

US EPA ARCHIVE DOCUMENT

3

I PROVISIONE Methods

RESEARCH REPORT

ADC Project #623-A

ANALYTICAL METHOD FOR DETERMINATION OF IPRIDIONE
AND ITS NONHYDROXYLATED METABOLITES IN BOVINE MILK

for

RHONE-POULENC CHEMICAL COMPANY
Agrochemical Division
P.O. Box 125 - Black Horse Lane
Monmouth Junction, NJ 08852

by

ANALYTICAL DEVELOPMENT CORPORATION
P.O. Box 429 - 1875 Willow Park Way
Monument, CO 80132

By: Larry D. Craig

February 10, 1982

ANALYTICAL DEVELOPMENT CORPORATION

FINAL REPORT COVER SHEET

TEST ARTICLE: Iprodione

STUDY TITLE: Iprodione Bovine Residue Feeding Study

ADC PROJECT NUMBER: 623

PURPOSE: To determine residue levels of parent compound and metabolites in tissues and milk of dairy cows fed four levels of test article.

SPONSOR: Rhone-Poulenc Chemical Co.

ADDRESS: P.O. Box 125, Black Horse Lane, Monmouth Junction, NJ 08852

STUDY MONITOR: Mr. Marc Buys, Mr. J. P. Wargo, Jr.

TEST FACILITY: Analytical Development Corporation

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STUDY DIRECTOR: Laurie C. Wilkes, Ph.D.

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Laura Servatius

SUBCONTRACT TEST

FACILITY: Metabolic Laboratory, Colorado State University

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STUDY DIRECTOR: Gerald M. Ward, Ph.D.

INVESTIGATOR(S): Brian P. Barry
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STARTING DATE: February 23, 1981

COMPLETION DATE: January 15, 1982

FINAL REPORT DATES: August 25, 1981 (animal husbandry)
February 10, 1982 (residue)

RECORD STORAGE: After approval of the final reports, all raw data and the final reports will be stored in the archives at ADC. The raw data is in the following ADC notebooks: 1-177, 1-177(A), 1-203, 1-213.

SPECIMEN STORAGE: The cow specimens are stored in the warehouse freezer at ADC.

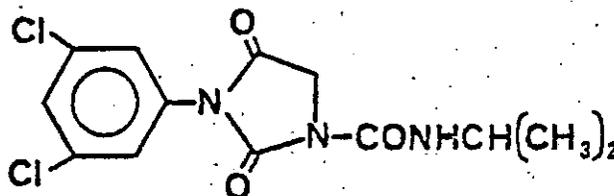
Heptafluorobutyric anhydride (HFBA), Pierce
Florasil, 60/100 mesh, Fisher

Hydrochloric acid, Electronic Grade, DuPont

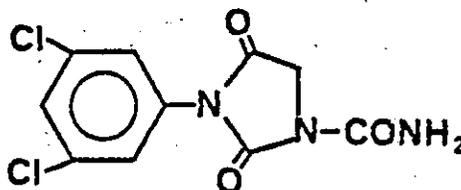
C. Reference Standards

1. Supplied by Rhone-Poulenc:

Iprodione (RP26019) 3-(3,5-dichlorophenyl)-
N-(1-methylethyl)-2,4-dioxo-1-imidazolidine
Carboxamide

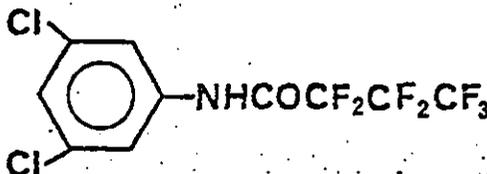


Iprodione Metabolite (RP32490)



2. Prepared by Analytical Development Corp.:

3,5-dichloroaniline-N-heptafluorobutyryl
derivative



The eluate was vacuum rotary evaporated to ~10 ml, then transferred with hexane to a volumetric flask for GC analysis.

IV. GAS CHROMATOGRAPHIC ANALYSIS

A. Instrumentation and Operating Parameters

Instrument: Hewlett-Packard HP 5710A, equipped with a linearized ^{63}Ni electron capture detector

Column: Pyrex glass, 6 ft x 4 mm i.d., coiled shape

Column Packing: 5% Carbowax 20M on 80/100 mesh Chromosorb W HP (Applied Science)

Carrier Gas: 10% methane in argon, flow rate at ~60 ml/min

Temperatures: Injection Port, 250°C
Column, 215°C Isothermal
Detector, 300°C

Recorder: 1 mV, 1/4"/min

B. Sample Injection and Quantitation of Results

The sample was injected from a 2-ml sample vial (sealed with a Teflon-lined septum) with an HP 7671A Automatic Sampler set to deliver ~2 μl ; the peak height (H) was measured in mm. Same volume injections were made of a corresponding standard solution (in hexane), containing a known quantity in $\mu\text{g/ml}$ (c) of the N-heptafluorobutyryl derivative of 3,5-dichloroaniline (HFB); the peak height (h) was measured in mm. For the sample, the concentration in $\mu\text{g/ml}$ (C) was calculated by:

$$C = \frac{H \times c}{h} \text{ in } \mu\text{g/ml}$$

The residue in the sample was expressed as ppm Iprodione equivalents by:

$$\text{ppm} = \frac{C \times 0.922^{(a)} \times \text{dilution volume (ml)}}{\text{sample weight (g)} \times \text{recovery factor}^{(b)}}$$

(a) $0.922 = \frac{\text{MW Iprodione}}{\text{MW HFB derivative}}$

(b) Recovery factor determined from average of fortified control samples run concurrently.

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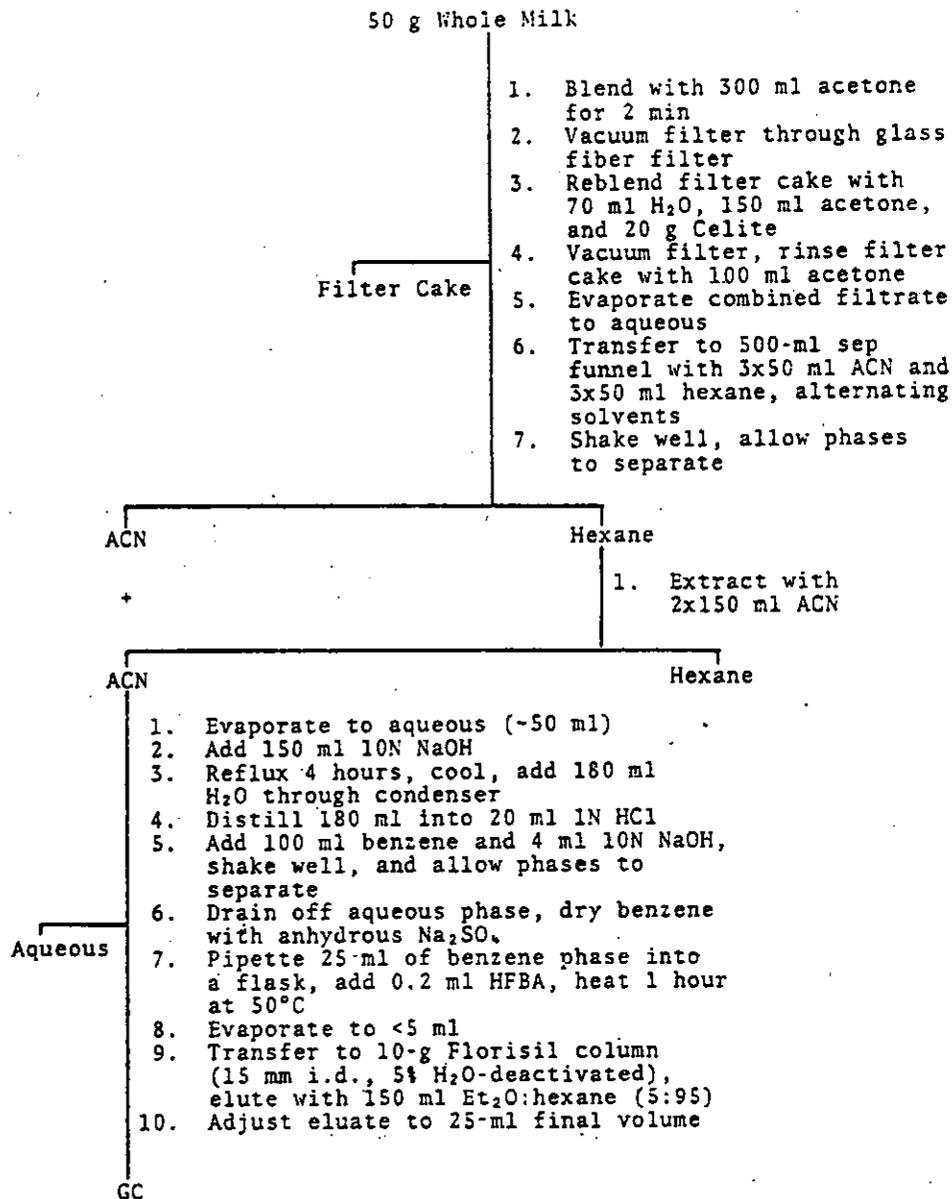
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Section Manager, R&D

