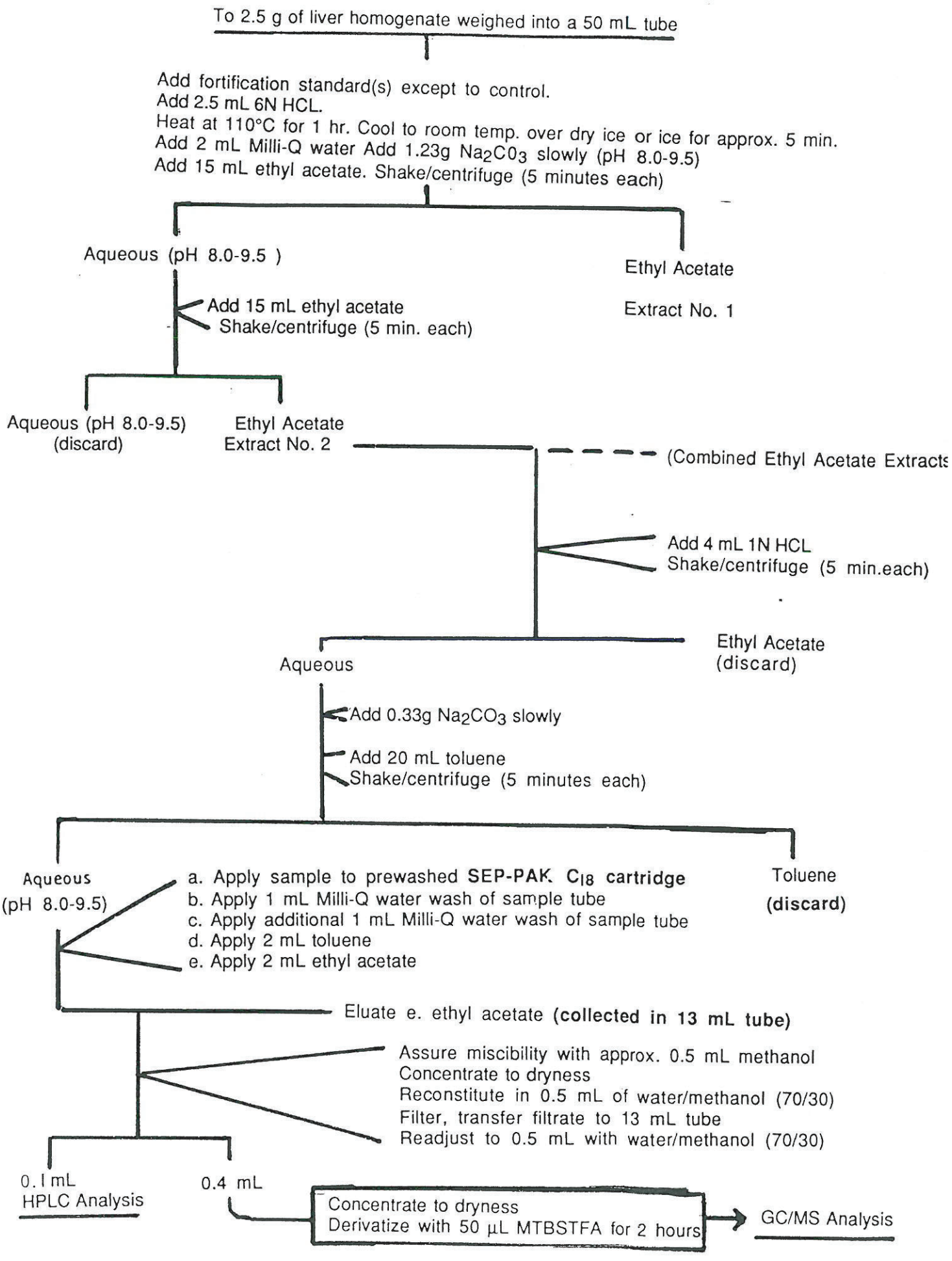
ALBENDAZOLE
IN
CATTLE LIVER
QUANTITATIVE PROCEDURE

QUANTITATIVE PROCEDURE FOR RESIDUES OF ALBENDAZOLE IN CATTLE LIVER

Introduction

This report describes a method for the determination and confirmation of albendazole residues in cattle liver by analyzing the marker chemical (SK&F 81038, S-(Propylsulfonyl)IH-benzimidazol-2-amine. The method is practical, reproducible. accurate and sensitive for determining and confirming the marker residue in liver from albendazole dosed cattle at concentrations of 100 to 400 ppb. Quantification of the marker residue is based on the internal standard method where SK&F 101437(5-(Butylsulfonyl)- IH-benzimidazol-2-am1ne) is employed as the internal standard. The determinative procedure utilizes High Performance Liquid Chromatography (HPLC) for quantifying the marker residue concentration. If the marker residue is determined in excess of the established tolerance, four fifths of the sample extract is used for confirmation by preparing the t-butyldimethylsilyl (t-BDMS) derivative followed by Gas Chromatographic/Mass Spectrometric/Multiple ion Detection (GC/MS/MID) analysis.

FLOW DIAGRAM OF EXTRACTION PROCEDURE



A. Apparatus:

Glass graduated centrifuge tube, 50 mL ground glass stoppered, Kimble 45176-50 or equivalent.

