UNIROYAL CHEMICAL COMPANY, INC.
74 Amity Road
Bethany, Connecticut 06525
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415382-C7

VOLUME 7

PROCURE 50W (400-UGR)

Triflumizole
PP #6F3372/FAP #6H5497

Study Title
Analytical Methods for the Determination of
Triflumizole and its Metabolites in Meat, Milk and Eggs

Data Requirement
Guideline 171-4(d)

Author
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Study Completed On
June, 1990

Performing Laboratory
NA

Laboratory Project ID
NA

CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B) or (C).

Company: Uniroyal Chemical Company, Inc.

Company Agent: Willard F. Cummings Date: 6-21-90

Title: U. S. Registration Manager Signature: [signature]

The information and data contained herein is considered proprietary and confidential material and therefore cannot be used or released without the written consent of Uniroyal Chemical Company, Inc.

However, no claims of confidentiality for this document are made, and the data are releasable, when submitted to the U.S. Environmental Protection Agency under FIFRA.

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICES

This report is a rewrite of the residue methods for determining triflumizole in meat, milk and eggs, and as such is not a study.

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- Proposed Enforcement Procedure for the Determination of Triflumizole and its Chlorotrifluoromethylaniline Metabolites in Meat, Milk and Eggs

- Proposed Enforcement Procedure for the Determination of FA-1-5, 2-Hydroxy-4-chloro-5-trifluoromethylaniline, and its Conjugates in Meat and Milk

UNIROYAL CHEMICAL

Uniroyal Chemical Company, Inc
World Headquarters
Middlebury, CT 06749

PROPOSED ENFORCEMENT PROCEDURE FOR THE DETERMINATION OF TRIFLUMIZOLE AND ITS CHLOROTRIFLUOROMETHYLANILINE METABOLITES IN MEAT, MILK, AND EGGS

1.0 INTRODUCTION

The analysis of FA-1-1, chlorotrifluoromethylaniline, as final hydrolysis product of triflumizole (1-[1[[4 chloro-2-(trifluoromethyl)phenyl)imino]-2-propoxyethyl]-1H-imidazole]) or any of its metabolites (FM-5-1, FM-8-1, FD-1-1, FD-2-1, or FM-8-1-S) is based on hydrolysis in an alkaline medium, and silica column cleanup.

2.0 REAGENTS AND APPARATUS

2.1 Sodium hydroxide pellets - Reagent grade (Fisher Scientific Cat No. S-318).

2.1.1 20% aqueous NaOH solution.

2.2 Antifoam - Dow Corning Antifoam B Emulsion (Dow Corning).

2.3 Hexane - Reagent Grade (Fisher Scientific Cat. No. H302-4) or equivalent.

2.3.1 Eluting solvent (hexane/methylene chloride/methanol) - Mix 900 mL hexane, 99.5 mL methylene chloride and 0.5 mL methanol.

2.4 Methanol - HPLC Grade (Fisher Scientific Cat. No. H452) or equivalent.

2.5 Methylene chloride - HPLC Grade (Fisher Scientific Cat. No. D143) or equivalent.

2.6 Sodium sulfate, anhydrous - (Mallinkrodt Cat. No. 8624 - granular ACS).

The sodium sulfate must be pretreated before use. Place one piece of Whatman #1 15.0 cm filter paper into a 170 mm OD Coors porcelain funnel. Pour in 450 grams sodium sulfate.

Add 400-500 ml of methanol and allow to soak in until there is an excess of methanol pooling at the top of the sodium sulfate. Drain the methanol through by vacuum filtration. Rinse the sodium sulfate twice more, adding the methanol prior to reapplication of the vacuum. Discard the methanol. Place the sodium sulfate in an oven and heat at 135-140°C for 12 hours. Store in a closed container.