LDC:ge

FIGURE 1

ANALYTICAL METHOD FOR DETERMINATION OF IPRODIONE AND ITS
NONHYDROXYLATED METABOLITES IN BOVINE MILK



6' x 4 mm i.d., 5% Carbowax 20M
on Chromosorb W HP 80/100;
215° Isothermal

B 82-28

CONFIDENTIAL

PDD NO.: 81/021
REF.NO.: 81/589/BHL/AG

RHÔNE-POULENC METHOD NO. 159
DETERMINATION OF HYDROXYLATED IPRADIONE (RP-26019) METABOLITES IN
COW MILK BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY

Reported by: A. A. Gemma
G. Heinzelmann
J. P. Wargo

Approved by: A. Guardigli

Rhône-Poulenc Chemical Company
Agrochemical Division
Monmouth Junction, New Jersey
December, 1981

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PDD REPORT NO.: 81/021

LAB REF. NO.: 81/589/BHL/AG

Issue Date: December, 1981

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ABSTRACT

Metabolism studies carried out in lactating cows and a goat (ADC #543B, 1981 and ADC #622, 1981) showed that the major metabolites in urine and milk were RP36114 and RP32490 while the residue in tissue samples consisted mainly of RP32490 and unchanged parent, RP26019. Specific and sensitive analytical methods suitable for enforcement purposes were developed to analyze for all dichloraniline moiety containing metabolites in bovine tissue and milk (ADC #623A, ADC #623B, 1981). These methods were validated by conventional and radiometric means (ADC #622 and ADC #543C, 1981) and used to analyze samples of tissue and milk from a bovine residue feeding study (ADC #623, 1981).

The method described in this report was developed to analyze samples of milk for metabolites containing the dichloroaminophen moiety since the milk samples from cows fed ¹⁴C Iprodione contained significant amounts of RP36114 as the principal hydroxy containing metabolite. This method can be used for enforcement purposes and will account for that residue which is not determined by the methods described above for the dichloraniline moiety containing metabolites. This method was developed for the analysis of bovine milk only because the tissue samples containing ¹⁴C residues had little or no hydroxy containing compounds present. This method was also validated by conventional and radiometric means (ADC #623C, 1981) and used to analyze milk samples from the bovine residue feeding study (ADC #623, 1981) described above.

TITLE: Determination of Hydroxylated Iprodione (RP-26019)
Metabolites in Cow Milk.

1.0 SCOPE:

This method describes the procedures for the determination of the RP-26019 hydroxylated metabolites in cow milk by gas chromatography. The method is sensitive to 0.01 ppm.

2.0 PRINCIPLE:

The hydroxylated metabolites¹ are extracted from the milk using acetone. The samples are cleaned-up using an aluminum sulfate precipitation and a hexane/acetonitrile partition. Any conjugated metabolites present are released using a mild acid hydrolysis. This is followed by methylation using diazomethane. All the hydroxylated metabolites are then converted to a common moiety, 4-methoxy-3,5-dichloro aniline, by means of basic hydrolysis. The aniline is then derivatized using HFBA to form the heptafluorobutyrate¹. The final derivative is cleaned up by Florisil Column Chromatography and the final determination is made by gas chromatography using electron-capture detection.

3.0 REAGENTS:

- 3.1 Acetone: Pesticide quality or equivalent.
- 3.2 Acetonitrile: Pesticide quality or equivalent.
- 3.3 Hexane: Pesticide quality or equivalent.
- 3.4 Benzene: Pesticide quality or equivalent.
- 3.5 Ethyl Ether: Reagent grade.
- 3.6 Ethyl Acetate: Pesticide quality or equivalent.
- 3.7 Diazomethane/Ether Solution: Prepared as described in note 7.5.
- 3.8 Heptafluorobutyric Anhydride: Pierce Chemical Co., Rockford, Ill.
- 3.9 Florisil: Floridin PR Grade 60/100 mesh. Before use, the Florisil is deactivated by adding 5 ml H₂O to 95g Florisil, shaking and sitting for 2 hrs.
- 3.10 Sodium Sulfate: Anhydrous, reagent grade.
- 3.11 Sodium Sulfate Solution: 1% w/w in H₂O.
- 3.12 Aluminum Sulfate Solution: 25% w/w in H₂O.
- 3.13 Iso-octane: Pesticide quality or equivalent.
- 3.14 Sodium Hydroxide: Reagent grade, 1.0N and 10N
- 3.15 Hydrochloric Acid: Reagent grade, 0.10N.
- 3.16 RP-36114: Analytical Standard, Rhone-Poulenc.
- 3.17 Heptafluorobutyryl Derivative: Analytical Standard, Rhone-Poulenc.

1. See Figure 1 for structural formulae of metabolites and derivative.

4.0 EQUIPMENT:

- 4.1 Rotary Flash Evaporator: Buchi Rotavapor-R or equivalent.
- 4.2 Blender: Waring or equivalent. Equipped with a 1 qt. jar.
- 4.3 Chromatographic Column: Glass 240mm X 10.5mm I.D. with a 200 ml reservoir.
- 4.4 Filter Paper: Whatman No. 1 - 7 & 11 cm.
Whatman Glass Fiber 934-AH - 7 & 11 cm.
- 4.5 Glass Wool
- 4.6 Buchner Funnels
- 4.7 General Laboratory Glassware
- 4.8 Special Indicator Sticks: Color pHast. MC/B Manufacturing Chemists, Inc. pH-5-10, pH-0-14.
- 4.9 Gas Chromatograph: Tracor Model 222 equipped with a ⁶³Ni EC detector.
- 4.10 Microsyringe: 10 microliter (10 μ l), Hamilton or equivalent.
- 4.11 Heating Mantles
- 4.12 Reflux Condensers

5.0 PROCEDURE: (see Figure 4 for Schematic Flow Diagram)

5.1 Sample Preparation

5.1.1 Milk: Mix well by shaking to ensure homogeneity before sub-sampling.

5.2 Extraction

5.2.1 Cow Milk

- 5.2.1.1. Weigh out a 50g sample in a Waring blender jar.
- 5.2.1.2 Blend for 5 min. with 200 ml. of acetone.
- 5.2.1.3 Vacuum filter through a Buchner funnel with filter paper.
- 5.2.1.4 Re-blend the filter pad with 100 ml. of acetone.
- 5.2.1.5 Vacuum filter through a Buchner funnel with filter paper.
- 5.2.1.6 Rinse the blender jar and filter cake with 100 ml. of acetone.
- 5.2.1.7 Combine all the acetone extracts.

5.3 Aluminum Sulfate Precipitation Step

- 5.3.1 Evaporate extract from 5.2.1.7 to aqueous.
- 5.3.2 Add 20 ml. of 25% $Al_2(SO_4)_3/H_2O$ to sample.
- 5.3.3 Adjust pH of sample to 4.5-6.0 using 10N NaOH. A milky precipitate will form.
- 5.3.4 Vacuum filter through a Buchner funnel with filter paper.
- 5.3.5 Rinse filter cake with 20ml H_2O and 50ml CH_3CN . Discard filter cake.

5.4 Hexane/Acetonitrile Partition

- 5.4.1 Transfer extract from 5.3.5 to a separatory funnel. Rinse flask with an additional 50ml of CH_3CN .
- 5.4.1 Partition aqueous/ CH_3CN with 2 X 50ml hexane.
- 5.4.2 Backwash the hexane with 50ml CH_3CN .
- 5.4.3 Combine the CH_3CN fractions and discard the hexane.