Glass graduated centrifuge tube, 13 mL ground glass stoppered, Kimble 45176-13 or equivalent.

Oven, Fisher Isotemp Model 176 equipped with thermometer, Fisher Scientific Co., or equivalent.

Mechanical shaker, Eberbach flatbed, A. H. Thomas 8287-E30 or equivalent.

Centrifuge, Sorvall Model GLC-I, or equivalent equipped with rotors for 50 mL and 15 mL tubes.

N-Evap, Model 112, Organomation Assoc., Inc., or equivalent.

Commercial Blender, Waring Model 31BL91, or equivalent.

Pipetter, Becton Dickinson Labware (BGL) Model 6606, or equivalent.

pH meter, Radiometer Model 22, or equivalent.

SEP-PAK ®C_{18} cartridges, Waters Assoc. (Part # 51910).

Vortex-Genie Mixer, Model K-550-G, Fisher SCIENTIFIC Co.

Filter assembly, MF-I Centrifugal Microfilter, Bioanalytical Systems Inc.

Filters, 0.2µm RC58 (Regenerated cellulose), Bioanalytical Systems Inc.

Balance, top loading Sartorius model **1219MP, or equivalent.**

Dry ice

A. Apparatus (Cont'd)

Pipet, 10 mL glass disposable, A. H. Thomas (7536-H85), Corning #7077, or equivalent.

Pipet, Pasteur disposable, A. H. Thomas 7760-A10, 7760-A26 or equivalent.

Pipet-Aid®, Drummond Scientific Co.

Syringe, 10 mL glass luer tip hypodermic, A. H. Thomas, 8932-050 or equivalent.

Electrobalance, Cahn, Model G-2 or equivalent.

HPLC column, μ-BONDAPAK™ C_{18} 30 cm x 3.9 mm I.D., Waters Associates (P/N 27324).

HPLC Microprocessor/controller, Model 420, Altex Scientific Inc., or equivalent.

Guard column, CO:PELL ODS, 2mm ID x 5cm, Whatman, Inc., or LiChrosorb 10 μm Brownlee Labs MPLC cartridge, or equivalent.

HPLC Pump, Model 100, Altex Scientific Inc., or equivalent

HPLC Injection valve, Model 7120 equipped with 20 μL loop,

Rheodyne, Inc., or equivalent, or automatic sampler.

Recording Integrator, Model 5880, Hewlett Packard, or equivalent.

HPLC Fluorescence Spectrophotometer, Model 204A, Perkin-Elmer Corp., or equivalent dual monochromator instrument.

Syringe Model LC-210, Pressure-lok, equipped with Rheodyne needle, Precision Sampling Corp., or equivalent.

Xenon Power Supply, Model 150, Perkin-Elmer Corp. Vacuum filter, 47 mm glass, Millipore Corp.

A. Apparatus (Cont'd)

Filter, Nylon-66 membrane 0.45µm, 47 mm diameter, Rainin Instrument Co., or equivalent.

Syringe, 10µL glass, Hamilton, Model 18O1 or equivalent.

Syringe, 50 µL glass Pressure-Lok®, Precision Sampling, Model C-160 or equivalent.

Fused silica capillary column, OB-5 liquid phase, 0.25µm film thickness, 30 m X 0.25 mm 1.d., J & W Scientific, Inc., or equivalent.

Gas Chromatograph/Mass Spectrometer, Finnigan Corporation, Model 4500 equipped with Model 9610 gas chromatograph, or equivalent.

B. Reagents and Chemicals

SK&F 81038, S-(Propyl)sulfonyl)-IH benzimidazol-2-amine, analytical standard, SmithKline Animal Health Products, 1600 Paoli Pike, West Chester, PA 19380.

SK&F 101437, S-(Butylsulfonyl)-IH benzimidazol-2-amine, analytical standard, SmithKline Animal Health Products, 1600 Paoli Pike, West Chester, PA 19380.

Hydrochloric acid, concentrate, J. T. Baker Chemical Co., #1-9535 or equivalent.

Dimethylsulfoxide, distilled in glass, Burdick & Jackson Laboratories, or equivalent.

Ethyl Acetate, distilled in glass, Burdick & Jackson Laboratories, or equivalent.

Methanol, distilled in glass, Burdick & Jackson Laboratories, or equivalent.

Toluene, distilled in glass, Burdick & Jackson Laboratories, or equivalent.

B. Reagents and Chemicals (Cont'd)

Acetonitrile, distilled in glass, Burdick & Jackson Laboratories, or equivalent.

Diethanolamine, Fisher Scientific Co., D-45 or equivalent.

Sodium Carbonate, anhydrous powder, Mallinckrodt, Inc. #7521, or equivalent.

Water, via Milli-Q/Milli-RO system, Millipore Corp., or equivalent.

Potassium Phosphate Monobasic, Fisher Scientific Co. P-285, or equivalent.

Potassium Phosphate Dibasic, **anhydrous, Mallinckrodt, Inc.** #7092, or equivalent.

Nitrogen, Prepurified grade, M. G. **Burdett Gas Products Co.,** or equivalent.

N-Methyl-N-(t-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), Regis Chemical Co.

C. Preparation of Standard Solutions

Stock Solutions :

1. Into a 100 mL volumetric flask, weigh 10.0 mg of SK&F 8103B analytical standard. Dissolve and take to mark with DMSO (100 µg/mL).