2.7 Bleidner tubes for continuous extraction of steam-volatile substances (Ace Glass Incorporated, Vineland, NJ, Cat. No. 6826-10, see Figure 1)

2.8 Flask, round-bottomed, Monel metal, 2-liter capacity, 29/42 TS.

2.9 Glass Wool (Fisher Scientific Cat. No. 3950).

2.10 Solid phase extraction column, silica, 3cc (Bakerbond NO. 7086-03).

2.11 1000 mL round-bottom flasks, 29 x 26 joint (Lab Glass).

2.12 1000 mL round-bottom flasks, 24 x 40 joint (Lab Glass).

2.13 Powder funnel, 45°

2.14 15 mL graduated centrifuge tube with corks.

2.15 Rotary evaporator with water bath (Brinkmann or Buchi or equivalent)

2.16 Nitrogen evaporator, model 112 (Organomation, Model 112 or equivalent)

2.17 Gas chromatograph (Tracor 560 or equivalent)

2.18 N-P detector (Tracor 702 or equivalent)

2.19 GC column - 2' x 2 mm ID Pyrex, 10% SP-2250 on 100-120 Supelcoport (or equivalent).

2.20 Analytical Standards (Uniroyal Chemical Co.)

3.0 PROCEDURE

3.1 Preparation of Standard Solutions

3.1.1 Stock Solutions (500 µg/ml):Weigh 50 mg of compound and transfer to a 100 ml volumetric flask with acetone. Dissolve and make up to volume with acetone.

3.1.2 Working Standard (2.5 µg/ml): Pipette 0.5 ml of the stock solution into a 100 ml volumetric flask and dilute to volume with acetone. The working standard is used for recovery studies by adding (pipette) standard solution to the appropriate amount of control sample.

3.2 Distillation-Extraction

3.2.1 Place 10 to 50 grams of representative tissue in a 2-liter Monel metal flask, and add several boiling chips, 2 teaspoonfuls of antifoam, and 500 ml of 20% sodium hydroxide solution.

3.2.2 Fill the U-tube of the distillation-extraction head to the level of the lower arm with distilled water and attach the flask to the lower arm.

3.2.3 Add 400 ml of hexane and a boiling chip to the 1-liter round-bottomed flask and attach to the upper arm.

3.2.4 With a 24-inch water-cooled condenser in position, apply heat to both flasks at such a rate that condensed hexane and water pass through the capillary in the form of small sausages. Allow digestion-extraction to proceed for 4 hours.

3.2.5 At the completion of the digestion-extraction period, cool the hexane and pass it through anhydrous sodium sulfate in a powder funnel into a 1000 mL round-bottom flask.

3.2.6 Evaporate off the hexane on a rotary evaporator at 30-35°C to a volume of 5-10 mL (never take to dryness or compound will be lost).

3.2.7 Prepare silica SPE column by rinsing with 5 mL of hexane. In all steps for column cleanup, a small amount of vacuum may be applied to facilitate elution. A commercial 12 port vacuum manifold may be used, e.g. Supelco Cat No. J-7030.

3.2.8 Load sample with 2-3 rinses (total hexane volume 10-15 mL), and discard the solvent.

3.2.9 Rinse the column with 2 mL eluting solvent and discard the solvent.

3.2.10 Place a centrifuge tube inside the filter flask so the end of the needle is inside the tube. Elute with 8 mL of eluting solvent.

3.2.11 Remove and cap the tube. Store at -20°C for analysis.

3.3 Analysis

3.3.1 Using the N-Evap (without water bath) bring sample to appropriate volume for GC (not to dryness).

3.3.2 GC Conditions for determination of FA-1-1 (These conditions should give a retention time of 2-3 minutes and may be varied as necessary):

Injection temp:	220°C
Column temp:	110°C
Detector temp:	250°C
Carrier flow:	Helium @ 10 cc/min.
Air flow:	70 psi
Hydrogen flow:	3.0 cc/min.
Chart speed:	1.0 cm/min.
Attenuation:	as needed

3.3.3 Calibration

3.3.3.1 Inject aliquots of standard solutions (1-10 µL) so that the highest standard injected does not exceed full scale deflection.

3.3.3.2 The range of concentrations may be varied depending on the range of concentrations found in the extracted samples.