5.5 Acid Hydrolysis

- 5.5.1 Take extract from 5.4.3 and evaporate off the acetonitrile.
- 5.5.2 To the remaining aqueous add 50ml of 0.10N HCl.
- 5.5.3 Reflux for 1 hour. Cool.
- 5.5.4 When cool, extract with 3 X 50ml of ethyl acetate.
- 5.5.5 Dry ethyl acetate fractions through a pad of anhydrous Na_2SO_4 . Combine ethyl acetate fractions. Discard aqueous fraction.

5.6 Methylation

- 5.6.1 Evaporate the extract from 5.5.5 to dryness.
- 5.6.2 Re-dissolve the extract in 20ml of iso-octane.
- 5.6.3 Methylate by adding 2-3ml of diazomethane solution to the iso-octane. React for 1 hour.
Note: Yellow color should remain. If not, additional diazomethane should be added.

5.7 Base Hydrolysis

- 5.7.1 After the methylation is completed, take the sample (5.6.3) and evaporate to dryness.
- 5.7.2 Add 100ml of 1N NaOH and reflux for 2 hours.
- 5.7.3 Cool to room temperature and rinse the condenser with 150ml of 1% $\text{Na}_2\text{SO}_4/\text{H}_2\text{O}$ Solution.
- 5.7.4 Extract the aqueous with 3 X 25ml of benzene.
- 5.7.5 Dry the benzene fractions over a pad of anhydrous sodium sulfate. Combine the benzene fractions. Discard the aqueous fraction.

5.8 HFBA Derivatization

- 5.8.1 To the benzene fraction (5.7.5) add 0.5ml of heptafluorobutyric anhydride. Stopper with a teflon stopper and shake.
- 5.8.2 Place sample in an oven at 60°C and react for 3 hours.
- 5.8.3 Cool sample to room temperature and evaporate to 25-50ml.

5.9 Florisil Column Clean-up

- 5.9.1 Place a glass wool plug at the bottom of the chromatographic column.
- 5.9.2 Add florisil (see 3.9) to a height of 10 cm. Add 2 cm of anhydrous Na₂SO₄ to the top of the florisil
- 5.9.3 Wash the column with 50 ml of hexane. Discard.
- 5.9.4 Add the extract from 5.8.3 to the column. When the sample has reached the top of the sodium sulfate rinse the flask with 20ml of 10% ethyl ether/hexane and add this to the column.
- 5.9.5 When the rinse has reached the top of the sodium sulfate, elute with an additional 50ml of 10% ethyl ether/hexane.
- 5.9.6 Collect all the fractions from the column. Samples are then diluted/concentrated to appropriate volume for final determination.
eg. - A 50g sample screened at 0.01 ppm would have a final volume of 50ml which is a concentration of 0.01µg/ml.

5.10 Final Determination

- 5.10.1 Calibrate the gas chromatograph by injecting a series of 5µl amounts of the heptafluorobutyryl derivative at 0.005, 0.01, 0.02, 0.025, and 0.03 µg/ml. GC conditions are given in Table I.
- 5.10.2 Construct a standard curve by plotting detector response (peak height) versus amount of standard injected. A typical standard curve for the heptafluorobutyryl derivative is shown in Figure 1. Typical chromatograms are shown in Figure 2.
- 5.10.3 Inject an appropriate aliquot of the sample dissolved in 10% ethyl ether/hexane from 5.9.6 into the gas chromatograph.
- 5.10.4 Directly compare the peak heights of unknown samples with the standard curve to obtain the amounts of the heptafluorobutyryl derivative contained in the injected aliquot.
- 5.10.5 Determine the residue results in terms of ppm Iprodione equivalents or RP-36114 equivalents in the sample by the following:

$$\text{ppm} = \left[\left(\frac{A}{B} \right) \times F \right] \div R, \text{ where}$$

A= Concentration extrapolated from standard curve, expressed as nanograms.

B= (g sample) X $\left(\frac{\text{microliters injected}}{\text{ml final volume}} \right)$ = mg injected

F= Molecular weight correction for derivative.
(F= 0.85 for Ipridione; 0.68 for RP-36114)

R= Recovery factor based on fortified controls carried through the procedure. R is expressed as a decimal (i.e. 100% = 1.00, 90% = 0.90, etc.)

5.10.6 Bracket every 3-5 samples analyzed with known amounts of standard to maintain a continual check for any shifts in sensitivity.

6.0 RECOVERY STUDIES:

Control milk samples were spiked at 0.01-0.20 ppm with stock solutions of RP-36114 prior to extraction. Recovery data are given in Table II and typical chromatograms are shown in Figure 3. A treated sample from the cow metabolic study (ADC #543) was also analyzed using the procedure described. The raw data and chromatograms for the treated sample are shown in Table III and Figure 3 respectively.

7.0 NOTES:

7.1 Preparation of Standard Solutions

7.1.1 RP-36114

7.1.1.1 Prepare a standard stock solution by weighing out 10.0 mg of RP-36114, quantitatively transferring to a 100ml volumetric flask, and diluting to volume with acetone. This solution contains 0.1mg/ml (100ug/ml). This is stock solution A. Stock solution A should be prepared every six months and stored in a freezer.

7.1.1.2 Transfer 10.0ml of stock solution A to a 100ml volumetric flask and dilute to volume with acetone. This solution contains 10ug/ml, (stock solution B). Stock solution B should be prepared fresh every 2 months and should be stored in a refrigerator.

7.1.1.3 Transfer 10.0ml of stock solution B to a 100ml volumetric flask and dilute to volume with acetone. This solution contains 1 ug/ml, (stock solution C). Stock solution C should be prepared fresh every 2 months and should be stored in a refrigerator.

7.1.2 Heptafluorobutyryl Derivative

- 7.1.2.1 Prepare a standard stock solution by weighing out 10mg of the derivative, quantitatively transferring to a 100ml volumetric flask, and diluting to volume with hexane. This solution contains 0.10 mg/ml. (100µg/ml). This is stock solution D. Stock solution D should be prepared every six months and stored in a freezer.
- 7.1.2.2 Transfer 2ml of stock solution D to a 100ml volumetric flask and dilute to volume with hexane. This solution contains 2 µg/ml, (stock solution E). Stock solution E should be prepared every six months and stored in a freezer.
- 7.1.2.3 Transfer 10ml of stock solution E to a 100ml volumetric flask and dilute to volume with hexane. This solution contains 0.20 µg/ml, (stock solution F). Stock solution F should be prepared every six months and stored in a freezer.
- 7.1.2.4 Dilutions of stock solution F can be made monthly for calibration of the gas chromatograph. The following dilutions were made:

<u>ml Stock Solution F</u>	<u>Final Dilution Volume</u>	<u>Final Concentrations (µg/ml ea. Stand</u>
1) 2.5	100ml	0.005
2) 5.0	100ml	0.010
3) 7.5	100ml	0.015
4) 10.0	100ml	0.020
5) 12.5	100ml	0.025
6) 15.0	100ml	0.030

- 7.2 Care should be taken not to evaporate the 4-methoxy-3,5-dichloro aniline or the heptafluorobutyryl derivative below 25ml. The water bath for the rotovap should not exceed 40°C.
- 7.3 Care should be taken while making the HFBA derivative. Teflon stoppers and flasks without visible cracks should be used.
- 7.4 Teflon sleeves should be used on all condensers used for the NaOH hydrolysis.

7.5 Diazomethane Preparation

Dissolve 2.3g of KOH in 2.3 ml of distilled water in a 125 ml flask. Cool the solution to room temperature and add 25 ml of diethylether. Cool the flask in a freezer. In a hood, add 1.5g of N-methyl-N'-nitro-N-nitroso-guanidine. The additions should be done slowly over a period of a few minutes. After each addition, gently swirl the flask. In a bottle, equipped with a "poly-seal" polyethylene liner cap, decant the ether layer from the aqueous slurry that has formed. Cap and store in a freezer. Do not use ground glass stoppered bottles. The diazomethane solution may be stored at -20° C for over a week if it is kept in a tightly capped bottle. This procedure gives approximately 16 ml of ether solution.