2. Into a 100 mL volumetric flask, weigh 10.0 mg of SK&F 101437 analytical standard. Dissolve and take to mark with DMSO (100 µg/mL).

C. Preparation of Standard Solutions (Cont'd)

Working Standards :

1. Pipet 2.0 mL of stock solution 1 and 8.0 mL of DMSO into a 13 mL glass stoppered centrifuge tube and vortex mix, to produce a 20 µg/mL solution. (50 µL fortified to 2.5 g of liver is equivalent to 400 ppb).

2. Pipet 5.0 mL of working standard 1 and 5.0 mL of DMSO into a 13 mL glass stoppered centrifuge tube and vortex mix, to produce a 10 µg/ml solution. (50 µL fortified to 2.5 g of liver is equivalent to 200 ppb).

3. Pipet 5.0 mL of working standard 2 and 5.0 mL of DMSO into a 13 mL glass stoppered centrifuge tube and vortex mix, to produce a 5 µg/mL solution (50 µL fortified to 2.5 g of liver is equivalent to 100 ppb).

4. Pipet 1.0 mL of stock solution 2 and 9.0 ml of DMSO into a 13 mL glass stoppered centrifuge tube and vortex mix, to produce a 10 µg/mL solution (50 µL fortified to 2.5 g of liver is equivalent to 200 ppb).

All standard solutions should be stored in a refrigerator.* Since DMSO solidifies under these conditions it is necessary to re-liquify the standard solutions by placing the tubes in a beaker of lukewarm water and vortex mixing the solutions prior to use.

*Standard solutions are stable in DMSO for a **minimum of six months.**

D. Extraction Procedure

1.Representative sections of cattle liver are thoroughly homogenized in a commercial Waring Blender until no visible pieces remain (approx. 3-5 minutes/100 g). Homogenates should be kept frozen at all times until analyzed.

D. **Extraction Procedure** (Cont'd)

2. Into the bottom of a 50 mL glass centrifuge tube (unstoppered), supported in a beaker tared on a balance, weigh 2.5 ±0.05 g of homogenized liver using a wide tip disposable pipet. Prepare four blank tissue samples, one for control and three for fortification. Add 100 µL of pure DMSO to the control. Add 50 µL of DMSO to the tissues suspected of containing albendazole residue. Add 50 µL of working standard solution d to each of the remaining three blank tissue samples and the tissue samples suspected of containing albendazole residue. Add 50 µL of working standard solutions a, b and c to separate blank tissue samples from the set of three already fortified with working standard solution d. (Note: fortification solutions should be added to the 50 mL tube just above the homogenate to insure solution when the HCl is added in the step 3.)

3. Add 2.5 mL of 6N HCl to each sample prepared in step 2, stopper each tube, place the sample tubes (covered with another tube rack to prevent stoppers from popping) in an oven preset to 110 + 4°C (at elevations ≤1000 ft above sea level)*.

*At elevations ≥ 5000 ft, hydrolysis for 30 minutes in a 15 psi pressure vessel is required to adjust for atmospheric pressure. Timing for hydrolysis should begin when the samples are placed Into the pressure vessel with the water already boiling. It is necessary to have the water boiling to reach pressure as quickly as possible (4≤ minutes). The hydrolysis time is critical and must be timed accurately.

4 After 1 hour, remove the samples from the oven and allow the tubes to cool to room temperature over ice for approx 5 min.

5. Add 2.0 mL of water (Milli-Q) top each sample. While vortex mixing the sample, slowly add enough (approximately 1.23 g) of sodium carbonate (approx. 4-5 minutes to avoid foaming and/or caking) to achieve a pH 8-9.5 when monitored with a pH meter. (Place the clean pH electrode directly into the sample. After recording the pH, dab any drop of solution from the electrode on the inside of the tube then completely remove the electrode from the tube, rinse it with water and dry it with a Kimwipe before monitoring the next sample). Note, samples at or near room temperature do not foam as much as colder samples. Therefore, the Na_2C0_3 can be added faster.

D. **Extraction Procedure** (Cont'd)

6 Add 15 mL of ethyl acetate to each sample and stopper the tubes. Shake the samples supported in a rack in a horizontal position for 5 minutes. Remove the stoppers and centrifuge the samples at approximately 400 g for 5 minutes.

7. Transfer as much as possible of the ethyl acetate extracts to separate 50 mL glass centrifuge tubes using a disposable pipet taking care not to transfer any of the aqueous phase (use of a Pipet-Aid in this step is beneficial).

8. Re-extract each sample a second time by repeating steps 6 and 7 and pooling the appropriate extracts in the respective 50 mL glass centrifuge tubes.

9. Add 4.0 mL of 1N HCl to each ethyl acetate pooled extract and stopper the tubes. Shake the samples supported in a rack in an horizontal position for 5 minutes. Remove the stoppers and centrifuge the samples for approximately 5 minutes.

10. Remove as much as possible of the ethyl acetate phase by aspiration, taking care not to remove any of the aqueous phase. Evaporate the remaining ethyl acetate (≤1 mL) from the aqueous phase in the N-Evap waterbath at 35 + 5°C. (If necessary the extraction can be stopped at this point.)