3.3.3.3 A calibration curve is calculated using a linear regression program. Peak heights of standards are entered versus ng injected.

3.3.3.4 A separate calibration curve should be plotted each day that samples are run or if GC conditions are changed during the day that affect retention time.

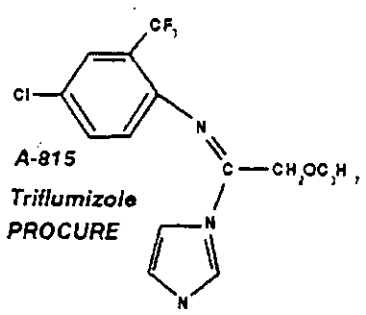
3.3.4 Sample Analysis - Inject aliquots of the residue extracts onto the column.

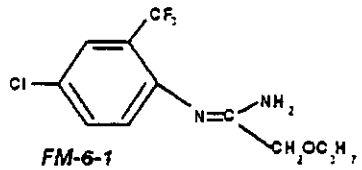
3.3.5 Calcalation of ppm FA-1-1:

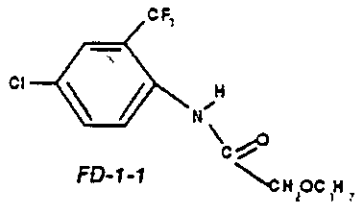
$$\text{ppm FA-1-1} = \frac{(\text{ng found from std. curve}) \times (\text{final volume (ml)})}{(\mu\text{L injected}) \times (\text{sample weight (g)}) \times (\text{conversion factor})}$$

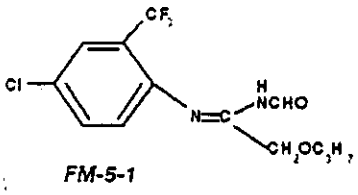
3.3.6 Calculation of ppm Triflumizole and other metabolites - if ppm equivalents of the original compound are desired, apply a conversion factor in the denominator of the equation in 3.3.5. Conversion factors are listed for the following compounds: (structures are shown in Figure 2)

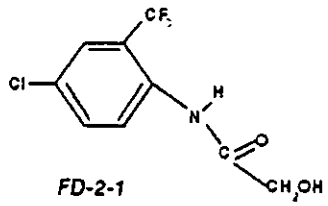
Compound	Molecular Weight	Conversion Factor
A-815 (Triflumizole)	345.5	0.566
FM-6-1	294.5	0.664
FD-1-1	295.5	0.662
FM-5-1	322.5	0.606
FD-2-1	253.5	0.771
FM-8-1	252.5	0.774
FM-8-1-S	289.5	0.675
FA-1-1	195.5	1.000

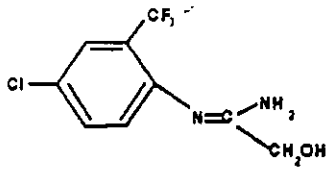


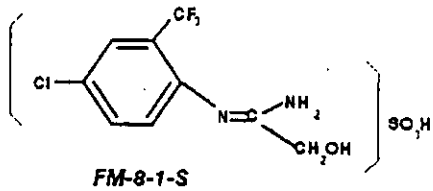












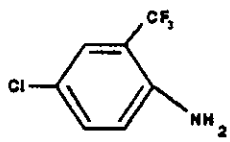


Figure 2. Structures of triflumizole and metabolites mentioned in text.