CAUTION: The diazoalkane may be prepared in a larger quantity by increasing the amounts of chemicals, but the proportions must not be changed. The diazoalkanes are toxic and potentially explosive. Do not allow the nitrosoguanidine or diazomethane solution to come in contact with the skin, as these compounds may cause skin rashes.

Etched or scratched glassware should be avoided. Use only diethylether as the solvent for diazomethane.

8.0 INDEX OF FIGURES:

Figure 1 Structural formulae of metabolites and derivative.

Figure 2 Typical standard curve of the heptafluorobutyryl derivative of 4-methoxy-3,5-dichloroaniline using a 63 Ni EC detector.

Figure 3 Typical chromatograms of standards of the heptafluorobutyryl derivative of 4-methoxy-3,5-dichloroaniline using a 63 Ni EC detector.

Figure 4 Typical chromatograms of cow milk; control, fortified controls, and 14 C feeding sample using a 63 Ni EC detector.

Figure 5 Schematic Flow Diagram.

9.0 LITERATURE REFERENCES

1. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #543-B) Metabolism of ^{14}C - Iprodione (RP26019) In The Dairy Cow.
2. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #622) Metabolism of ^{14}C - Iprodione (^{14}C RP26019) In The Lactating Goat.
3. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #623A) Analytical Method For Determination of Iprodione and Its Nonhydroxylated Metabolites in Bovine Milk.
4. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #623B) Analytical Method For Determination of Iprodione and Its Nonhydroxylated Metabolites in Bovine Tissue.
5. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #622) Validation of Analytical Method For Determination of Iprodione and Its Nonhydroxylated Metabolites in Bovine Tissue.
6. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #543C) Validation of Analytical Method For Iprodione and Its Nonhydroxylated Metabolites In Milk.
7. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #623) Analysis of Milk and Tissues of Treated Dairy Cattle For Iprodione and Its Metabolites.
8. A.D.C. (1981); Scientific Report From Analytical Development Corporation; Monument, Colorado (Confidential Communication ADC Project #623C) Validation of Analytical Method For Determination of Hydroxylated Metabolites of Iprodione In Bovine Milk.

TABLE I

GAS CHROMATOGRAPHIC CONDITIONS

Column:	4' X 1/4" Glass. 2.5% DEGS on Chromoso WHP 80/100 mesh.
Column Temperature:	185°C
Inlet Temperature:	225°C
Detector Temperature:	310°C
Carrier Gas:	10% Methane/Argon 70ml/min.
EC Voltage:	45 Volts
Pulse Rate:	180 μ sec.
Pulse Width:	4 μ sec.
Attenuation:	10^2 X 16
Chart Speed:	0.25 in./min.
Minimum Detectable Amount:	25 Picograms
Retention Time (Approx.):	2.5 min.

TABLE II

RECOVERY DATA FOR RP-36114 FROM COW MILK

<u>No.</u>	<u>Fortification Level (ppm)</u>	<u>% Recovery</u>
1)	0.01	71%
2)	0.01	62%
3)	0.02	63%
4)	0.02	73%
5)	0.10	62%
6)	0.10	69%
7)	0.10	76%
8)	0.10	64%
9)	0.10	70%
10)	0.20	72%
11)	0.20	67%
12)	0.20	66%

$$X = 67.9\%$$

$$\text{Std. deviation} = 4.64$$

$$\text{Coeff. of variation} = 6.83\%$$

TABLE III

RAW DATA OBTAINED FROM THE ANALYSIS OF ¹⁴C IPRODIONE
TREATED COW MILK FOR THE RESIDUES OF IPRODIONE
HYDROXYLATED METABOLITES

<u>Test No.</u>	<u>Sample No.</u>	<u>Daily Feeding Level</u>	<u>Iprodione Equivalent Residues Found (ppm)</u>
ADC-543	620-11 081580	60 ppm	0.165
ADC-543	620-11 081580	60 ppm	0.163
ADC-543	620-11 081580	60 ppm	0.100
ADC-543	620-11 081580	60 ppm	0.161

$$X = 0.147\text{ppm}$$

* Residues corrected for average recoveries.

Figure 1

STRUCTURAL FORMULAE OF METABOLITES
AND DERIVATIVES

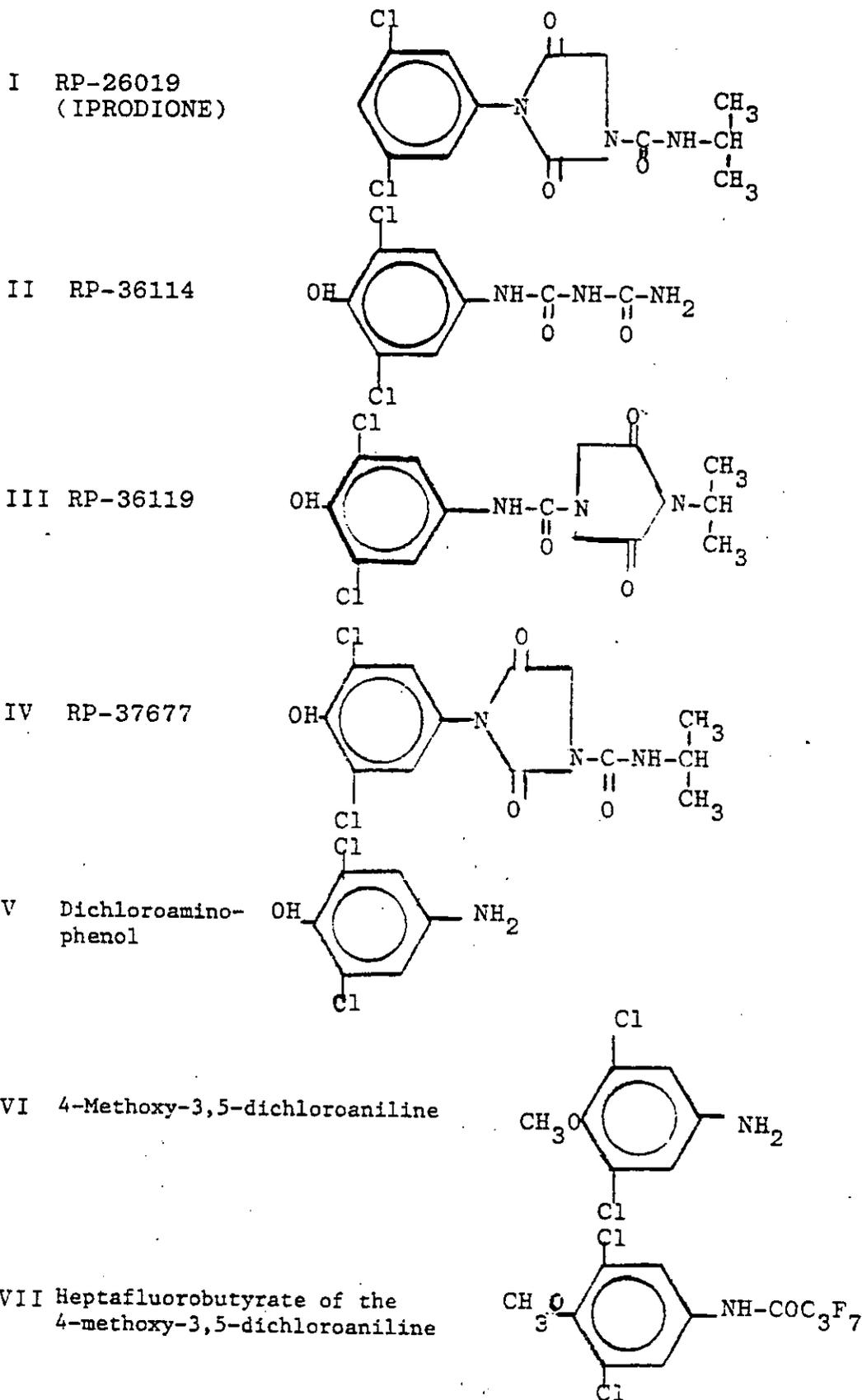
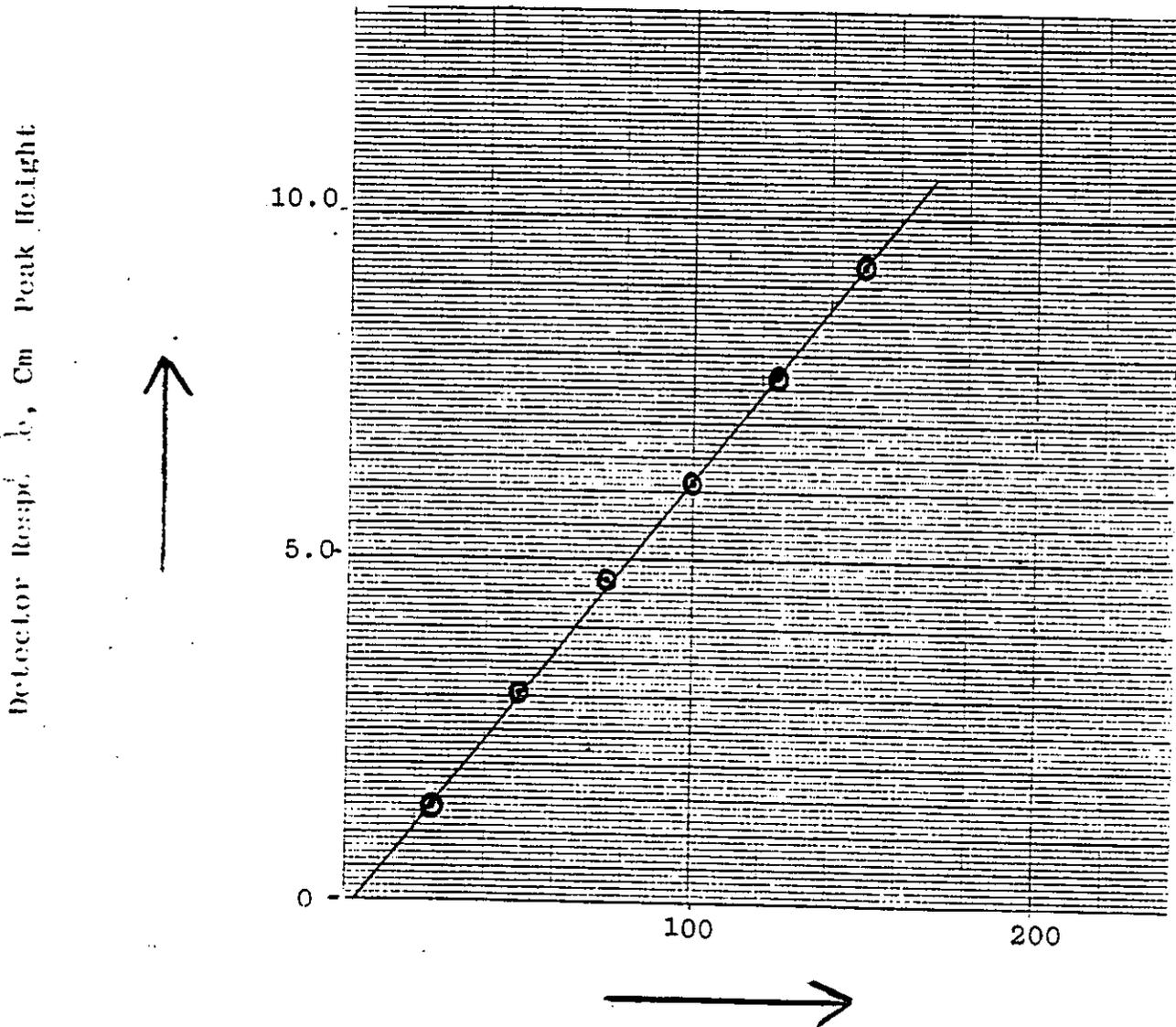