11. While vortex mixing each sample add enough (approximately 0.33 g) of sodium carbonate slowly (approx. 1-2 minutes to avoid foaming and/or caking). Each sample should be at pH 8-9.5 monitored with a pH meter.

12. Add 20 mL of toluene to each sample and stopper tubes. Shake the samples supported in a rack in a horizontal position for 5 minutes. Remove the stoppers and centrifuge the samples for approximately 5 minutes.

13. Remove as much as possible of the toluene phase by aspiration taking care not to remove any of the aqueous phase. Evaporate the remaining toluene (≤1 mL) from above the aqueous phase in the N-EVAP® waterbath at 35 ± 5°C.

D. **Extraction Procedure** (Cont'd)

14. Attach a SEP-PAK®C_{18} cartridge to the luer tip of a 10 mL glass syringe secured to a ring stand with a clamp for each sample (cylinder barrel removed).

15. Prewash each cartridge with 2 mL of methanol followed by 2 mL of 0.2M potassium phosphate buffer pH 8.0. Each reagent is transported through the cartridge by means of gentle nitrogen pressure applied through a rubber stopper fitted into the top of the syringe after addition of the respective reagent. The nitrogen pressure is removed when nitrogen begins to exit the cartridge. Flow rate approx. 1-2 mL/min. Discard eluates.

16. Transfer the aqueous phase from each sample from step 13 to a separate prewashed syringe cartridge unit. Elute this phase from the cartridge using nitrogen as in step 15 then transfer a 1 mL water (Milli-Q) wash of each sample tube (vortex-mixed) to the respective syringe-cartridge unit in a similar manner using the respective pasteur pipet. Discard all eluates.

17. Add an additional 1 mL water wash of each sample tube (vortex mixed) to the respective syringe-cartridge unit and elute using nitrogen as in step 15. Discard the eluates.

18. Add 2 mL of toluene to each syringe-cartridge unit and elute using nitrogen as in step 15. Discard eluates.

19. Place a 13 mL glass centrifuge tube beneath each syringe cartridge unit. Add 2 mL of ethyl acetate to each unit and elute using nitrogen as in step 15. This fraction contains the compounds of interest.

20. Add about 0.5 mL of methanol to each eluate from step 19 and vortex mix to obtain a homogeneous solution.

21. Concentrate the eluates from step 20 to dryness under dry nitrogen in an N-EVAP® waterbath at 35 + 5°C.

D. **Extraction Procedure** (Cont'd)

22. After reaching dryness add 0.5 mL of the water/methanol (70/30) solution and vortex mix to reconstitute the residue.

23. After @ 30 minutes apply each of the reconstituted samples to a separate Bioanalytical System filter unit fitted with an 0.2 µm regenerated cellulose filter. Centrifuge each filter unit containing a sample extract at approximately 5-10 minutes.

24. Transfer the filtrates to separate 13 mL glass stoppered centrifuge tubes* and adjust the volume of each filtrate to 0.5 mL with water/methanol (70/30). After vortex mixing remove 0.1 mL for HPLC analysis. Use the remaining liquid to conduct confirmation analyses of the residues. Refer to Method II for procedures.

* Use of silanized tubes is recommended.

E. Method Comments (Cont'd)

samples (DMSO only) should produce either no or only a negligible response at the retention time corresponding to the internal standard as compared to the response observed for a known control sample fortified at 200 ppb. Providing this background is negligible, calculate the background at the retention time corresponding to the albendazole marker in the control samples fortified with internal standard. If a response is observed calculate the peak area (height) ratio. If either no response or a peak area (height) ratio less than 5X of that calculated for a known control fortified at 200 ppb is determined, the cattle liver is considered a control.

F. Method Time Requirements

A time estimate for each step of the method assuming analysis of 8 samples (control liver, control liver fortified at 1/2 Rm*, Rm and 2 Rm and 4 samples) follows: steps 1-23 are performed on the first day; step 24, 25 and both HPLC and GC/HS analysis are performed the next day for a total assay time of two days.

Day 1

Step 1 - 30 minutes
Step 2 - 30 minutes
Step 3 - 65 minutes
Step 4 - 10 minutes
Step 5 - 30 minutes
Step 6 - 20 minutes
Step 7 - 15 minutes
Step 8 - 30 minutes
Step 9 - 15 minutes
Step 10- 20 minutes **
Step 11 - 15 minutes
Step 12 - 20 minutes
Step 13 -15 minutes
Step 14 & 15 - 15 minutes**
Steps 16-20 - 40 minutes
Step 21 - 20 minutes
Steps 22 & 23 - 35 minutes

Day 2

Step 24 1 - 1.5 hours
HPLC Analysis
(determinative procedure)
1.5 hours

Step 25. 2 hours
GC/MS Analysis(Confirm-atory Procedure: 3-4 hrs)

*NOTE: Rm = Marker residue concentration at withdrawl time.

** Extractions can be stopped at this point overnight!

E. Method Comments

If frozen cattle liver is used, it must be kept frozen until assay. Liver homogenate stored in small vials is thawed under lukewarm water prior to each assay. Brief thawing of either the frozen liver or frozen liver homogenate is acceptable, if re-frozen as soon as possible.

The Pipet-Aid® manufactured by Drummond Scientific Inc. is a convenient time-saving device for transferring or aspirating solutions.