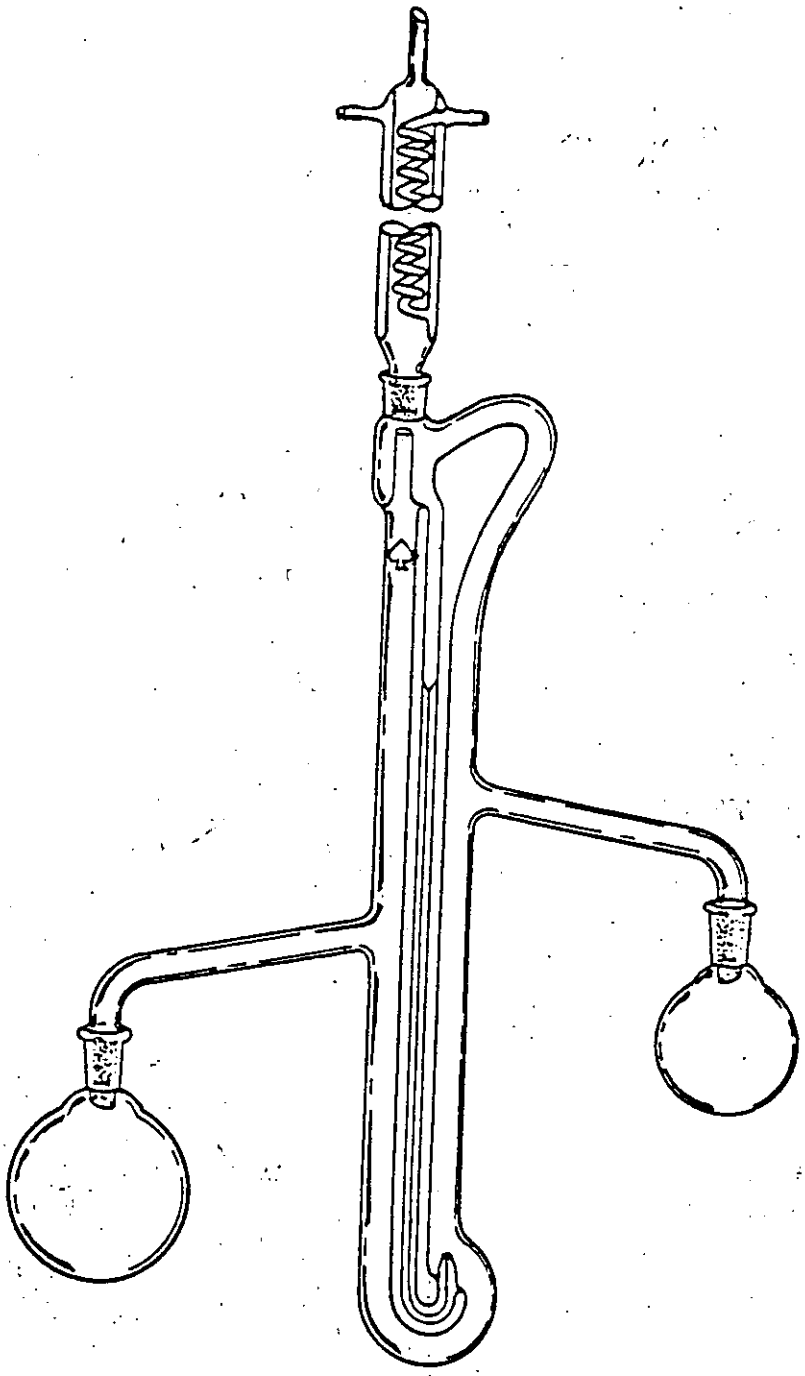


Figure 1. Extraction apparatus

ENVIRO-BIO-TECH, LTD.
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(215) 488-7664

VOLUME 4 PROCURE 50W (400-UGR)
Triflumizole
PP #6F3372/FAP #6H5497

415382-D4

Study Title

Analysis of FA-1-1 in Cow Muscle and Milk from 2, 4, and 6 Hour Refluxes

Data Requirement: Guideline #171-4(j)

Study Director

James Eckert

Report Issued

MARCH 23, 1990

Performing Labortatory

Enviro-Bio-Tech, Ltd.
Bernville, PA 19506

Laboratory Project ID

UR-02-89

Sponsor

Uniroyal Chemical Co.
Naugatuck, CT 06770

CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B) or (C).

Company: Uniroyal Chemical Company, Inc.

Company Agent: Willard F. Cummings Date: 6-21-90

Title: U. S. Registration Manager Signature: Will Cummings

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However, no claims of confidentiality for this document are made, and the data are releasable, when submitted to the U.S. Environmental Protection Agency under FIFRA.