Figure 2

TYPICAL STANDARD CURVE OF THE HEPTAFLUOROBUTYRYL
DERIVATIVE OF 4-METHOXY-3,5 - DICHLORO-ANILINE
USING A ^{63}Ni EC DETECTOR



Heptafluorobutyryl derivative standard, picograms injected

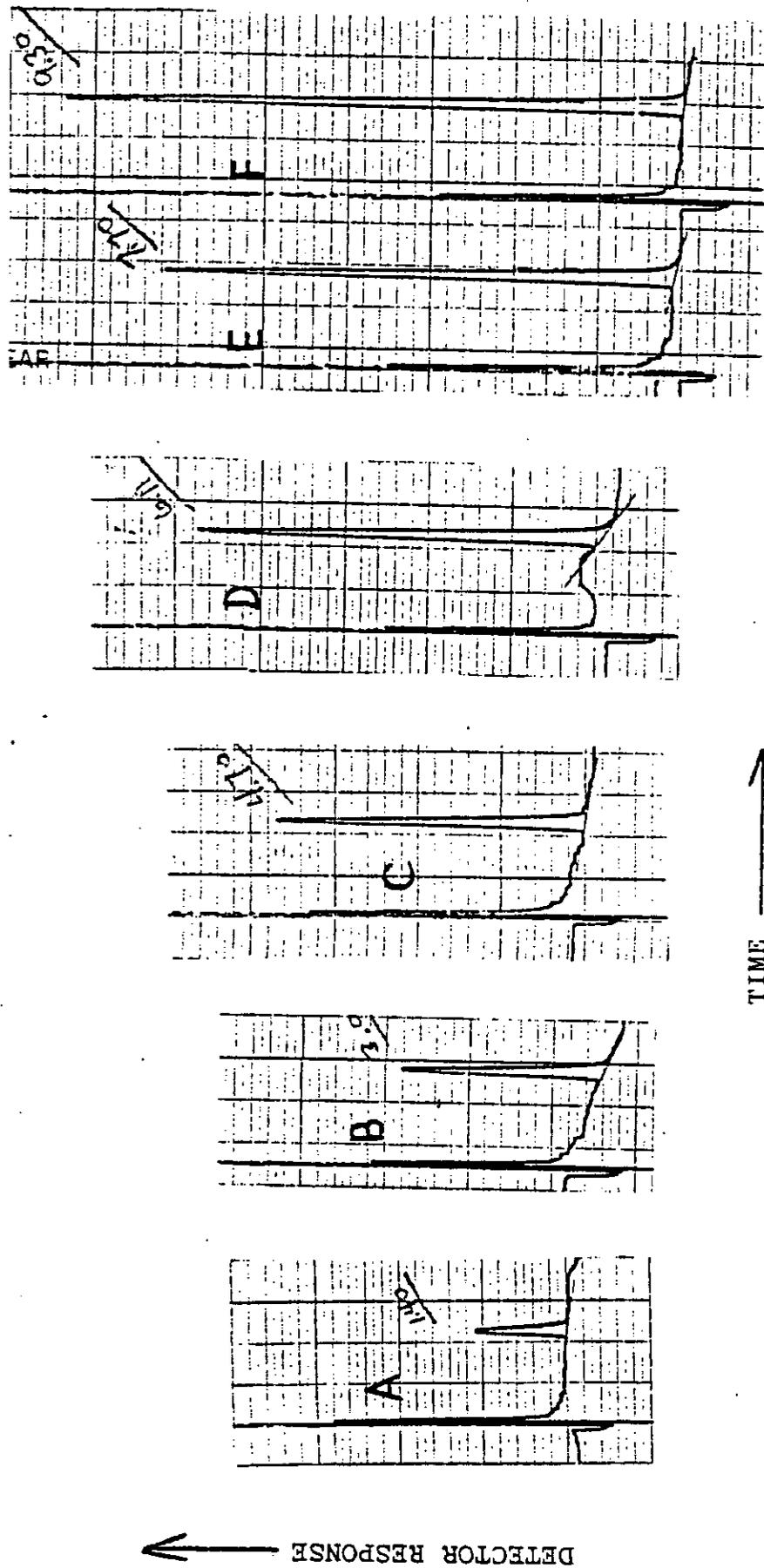
Slope = 0.0626

Intercept = -0.095

Correlation = 0.9998

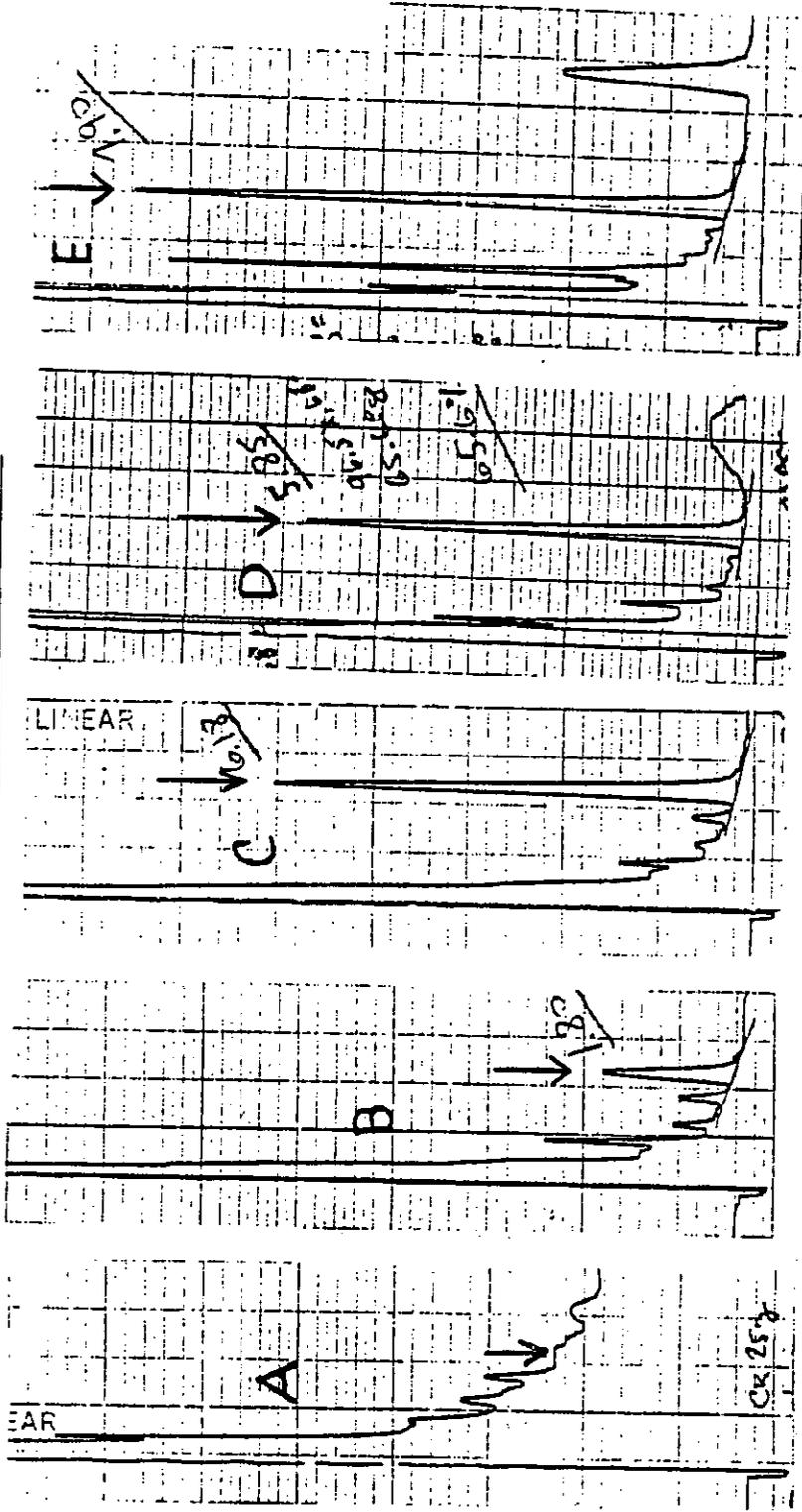
Figure 3

TYPICAL CHROMATOGRAMS OF STANDARDS OF THE HEPTAFLUOROBUTYRYL DERIVATIVE OF 4-METHOXY-3,5-DICHLORO ANILINE USING A ^{63}Ni EC DETECTOR