A ≥ 75% method recovery is achieved by taking care to transfer as much as possible of the extraction solvents (steps 6, 7, and B). In cases where the solvent is discarded and the aqueous containing the compounds of interest is retained, it is imperative to avoid removing any of the aqueous layer (steps 10 and 13). Transferring of the 4 mL aqueous phase in step 16 should be done quantitatively using a pasteur pipet. The 1 mL washes of the sample tube are transferred to the SEP-PAK®C_{18} cartridge using the same pasteur pipet. When transferring solutions, separate disposable and pasteur pipets are used for each sample to avoid contamination.

The sample extracts contained in water/methanol (70/30) are stable for at least a month and if reanalyzed provide data similar to the previous HPLC analysis.

The sample extracts concentrated to dryness for derivatization and subsequent GC/MS analysis are stable for at least a month when stored in a refrigerator.

Verification of Control Cattle-Liver

To determine that a cattle liver can be used as control proceed as follows. Prepare four 2.5 g liver homogenate samples. Fortify two aliquots with 100 µL of DMSO and the other two aliquots with 50 µL of DMSO and 50 µL of the internal standard solution (200 ppb). The samples are processed through the extraction method and analyzed using the HPLC procedure. The non-fortified control

G. Hiqh Performance Liquid Chromatoqraphic Analysis

Column: 30 cm x 3.9 mm I.D. μBondapak C_{18}
Flow Rate: I.8 mL/min
Mobile Phase: 68% 0.02M KH_2P0_4;0.01M diethanolamine (200 mL O.1M KH_2P0_4 + 1.05 g diethanolamine taken to 1 liter with water)

20% methanol

12% acetonitrile

This solution is prepared daily, filtered through an 0.45 μm Nylon-66 filter and degassed under vacuum prior to use.

Fluorescence Detector:

Initially set the excitation and emission wavelengths at 300 nm and 320 nm, respectively, with a slit width of @10 nm for both. Inject an appropriate volume of sample (20 μL) and observe the intensity of the response for both SK&F 81038 and SK&F 101437 with a reasonable baseline sensitivity setting. If additional sensitivity is required, proceed as follows:

While observing the detector absorption response, adjust the excitation wavelength @ 5 nm to reduce the zero suppression. Reinject the same sample as above and determine if the signal response for the compounds of interest increase. Continue this procedure of optimizing both the excitation and emission wavelengths to obtain an adequate signal to noise ratio for the analysis (s/n ≥ 25/l).

Temperature:

Column, mobile phase and detector temperature ~ ambient (70 ± 2°F)

Approximate Retention Time: Albendazole marker (SK&F 81038 approx. 4.3 minutes)

Internal Standard (SK&F 101437 approx. 7.0 minutes)

G. High Performance Liquid Chromatographic Analysis (Cont'd)

Data Computation

If an integrator is unavailable, peak height measurements and peak height ratio data can be substituted for peak area and peak area ratio data in the following discussion.

Divide the peak area of the albendazole marker SK&F 81038 by the peak area of the internal standard SK&F 101437 from the HPLC data for each sample extract to obtain peak area ratios.

$$\text{peak area ratio} = \frac{\text{area of albendazole marker (SK\&F 81038)}}{\text{area of internal standard peak (SK\&F 101437)}}$$

Construct an analytical curve by plotting, on linear axes, the peak area ratio versus the respective ppb value for each fortified control sample. Using the regression equation for the analytical curve, directly calculate the ppb level of albendazole marker in the unknown samples from their peak area ratios using the following equation.

$$x = \frac{y - b}{m}$$

where,
- x = ppb concentration in samples
- y = peak area ratio of sample extract
- b = intercept from regression equation
- m = slope from regression equation

ALBENDAZOLE
IN
CATTLE LIVER
CONFIRMATORY PROCEDURE

GC/MS Confirmatory Method for Albendazole Residues in Cattle Liver

Introduction

The methods used for the determination and confirmation of albendazole residues in cattle liver analyzes the marker chemical (SK&F 81038, S-(Propylsulfonyl)IH-benzimidazol-2-amine. The method is practical, reproducible. accurate and sensitive for determining and confirming the marker residue in liver from albendazole dosed cattle at concentrations of 100 to 400 ppb. Quantification of the marker residue is based on an internal standard method where SK&F 101437(5-(Butylsulfonyl)-IH-benzimidazol-2-amine) is employed as the internal standard. The determinative procedure utilizes High Performance Liquid Chromatography (HPLC) for quantifying the marker residue concentration. If the marker residue is determined in excess of the tolerance, four fifths of the sample extract is used for confirmation by preparing the t-butyl-dimethylsilyl (t-BDMS) derivative followed by Gas Chromato-graphic/Mass Spectrometric/Multiple ion Detection (GC/MS/MID) analysis.

A. Apparatus Refer to Method I

B. Reagents and Solution Refer to Method I

C. Preparation of Standard Solutions for GC/MS Analysis

Prepare standard solutions for GC/MS as just described in Part C (Method I) using methanol rather than DMSO as the solvent.

Into separate 13 mL glass centrifuge tubes, prepare standards equivalent to 100, 200 and 400 ppb based upon a 2.5 g tissue sample weight as follows:

> Pipet 50 µL of working standard 4 into each of three 13 mL glass tubes. Pipet 50 µL of each of the working standards 1, 2 and 3 into a separate 13 mL tubes containing working standard 4

D. Extraction Procedure

For the extraction of any potential residues of albendazole and it's metabolites from tissues, refer to Method I,steps 1 thru 24. The information relative to the derivatization process is explained below.