GOOD LABORATORY PRACTICES STATEMENT

This study was conducted in conformance with Good Laboratory Practices established by the United States Environmental Protection Agency, published in the United States EPA Good Laboratory Practices Standard; Pesticides Programs (40 CFR 160).

Signatures:

Study Director : _____ Date: 12/15/89
James Eckert

Technician : _____ Date: 12/15/89
Kristin Schlegel

Submitter* _____ 6-21-90
Uniroyal Chemical Company, Inc.

Sponsor* _____ 6-21-90
Uniroyal Chemical Company, Inc.

*This study was contracted to be conducted according to EPA FIFRA Good Laboratory Practice Standards (40 CFR 160). Based on the signature above, the study did follow these guidelines.

Introduction:
Residue chemistry data on the magnitude of the residues is required under the Federal Insecticide, Fungicide and Rodenticide Act to support the registration of any pesticide intended for use on food or feed crops.

Test Article:

Compound:	FA-1-1
Chemical Name:	2-Trifluoromethyl-4-chloroaniline
Analytical Standard:	Analytical reference standard of FA-1-1 were supplied by Uniroyal Chemical Company.
Storage conditions:	Store frozen until use.
Purity:	FA-1-1 (Uniroyal Lot #874-65) at 99% purity.

Analysis:
The analysis is performed according to SOP EBT-233.00 (Standard Operating Procedure for the Determination of FA-1-1 (Total Aniline) for Triflumizole and its Intermediate Metabolites in Soil, Crops and Tissues). The compounds are extracted from the tissue or milk sample by alkaline hydrolysis. The extract is concentrated by roto-evaporation and added to a silica cleanup column. The column is then eluted with hexane/methylene chloride/methanol (900/99.5/0.5, v/v/v).
The resulting sample extracts are analyzed by gas chromatography with a nitrogen-phosphorous detector. External standards of various concentrations of A-815 and FA-1-1 in acetone are analyzed with the samples. The measured peak heights are used to set up a linear regression program on a TI-60 calculator over the range of interest. By using the appropriate dilution factor for sample size injected, results of each analysis are reported on a ppm (µg/g) basis.
In this study, samples were refluxed at different time intervals (2, 4, and 6 hours) to determine the shortest time needed for acceptable recoveries.

Gas chromatographic conditions:

Instrument:	Tracor 560 GC
Detector:	Tracor 702 N-P
Column:	2' X 2mm ID Pyrex 10% SP-2250 on 100-120 Supelcoport
Column temp:	110°C (FA-1-1)
Injection Temp.	220°C

Detector Temp:	250°C
Carrier flow:	Helium 25 cc/min
Hydrogen flow:	3.0 cc/min
Attenuation:	10X1
Chart speed:	1.0 cm/min

Calculations:

$$\text{PPM FA-1-1} = \frac{(\text{ng found}) \times (\text{final volume})}{(\mu\text{L injected}) \times (\text{g sample})}$$

Fortification: The FA 1-1 standard was added to the sample before hydrolysis and the sample was taken through the procedure.

SUMMARY OF DATA

MILK

Hours	µg Added	ppm Added	ppm Found	% Recovery
2	2.0	0.2	0.122	61
2	2.0	0.2	0.175	88
4	2.0	0.2	0.100	50
4	2.0	0.2	0.16	80
6	2.0	0.2	0.080	40
6	2.0	0.2	0.16	80
6-check	-	-	<0.2	-

MUSCLE

Hours	µg Added	ppm Added	ppm Found	% Recovery
2	2.0	0.2	0.172	86
2	2.0	0.2	0.158	79
4	2.0	0.2	-	- (sample lost-boiled
4	2.0	0.2	0.198	99 over)
6	2.0	0.2	-	-
6	2.0	0.2	0.204	102
6-check	-	-	<0.2	-

