

ETHEPHON -03

CORNELL ANALYTICAL LABORATORIES—RESIDUE METHOD SERIES
CORNELL UNIVERSITY
GENEVA, NEW YORK 14456

I. TITLE

Residue Analyses of Ethephon by GC/FPD-Phosphorus Mode. Version: #1

Prepared by: A. Roloson Date: 3/17/93

Verified by: G. Helfman Date: 6/7/93

Commodity(s) in this version: Figs (Freeze Dried)

II. REFERENCES

Detailed Method of Analysis for Residues of (2-chloroethyl) Phosphonic Acid (Ethephon) in a Variety of Sample Types. Rhone-Poulenc Ag Company, SOP-90070, Issue 1.3, May 13, 1990.

III. STANDARDS

A. C.R.M.:

#209 Ethephon, Chem Service, 98.0% pure

B. Stock Solutions:

#209 Ethephon, 1000 µg/mL in MeOH from which the 100 µg/mL, 10 µg/mL and 1.0 µg/mL standards were prepared.

C. Working Standards:

#209 Ethephon was converted to Dimethyl Ethephon by taking 1000 µg/mL and adding diazomethane. After the reaction the solution was brought up to 100 mL for a final concentration of 10.0 µg/mL (as Ethephon). From this solution, working standards G.C. consisting of 1.0, 0.50, 0.20, 0.10, 0.050 and 0.020 µg/mL were created. All dilutions were done with Acetone.

IV. REAGENTS

- 1) MeOH - Fisher - HPLC
- 2) Ethyl Ether - ACS (Redistilled) but not necessary
- 3) Acetone - (Redistilled in-house)
- 4) HCL - Fisher ACS
- 5) Diazomethane -- Prepared in-house
 - a) Diazald - Aldrich
 - b) 2-(2-ethoxyethoxy)ethanol - Aldrich
 - c) Ethyl Ether - Fisher ACS
 - d) H₂O - Nanopure HPLC in-house
 - e) Potassium hydroxide - Bake Adamson

V. EQUIPMENT

- 1) Whatman 22 mM x 80 mM Thimbles - Fisher
- 2) Freeze-dryer — in-house
- 3) 15 mL Polypropylene Centrifuge tubes - VWR
- 4) 100 mL Polypropylene - Nalgene
- 5) 10 - 1 mL Polypropylene pipettes - Nalgene
- 6) Goldfish Fat/Oil Extractor - Labconco
- 7) Glass stoppered graduated cylinder (50 mL)
- 8) 0.5 mL glass pipette
- 9) Table top centrifuge
- 10) Nitrogen blow down w/Heated base.
- 11) Aldrich - Diazald Kit
- 12) 10 μ L syringe

VI. INSTRUMENTATION AND PARAMETERS

- 1) Tracor 222 Gas Chromatograph
- 2) Tracor Flame Photometric Detector, Phosphorus filter
- 3) Column 8% QF - 1 + 3% OV-17 gas chrom Q, 80/100, 6' x 1/8" (#127)
- 4) Spectrum Filter (electronics)
- 5) Fisher chart recorder

Parameters

Oven: 150°C
Inlet: 230°C
FPD: 240°C
Carrier: Nitrogen, Roto 6, Roto 3 (when determining fortifications)
Air: Roto 150
Hydrogen: Roto 100
Input Atten: 10³
Bucking Range: 5
Filter: Gain 1
c.o.f. 0.01
Atten: 4
Chart speed: 1 cm/min.

VII. PROCEDURE

A. Sample preparation and storage:

- 1) Samples were weighed wet, freeze dried, and weighed again before being stored.

B. Extraction:

- 1) Five grams of dry weight were placed in thimble and covered w/cotton.
- 2) Extract w/50 mL of methanol for 4 hours.
- 3) Add 0.5 mL of 10% methanolic HCl to the methanol extract.
- 4) Transfer extract to a 50 mL graduated cylinder, rinse w/MeOH and bring up to 50 mL.