Concentrate the remaining 0.4 mL of the liver extract samples (Method I) along with the standard samples prepared (Part D) to dryness in a N-EVAP® waterbath at 35 ± 5°c. If necessary reconstitute the residue in 50 µL of methanol and concentrate the samples to dryness such that the majority of residue is at or below the 100 µL mark on the tube.

The samples should be concentrated using dry air. Nitrogen may be used in place of air but the samples should be flushed with air momentarily if immediately proceeding to the next step.

Add 50 µL of MTBSTFA reagent to each concentrated sample, stopper tubes and vortex mix the samples. Place the stoppered sample tubes (covered with another tube rack to prevent stoppers from popping) in an oven preset to 100 ± 4°C to assure complete derivatization. Remove the samples from the oven approximately every half hour for vortex mixing then immediately return the tubes to the oven. After a total derivatization time of 2 hours, remove the samples from the oven for GC/MS analysis. (The samples may remain in the oven longer. Two hours derivatization is a minimum.) If a large number of samples are prepared for analysis, it is recommended that each sample remain in the oven until analysis. initial day of derivatization.

Note: Sample extracts concentrated to dryness for derivatization and subsequent GC/MS analysis are stable for at least one month when stored in a refrigerator.

E. Gas Chromatograph/Mass Spectrometer Analysis

Gas Chromatograph Conditions:

Column: Fused Silica Capillary, 30 m x 0.25 mm i.d., DB-5 (0.25 µm film thickness)
Injection Port Temperature = 260°C
Column Oven Temperature Program:
Initial Temperature = 200°C (hold 1 minute)
Final Temperature = 300°C (hold for duration of analysis)
Temperature Program Rate = 20°C/minute
Injection Mode: Grob Splitless, split vent timing
0.6-1 minute vent flow 100 mL/minute
Carrier Gas:Helium, 30-40 psi
Linear Velocity = 60-70 cm/second
Approximate Retention Times (t_R)
Marker Residue (SK&F 81038) approx. 8 minutes
Internal Standard (SK&F 101437) approx. 9 minutes
Injection Volume: 1.0-3.0 µL.

Mass Spectrometer Conditions:

Temperatures:
Source = 140°C (Model 4500),
= 225°C (Model 4000 dial setting 30)
Separator = 275 ± 5°C
Transfer Line = 275 ± 5°C
Manifold = 120°C(Model 4500),
= 100 ± 5°C Model 4000)

Multiplier: Gain = 5×10^4 to 1×10^5
Voltage = 2000 to 2400 ev

Election Energy = 70 ev

Calibration Standard: FC-43 (Perfluorotributylamine)

Emission Current = -0.30

Preamplifier Sensitivity = 10^{-7} to 10^{-8}

F. Gas Chromatograph/Mass Spectrometer Procedure:

After initial instrument calibration to ≥ 500 amu perform partial scan analysis (mass range 45-500 amu) with an appropriate amount (3.0 µL) of a standard sample containing 10 µg of SK&F 81038 and 10 µg of SK&F 101437 as the t-butyldimethylsilyl (t-BDMS) derivatives in 50 µL of derivatizing reagent. Determine the centroid mass assignments of the ions 189, 354, 410, 467 for SK&F 81038-t-BDMS and 189, 368, 424 and 481 for SK&F 101437-t-BDMS by averaging the observed centroid masses over approximately five scans of the respective chromatograhic peaks or by using a procedure applicable to the particular data system employed. The instrument should be calibrated to obtain a minimum relative intensity of 10% for each ion monitored. Set the multiple ion detection mode to monitor these seven ions. Select a beginning and end mass range for each ion and scan time appropriate for the particular instrument employed.

Proceed as follows for the Multiple Ion Detection (MID) Analysis:

1. Perform three analyses of the derivatized standard samples from Part D (equivalent to 100, 200 and 400 ppb) with an appropriate injection volume (approx. 3.0 µL). From these analyses determine the relative ion intensity reproducibility for the instrument employed.

2. Analyze an aliquot (approx. 3.0 µL) of the derivatization reagent (MTBSTFA) to insure there is no ghosting interference with the monitored ions at the retention times corresponding to the components of interest.

3. Analyze an appropriate amount (approx. 3.0 µL) of the derivatized control and fortified control (100, 200 and 400 ppb) cattle liver extracts, respectively. Determine the relative ion intensity reproducibility from these samples. The relative ion intensity for a particular ion should not vary by more than + 10%.

4. Repeat step 2 above.

F. GAS CHROMATOGRAPH/MASS SPECTROMETER PROCEDURE (CONT'D)

5. Providing the relative ion intensity is reproducible to within + 10% and no interference is apparent due to ghosting, proceed to analyze the derivatized cattle liver extracts suspected of containing albendazole residues. Alternate injections of each sample with injections of the derivatization reagent (step 2) to insure that there is no interference due to ghosting between analyses of the suspect samples.