MUSCLE - 4 HOURS

Hours	µg Added	ppm Added	ppm Found	% Recovery
4	2.0	0.2	0.208	104
4	2.0	0.2	0.174	87
4	2.0	0.2	0.174	87
4	2.0	0.2	0.141	70
4	2.0	0.2	0.188	94
4	2.0	0.2	0.160	80
4-check	-	-	<0.2	-

Average = 87%

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SOP EBT-233.00
Date: 6-2-88

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF FA-1-1 (TOTAL ANILINE) FOR TRIFLUMIZOLE AND ITS INTERMEDIATE METABOLITES IN SOIL, CROPS, AND TISSUES

REVISION NUMBER	DATE	PREPARED OR REVISED BY	COMMENTS	PAGE(S) REPLACED	DATE OF APPROVAL	APPROVED BY
.00	6/2/88	James Eckert				

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SOP EBT-233.00
Date: 6-2-88

STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF FA-1-1 (TOTAL ANILINE) FOR TRIFLUMIZOLE AND ITS INTERMEDIATE METABOLITES IN SOIL, CROPS, AND TISSUES

1.0 INTRODUCTION

The analysis of FA-1-1 (total aniline) as final hydrolysis product of triflumizole ([1-[1[[4 chloro-2-(trifluoromethyl) phenyl] imino]-2-propoxyethyl]-1H imidazole]) or any of its metabolites (FM-5-1, FM-6-1, FM-8-1, FD-1-1, FD-2-1, or FM-8-1-S) is based on hydrolysis in an alkaline medium, and silica column cleanup.

2.0 REAGENTS AND APPARATUS

2.1 Sodium hydroxide pellets - Reagent grade (Fisher Scientific Cat No. S-318).

2.1.1 20% aqueous NaOH solution.

2.2 Antifoam - Dow Corning Antifoam B Emulsion (Dow Corning).

2.3 Hexane - Reagent Grade (Fisher Scientific Cat No. H302-4) or equivalent.

2.5.1 Eluting solvent (hexane/methylene chloride/methanol) - Mix 900 mL hexane, 99.5 mL (100 mL) methylene chloride and 0.5 mL methanol.

2.4 Methanol - HPLC Grade (Fisher Scientific Cat. No. H452) or equivalent.

2.5 Methylene chloride - HPLC Grade (Fisher Scientific Cat. No. D143) or equivalent.

2.6 Sodium sulfate, anhydrous - (Mallinkrodt Cat. No. 8624 - granular ACS).

The sodium sulfate must be pretreated before using it. Place one piece of Whatman #1 15.0 cm filter paper into a 170 mm OD Coors porcelain funnel. Pour in 450 grams sodium sulfate. Add 400-500 ml of methanol and allow to soak in until there is an excess of methanol pooling at the top of the sodium sulfate. Drain the methanol through by vacuum filtration. Rinse the sodium sulfate twice more, adding the methenol prior to reapplication of the vacuum. Discard the methanol. Place the sodium sulfate in an oven and heat at 135-140°C for 12 hours. Store in a closed container.

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2.7 Blighton tubes for continuous extraction of steam-volatile substances (see figure 1).

2.8 Flask, round-bottomed, Monel metal, 2-liter capacity, 29/42 TS.

2.9 Glass Wool (Fisher Scientific Cat. No. 3950).

2.10 Silica column, 3 cc (Bakerbond NO. 7086-03).

2.11 1000 mL round-bottom flasks, 29 x 26 joint (Lab Glass).

2.12 1000 mL round-bottom flasks, 24 x 40 joint

2.13 Powder funnel, 45°.

2.14 15 mL Graduated centrifuge tube with corks.

2.15 Rotary evaporator with water bath (Brinkmann or Buchi).

2.16 Nitrogen evaporator, model 112 (Organomation).

2.17 Gas chromatograph (Tracor 560).

2.18 N-P detector (Tracor 702).

2.19 GC column - 2' x 2mm ID Pyrex, 10% SP-2250 on 100-120 Supelcoport (or equivalent).

2.20 Analytical Standards (Uniroyal Chemical Co.).

3.0 PROCEDURE

3.1 Preparation of Standard Solutions

3.1.1 Stock Solutions (500 µg/ml): Weigh 50 mg of compound and transfer to a 100 ml volumetric flask with acetone. Dissolve and make up to volume with acetone.