- All injections are 5 μl
- A. 0.005 $\mu\text{g}/\text{ml}$, (25pg)
 - B. 0.010 $\mu\text{g}/\text{ml}$, (50pg)
 - C. 0.015 $\mu\text{g}/\text{ml}$, (75pg)
 - D. 0.020 $\mu\text{g}/\text{ml}$, (100pg)
 - E. 0.025 $\mu\text{g}/\text{ml}$, (125pg)
 - F. 0.030 $\mu\text{g}/\text{ml}$, (150pg)

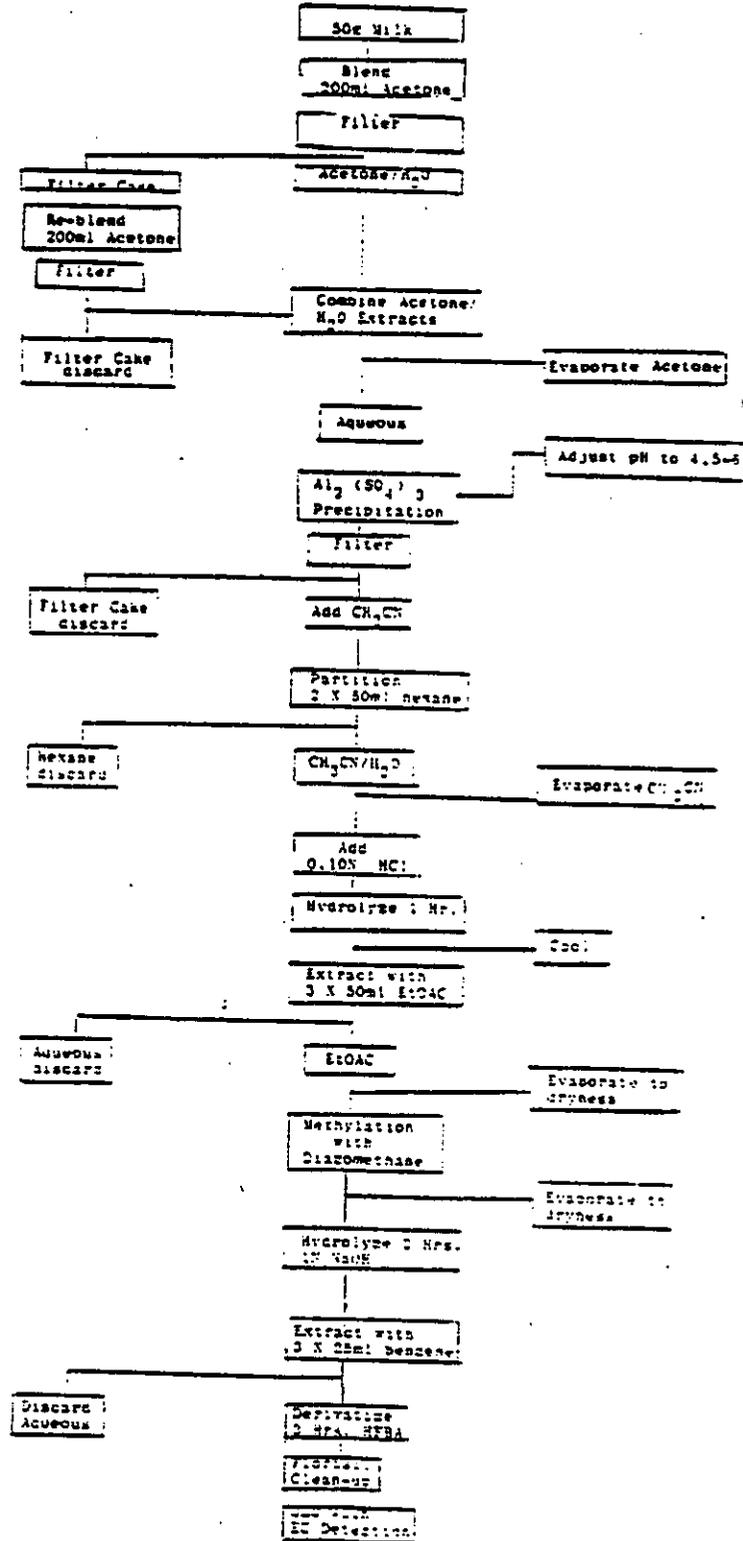
TYPICAL CHROMATOGRAMS OF CO. MILK: CONTROL, FORTIFIED CONTROLS, AND FEEDING SAMPLE USING A ⁶³Ni EC DETECTOR