Record the relative intensity data for ions 189, 354, 410 and 467 for SK&F 81038-t-BDMS and 189, 368, 424 and 481 for SK&F 101437-t-BDMS from each analysis. Confirmation of the albendazole marker residue in the suspect cattle liver samples consists of: 1) identifying a response at the retention time corresponding to the albendazole marker t-BDMS derivative, 2) this response containing the four characteristic mass ions (m/e = 189, 354, 410 and 467), and 3) the relative ion intensity of each ion reproducible to within ± 10% of that obtained for these ions from the derivatized control cattle liver extracts fortified with the albendazole marker chemical.

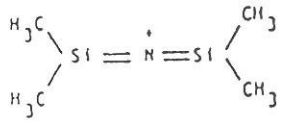
G. Supplemental Mass Spectral Information

A summary of the most reasonable exact mass assignments and elemental compositions of the ions of interest for the electron impact mass spectrum of the albendazole marker t-BDMS derivative in Figure 1 is listed in Table 1. These data were obtained with a high resolution VG Model 7070 magnetic sector instrument. The m/e 354 ion was saturated deliberately to enhance several of the weaker ions. The structures for the elemental compositions in Figure 2 are only hypothetical and should be regarded as such without further data on the sequence of fragmentation. These data are presented merely as supplemental information to support the rationale for choosing the ions to monitor.

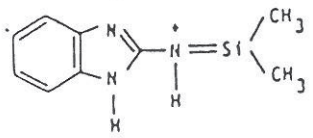
FIGURE 2

POSSIBLE STRUCTURES FOR THE MAJOR IONS FROM THE EI MASS SPECTRA OF ALBENDAZOLE MARKER t-BDMS DERIVATIVE

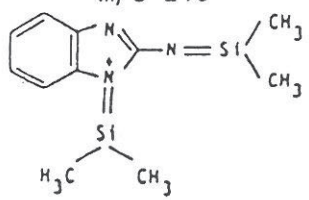
m/e=130



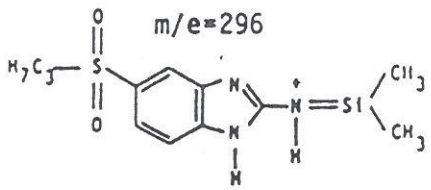
m/e=189



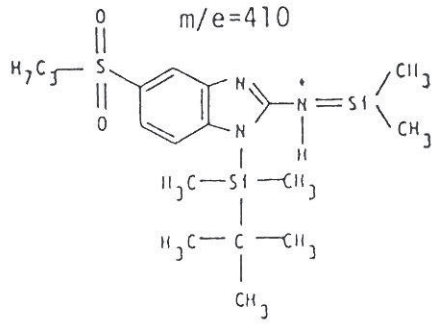
m/e=246



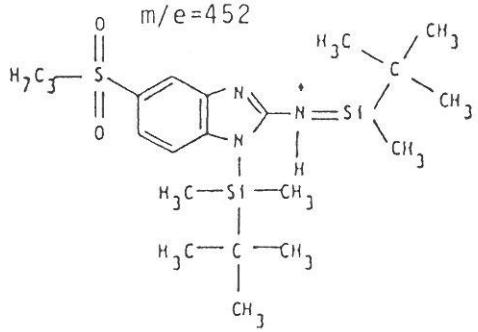
m/e=296



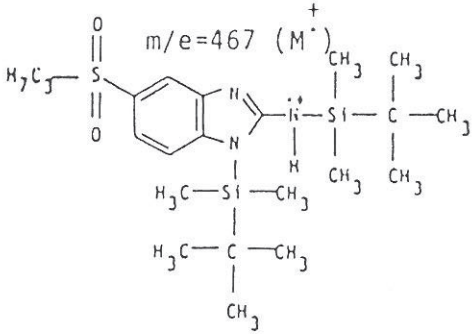
m/e=410



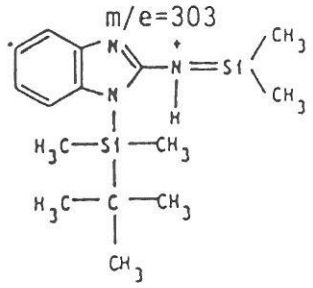
m/e=452



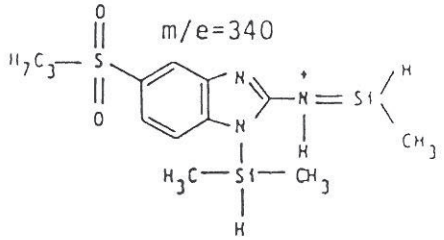
m/e=467 (M^+)