3.1.2 Working Standard (2.5µg/ml): Pipette 0.5 ml of the stock solution into a 100 ml volumetric flask and dilute to volume with acetone. The working standard is used for recovery studies by adding (pipette) standard solution to the appropriate amount of control sample.

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3.2 Distillation-Extraction

3.2.1 Place 10 to 50 grams of representative tissue in a 2-liter Monel metal flask, and add several boiling chips, 2 teaspoonfuls of antifoam, and 500 ml of 20% sodium hydroxide solution.

3.2.2 Fill the U-tube of the distillation-extraction head to the level of the lower arm with distilled water and attach the flask to the lower arm.

3.2.3 Add 400 ml of hexane and a boiling chip to the 1-liter round-bottomed flask and attach to the upper arm.

3.2.4 With a 24-inch water-cooled condenser in position, apply heat to both flasks at such a rate that condensed hexane and water pass through the capillary in the form of small sausages. Allow digestion-extraction to proceed for about 12 hours (overnight is convenient).

3.2.5 At the completion of the digestion-extraction period, cool the hexane and pass it through anhydrous sodium sulfate in a powder funnel into a 1000 mL round-bottom flask.

3.2.6 Evaporate off the hexane on a rotary evaporator at 30-35°C to a volume of 5 - 10 mL (never take to dryness or compound will be lost).

3.2.7 Prepare silica cleanup column by rinsing with 5 mL of hexane. In all steps for column cleanup, a small amount of vacuum may be applied to facilitate elution. This is done by connecting the vacuum hose to a filter flask (500 mL) with the stopcock off. The column is fitted to a needle from the Organomation 112 N-Evap. The needle is pierced through a rubber stopper fitting on the flask. By quickly opening and closing the stopcock, a vacuum can be created which can be controlled to prevent the column from sucking dry by removing the column from the needle just as the liquid reaches the top of the column.

3.2.8 Load sample with 2-3 rinses (total hexane volume 10-15 mL), elute, and discard the solvent.

3.2.9 Rinse the column with 2 mL eluting solvent and discard the solvent.

3.2.10 Place a centrifuge tube inside the filter flask so the end of the needle is inside the tube. Elute with 8 mL of eluting solvent.

3.2.11 Remove and cap the tube. Store at -20°C for analysis.

3.3 Analysis

3.3.1 Using the N-Evap (without water bath) bring sample to appropriate volume for GC (not to dryness).

3.3.2 GC Conditions for determination of FA-1-1 (These conditions should give a retention time of 2-3 minutes and may be varied as necessary):

Injection temp.:	220°C
Column temp.:	110°C
Detector temp.:	250°C
Carrier flow:	Helium @ 10 cc/min.
Air flow:	70 psi
Hydrogen flow:	3.0 cc/min.
Chart speed:	1.0 cm/min.
Attenuation:	as needed

3.3.3 Calibration

3.3.3.1 Inject aliquots of standard solutions (1-10 µL) so that the highest standard injected does not exceed full scale deflection.

3.3.3.2 The range of concentrations may be varied depending on the range of concentrations found in the extracted samples.

3.3.3.3 A calibration curve is calculated on a calculator (such as a Texas Instruments TI-60) using a linear regression program. Peak heights of standards are entered versus ng injected.

3.3.3.4 A separate calibration curve should be plotted each day that samples are run or if GC conditions are changed during the day to affect retention time.