- A. Control: 25g; 1.25mg injected; <0.02ppm.
- B. Control + 0.02ppm; 25g; 1.25mg injected; 73% recovery.
- C. Control + 0.10ppm; 25g; 1.0mg injected; 70% recovery.
- D. Control + 0.20ppm; 25g; 0.5mg injected; 66% recovery.
- E. 60ppm; feeding sample; 50g; 1.0mg injected; 0.161ppm found (corrected) (sample 620-11, 081580, ADC No. 543)

FIGURE 5

FLOW DIAGRAM



10/25

RESEARCH REPORT

ADC Project #623-B

ANALYTICAL METHOD FOR DETERMINATION OF IPRADIONE
AND ITS NONHYDROXYLATED METABOLITES IN BOVINE TISSUES

for



RHONE-POULENC CHEMICAL COMPANY
Agrochemical Division
P.O. Box 125 - Black Horse Lane
Monmouth Junction, NJ 08852

by

ANALYTICAL DEVELOPMENT CORPORATION
P.O. Box 429 - 1875 Willow Park Way
Monument, CO 80132

By: Laura Servatius
Larry D. Craig

February 10, 1982

ANALYTICAL DEVELOPMENT CORPORATION

FINAL REPORT COVER SHEET

TEST ARTICLE: Iprodione

STUDY TITLE: Iprodione Bovine Residue Feeding Study

ADC PROJECT NUMBER: 623

PURPOSE: To determine residue levels of parent compound and metabolites in tissues and milk of dairy cows fed four levels of test article.

SPONSOR: Rhone-Poulenc Chemical Co.

ADDRESS: P.O. Box 125, Black Horse Lane, Monmouth Junction, NJ 08852

STUDY MONITOR: Mr. Marc Buys, Mr. J. P. Wargo, Jr.

TEST FACILITY: Analytical Development Corporation

ADDRESS: 1875 Willow Park Way, Monument, CO 80132

STUDY DIRECTOR: Laurie C. Wilkes, Ph.D.

INVESTIGATOR(S): Larry D. Craig
Laura Servatius

SUBCONTRACT TEST

FACILITY: Metabolic Laboratory, Colorado State University

ADDRESS: Fort Collins, CO 80523

STUDY DIRECTOR: Gerald M. Ward, Ph.D.

INVESTIGATOR(S): Brian P. Barry
Lorence Raim

STARTING DATE: February 23, 1981

COMPLETION DATE: January 15, 1982

FINAL REPORT DATES: August 25, 1981 (animal husbandry)
February 10, 1982 (residue)

RECORD STORAGE: After approval of the final reports, all raw data and the final reports will be stored in the archives at ADC. The raw data is in the following ADC notebooks: 1-177, 1-177(A), 1-203, 1-213.

SPECIMEN STORAGE: The cow specimens are stored in the warehouse freezer at ADC.

ANALYTICAL METHOD FOR DETERMINATION OF IPRODIONE
AND ITS NONHYDROXYLATED METABOLITES IN BOVINE TISSUES

I. INTRODUCTION

A method was developed by Analytical Development Corporation to detect Iprodione and its nonhydroxylated metabolites in bovine tissues. The nonhydroxylated species were converted to the N-heptafluorobutyl derivative of 3,5-dichloroaniline, which was then quantitated by electron-capture gas chromatography; the lower limit of detection was 0.05 ppm as Iprodione equivalents.

II. MATERIALS

A. Apparatus

Assorted laboratory glassware

Buchner funnel

Vacuum filtering flask

Separatory funnels with Teflon stopcocks

Polytron PT10ST homogenizer

Rotary vacuum evaporator, all glass system, Büchler

Chromatography column, 15 mm i.d., 200-ml reservoir

Reflux condenser, Liebig

Teflon boiling chips, Chemplast

Glass fiber filters, Whatman 934-AH

Glass wool

Gas chromatograph (HP 5710A) equipped with electron capture detector (^{63}Ni) and automatic sampler (HP 7671A)

Heating mantle, Glas-Col

Variable transformer, Staco

B. Reagents

Deionized water

Solvents, Fisher Certified Pesticide Grade, Mallinckrodt Nanograde, Baker Resi-Analyzed, or Burdick and Jackson Distilled in Glass

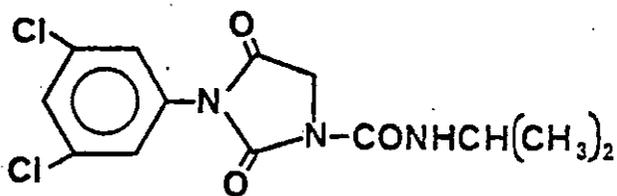
Sodium sulfate, anhydrous, granular, Certified ACS, Fisher; prewashed with hexane, then heated at 120°C for at least 1 hour

Sodium hydroxide, Certified ACS, Fisher
Heptafluorobutyric anhydride (HFBA), Pierce
Florisol, 60/100 mesh, Fisher
Hydrochloric acid, Electronic Grade, DuPont

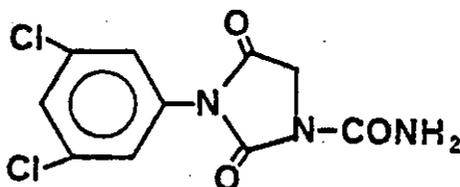
C. Reference Standards

1. Supplied by Rhone-Poulenc:

Iprodione (RP26019) 3-(3,5-dichlorophenyl)-
N-(1-methylethyl)-2,4-dioxo-1-imidazolidine
Carboxamide

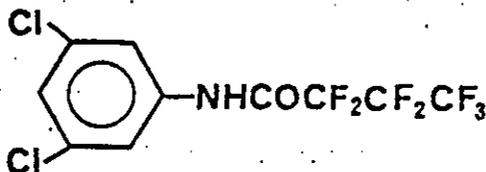


Iprodione Metabolite (RP32490)



2. Prepared by Analytical Development Corp.:

3,5-dichloroaniline-N-heptafluorobutyryl
derivative



III. METHODS (Figure 1 for Muscle, Kidney, and Fat;
Figure 2 for Liver)

A. Acetone Extraction (Muscle, Kidney, Fat, Liver)

Ten grams of tissue was blended in a 250-ml brown bottle with 150 ml of acetone and 10 ml of 1N HCl with the Polytron PT10ST homogenizer at medium speed for at least 2 minutes. The acetone extract was filtered under vacuum through a Buchner funnel with a glass fiber filter into a 1000-ml filtering flask, followed by two 25-ml acetone rinses. The filter cake and filter were reblended in the same bottle with 150 ml of acetone and 10 ml of 1N NaOH at medium speed for 4 minutes. The acetone extract was filtered under vacuum into the same filtering flask, followed by two 50-ml acetone rinses. The combined acetone filtrate was transferred to a 1000-ml flat bottomed flask and was vacuum rotary evaporated to the residual aqueous solution (~20 ml, Extract A).

B. Acetonitrile/Hexane Partition

1. Muscle, Kidney, and Fat

Fifteen milliliters of deionized water was added to Extract A; the pH was checked and adjusted to <5 with 1N HCl, if necessary. The solution was transferred to a 500-ml separatory funnel with three 50-ml rinses each of acetonitrile (ACN) and hexane, alternating the solvents. The separatory funnel was shaken vigorously for 1 minute, then the phases were allowed to separate. The hexane layer was extracted two more times with 150 ml of ACN. The combined ACN extract was vacuum rotary evaporated to the residual aqueous solution (~35 ml, Extract B).

2. Liver

Twenty milliliters of 25% $Al_2(SO_4)_3$ in water was added to Extract A and the pH was adjusted to 5-7 with 10N NaOH. The solution was filtered under vacuum through a Buchner funnel with two glass fiber filters into a 500-ml filtering flask. The filter cake was rinsed with ~20 ml of H_2O , followed by 50 ml of ACN. The filtrate was then transferred to a 500-ml separatory funnel and extracted as described above (Extract B).