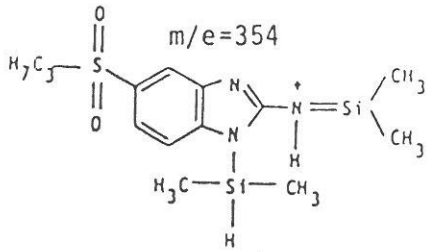
m/e=303



m/e=340



m/e=354



m/e=396

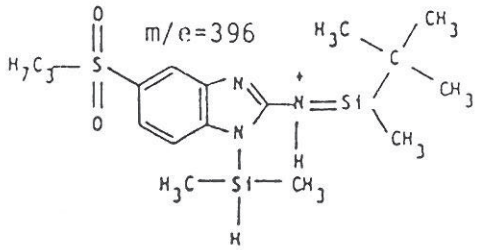


FIGURE 1

EI MASS SPECTRUM OF ALBENDAZOLE MARKER t-BDMS DERIVATIVE

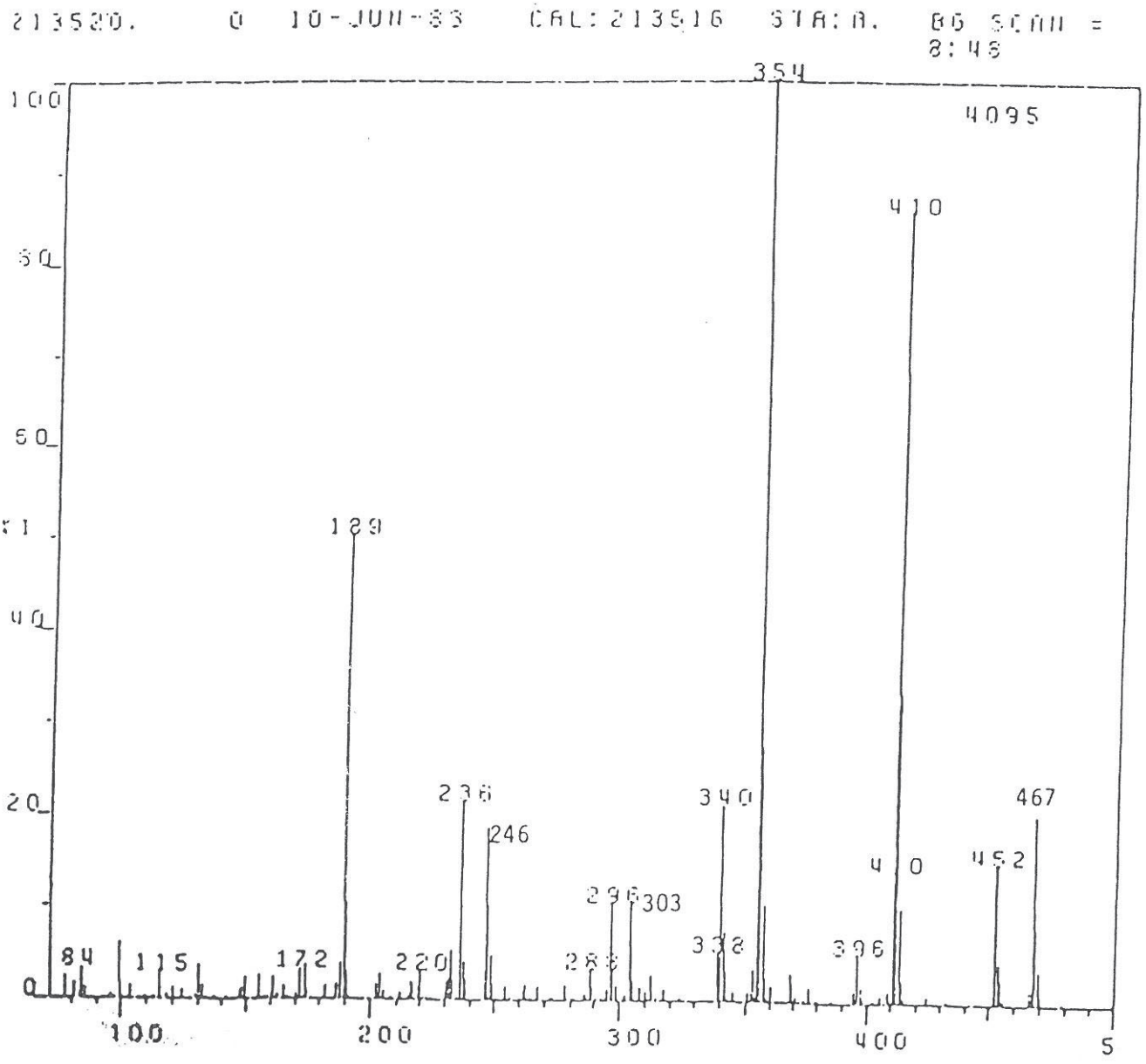


TABLE 1

EXACT MASSES AND ELEMENTAL COMPOSITIONS OF IONS
FOR ALBENDAZOLE MARKER t-BDMS DERIVATIVE

Nominal Mass	Exact Mass	Elemental Composition	Error (ppm)
130	130.0507	$C_4H_{12}NSi_2$	1.6
189	189.0721	$C_9H_{11}N_3Si$	4.2
236[a]	--	--	--
246	246.0881	$C_{11}H_{16}N_3Si_2$	2.0
296	296.0886	$C_{12}H_{18}N_3O_2SSi$	-0.2
303	303.1585	$C_{15}H_{25}N_3Si_2$	0.6
340	340.0969	$C_{13}H_{22}N_3O_2SSi_2$	1.4
354	354.1126	$C_{14}H_{24}N_3O_2SSi_2$	7.4
396	396.1596	$C_{17}H_{30}N_3O_2SSi_2$	-7.1
410	410.1752	$C_{18}H_{32}N_3O_2SSi_2$	-4.2
452	452.2221	$C_{21}H_{38}N_3O_2SSi_2$	-10.1
467	467.2455	$C_{22}H_{41}N_3O_2SSi_2$	-11.5

[a]No reasonable compositions