3.3.4 Sample Analysis - Inject aliquots of the residue extracts onto the column.

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3.3.5 Calculation of ppm FA-1-1:

$$\text{ppm FA-1-1} = \frac{(\text{ng found from std. curve}) \times (\text{final volume (ml)})}{(\mu\text{L injected}) \times (\text{sample weight (g)}) \times (\text{recovery factor})}$$

3.3.6 Calculation of ppm Triflumizole and other metabolites - If ppm equivalents of the original compound are desired, apply a conversion factor in the denominator of the equation in 3.3.5. Conversion factors are listed for the following compounds:

Compound	Molecular weight	Conversion factor
A-815 (Triflumizole)	345.5	0.566
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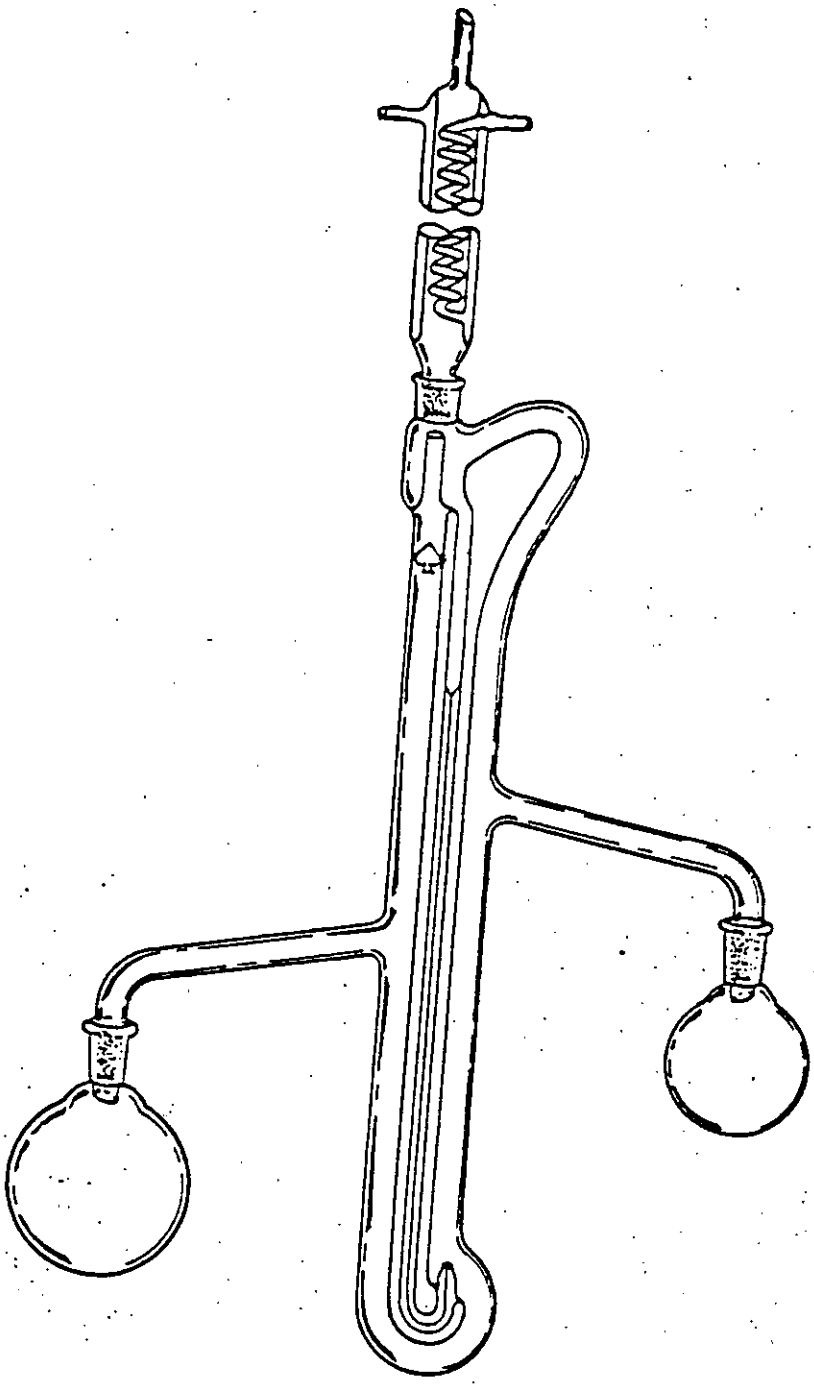

Figure 1. Extraction apparatus