C. Hydrolysis, Benzene Partition, and Derivatization

One hundred and fifty milliliters of 10N NaOH and several Teflon boiling chips were added to Extract B, which was then refluxed for 8 hours (6 PM - 2 AM) at a setting of 40 on the variable transformer. The condenser was rinsed with 150 ml of 1% Na₂SO₄ in water, followed by 50 ml of benzene. The hydrolysate was transferred to a 1000-ml separatory funnel and extracted three times with 50 ml of benzene. The benzene extracts were successively filtered through a pad of anhydrous Na₂SO₄ into a 500-ml flat bottomed flask. The separatory funnel and Na₂SO₄ pad were rinsed with ~30 ml of benzene.

The benzene extract was derivatized with 0.2 ml of heptafluorobutyric anhydride for 1 hour in a sandbath at 50°C. The derivatized solution was vacuum rotary evaporated to ~20 ml.

D. Florisil Column Chromatography

1. Preparation of Deactivated Florisil

Florisil was activated at ~120°C for at least 24 hours. The cooled Florisil was deactivated by adding deionized H₂O (5%, w/w). The mixture was shaken vigorously for ~4 min, then allowed to equilibrate for 2 hours with occasional shaking.

2. Column Preparation and Sample Cleanup

A plug of glass wool was placed in the bottom of a glass chromatographic column (15 mm i.d.). Thirty milliliters of ethyl ether:hexane (5:95) was added to the column; air bubbles were dislodged with a glass rod. Ten grams of 5%-deactivated Florisil was slowly added, while gently tapping the sides of the column. A few grams of anhydrous Na₂SO₄ was layered on top of the Florisil. The solvent was drained to the top of the Na₂SO₄; the eluate was discarded.

The sample was quantitatively transferred to the Florisil column with three 2-ml rinses of ethyl ether:hexane (5:95); the solvent was drained to the Na₂SO₄ layer after each addition and the eluates were collected in a 250-ml flat bottomed flask. The column was then washed

with 150 ml of ethyl ether:hexane (5:95); the eluate was collected in the same flask as above. The combined eluate was vacuum rotary evaporated to ~5 ml, then transferred to an appropriate volumetric flask with hexane.

IV. GAS CHROMATOGRAPHIC ANALYSIS

A. Instrumentation and Operating Parameters

Instrument: Hewlett-Packard HP 5710A equipped with a linearized ^{63}Ni electron capture detector

Column: Pyrex glass, 6 ft x 4 mm i.d., coiled shape

Column Packing: 5% Carbowax 20M on 80/100 mesh Chromosorb W HP (Applied Science)

Carrier Gas: 10% methane in argon, flow rate at ~60 ml/min

Temperatures: Injection Port, 250°C
Column, 200°C or 215°C Isothermal
Detector, 300°C

Recorder: 1 mV, 1/4"/min

B. Sample Injection and Quantitation of Results

The sample was injected from a 2-ml sample vial (sealed with a Teflon-lined septum) with an HP 7671A Automatic Sampler set to deliver ~2 μl ; the peak height (H) was measured in mm. Same volume injections were made of a corresponding standard solution (in hexane), containing a known quantity in $\mu\text{g/ml}$ (c) of the N-heptafluorobutyryl derivative of 3,5-dichloroaniline (HFB); the peak height (h) was measured in mm. For the sample, the concentration of the derivative in $\mu\text{g/ml}$ (C) was calculated by:

$$C = \frac{H \times c}{h} \text{ in } \mu\text{g/ml}$$

The residue detected in the sample was expressed as ppm Iprodione equivalents by:

$$\text{ppm} = \frac{C \times 0.922(a) \times \text{dilution volume (ml)}}{\text{sample weight (g)} \times \text{recovery factor}(b)}$$

$$(a) \ 0.922 = \frac{\text{MW Iprodione}}{\text{MW HFB derivative}}$$

(b) Recovery factor determined from average of fortified control samples run concurrently.

ANALYTICAL DEVELOPMENT CORPORATION

By: Laura J. Servatius
Laura J. Servatius
Research Assistant

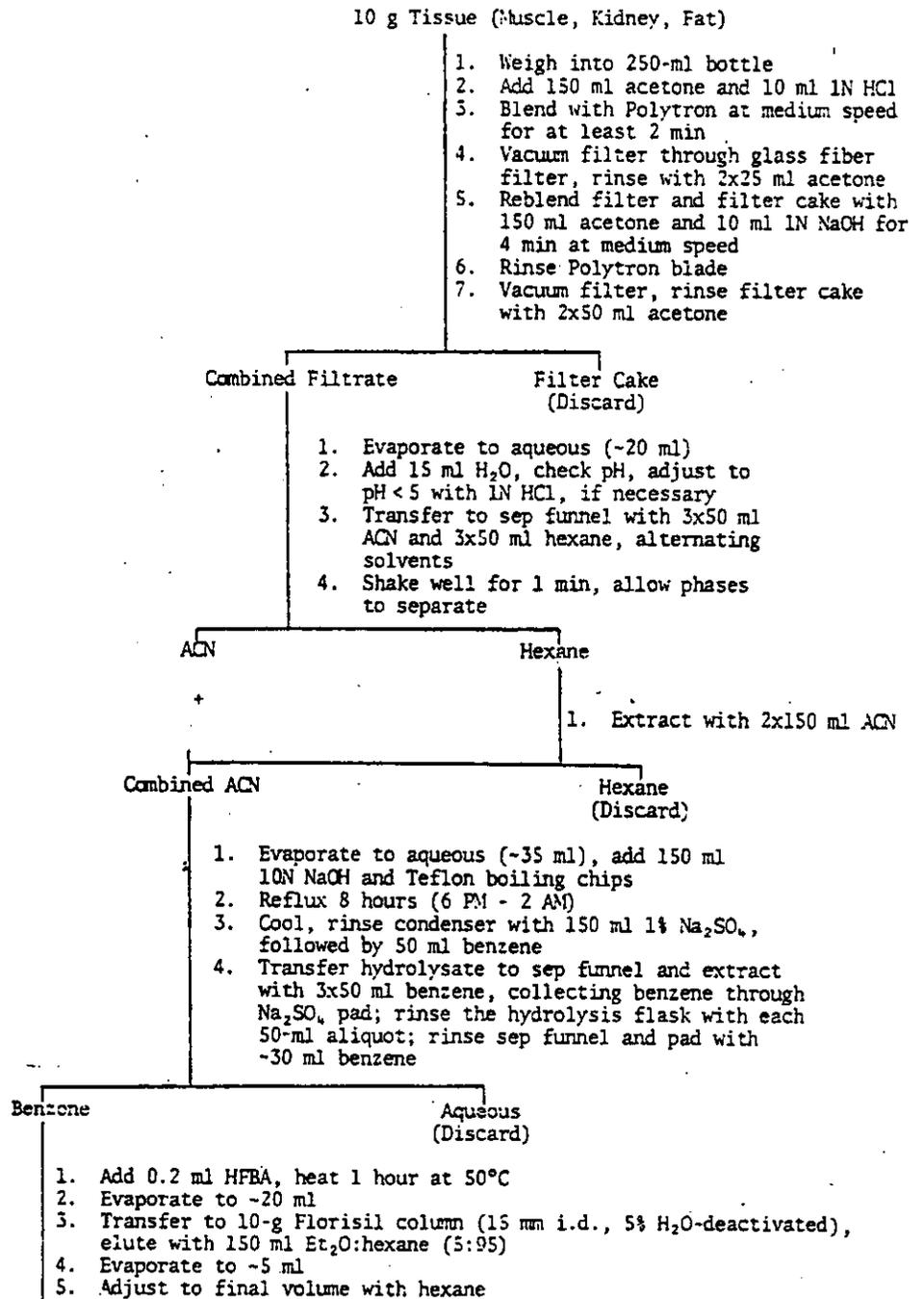
Approved by: Larry D. Craig
Larry D. Craig
Research Scientist

& Laurie C. Wilkes
Laurie C. Wilkes, Ph.D.
Section Manager, R&D

dka

FIGURE 1

ANALYTICAL METHOD FOR DETERMINATION OF IPRADIONE AND ITS
NONHYDROXYLATED METABOLITES IN BOVINE MUSCLE, KIDNEY, AND FAT



GC
6' x 4 mm i.d., 5% Carbowax 20M on Chromosorb W HP
80/100; 215°C Isothermal

FIGURE 2

ANALYTICAL METHOD FOR DETERMINATION OF IPRODIONE AND ITS
NONHYDROXYLATED METABOLITES IN BOVINE LIVER