- 5) Transfer 1/10 of Extract (5 mL) to a 15 mL polypropylene graduated centrifuge tube.
- 6) Concentrate to about 1.5 mL, using gentle stream of dry nitrogen in a 30-35°C water bath.
- 7) Add 0.5 mL 10% methanolic HCl.
- 8) Add 8 mL ethyl ether, mix well and let stand for about 10 min., shake again and then centrifuge for about 10 min.
- 9) Decant supernatant to a clean polypropylene graduated centrifuge tube.
- 10) Rinse residue with 2 - 1 mL ethyl ether and add rinsings to extract.
- 11) Concentrate to 1 - 1.2 mL using a gentle stream of dry nitrogen at 30-35°C.
- 12) In a fume hood add about 5 mL diazomethane.
- 13) Cap tightly and let stand for 15 minutes.
- 14) Concentrate to about 2 mL using nitrogen.
- 15) Bring up to 5 mL w/Acetone, ready for G.C.

C. Quantitation: Sample Calculation.

3.8 cm \approx 0.4 μ g/mL (from std. curve)

0.4 μ g/mL x 5 mL Total Volume = 2.0 μ g

2.0 μ g \div 0.5g Aliquot = 4.0 ppm based on Freeze Dry Weight (determined for each sample)

4.0 ppm x 0.8300 (moisture factor) = 3.3 ppm Ethephon based on Wet Weight

VIII. SENSITIVITY

Using at least 3 points a standard curve is prepared based on peak heights. Concentration is calculated in ppm and a moisture factor is used to obtain ppm based on wet weight.

A. Points on Standard Curve:

0.050, 0.10, 0.20, 0.50, 1.0 μ g/mL (as Ethephon)

B. Lowest concentration detected:

0.54 ppm based on wet weight

0.90 ppm based on dry weight

Limit of Detection:

0.50 ppm based on dry weight

Highest concentration detected:

3.8 ppm based on dry weight

3.3 ppm based on wet weight

X. RECOVERIES

A. Spikes: Ave. (Based on dry weight)

20 ppm 88%

2.0 ppm 86%

1.0 ppm 96%

NOTE: Samples were spiked just before the 4 hr extraction with MeOH.

B. Fortifications:	<u>Ave.</u>	
1.0 ppm	82%	
0.20 ppm	100%	Based on 20g sample (wet weight)
0.20 ppm	<0.5 ppm	Based on 10g sample (wet weight) (below limit of detection)

XI. COMMENTS

NOTE:

For the analysis of the 20.0g wet weight fortification, the sample was cut in two portions and ran in 2 thimbles w/50 mL of MeOH in each. The extracts were combined to a total volume of 100 mL. Then 5 mL of this volume makes for a 1/20 aliquot. The sample was then treated the same from this point on as the others.

CORNELL ANALYTICAL LABORATORIES—RESIDUE METHOD SERIES
CORNELL UNIVERSITY
GENEVA, NEW YORK 14456

I. TITLE

Residue Analyses of Ethepon by GC/FPD-Phosphorous Mode. Version: #2

Prepared by: A. Roloson Date: 4/5/93

Verified by: George Helfman Date: 6/10/93

Commodity(s) in this version: Cranberry (Freeze Dried)

II. REFERENCES

Detailed Method of Analysis for Residues of (2-chloroethyl) Phosphonic Acid (Ethepon) in a Variety of Sample Types. Rhone-Poulenc Ag Company, SOP-90070, Issue 1.3, May 13, 1990.

III. STANDARDS

A. CRM:

#209 Ethepon, Chem Service, 98.0% pure

B. Stock Solutions:

#209 Ethepon, Prepared 1/18/93 1000 µg/mL in MeOH from which the 100 µg/mL, 10 µg/mL and 1.0 µg/mL standards were prepared.

C. Working Standards:

#209 Ethepon was converted to Dimethyl Ethepon by taking 1000 µg/mL and adding diazomethane. After the reaction the solution was brought up to 100 mL for a final concentration of 10.0 µg/mL (as Ethepon). From this solution working standards consisting of 1.0, 0.5, 0.2, 0.1, 0.05, 0.02 µg/mL were created. All dilutions were done with Acetone.

IV. REAGENT and MATERIALS

- 1) MeOH - Fisher - HPLC
- 2) Ethyl Ether - ACS (Redistilled) but not necessary
- 3) Acetone - (Redistilled in-house)
- 4) HCL - Fisher ACS
- 5) Diazomethane -- Prepared in-house
 - a) Diazald - Aldrich
 - b) 2-(2-ethoxyethoxy)ethanol - Aldrich
 - c) Ethyl Ether - Fisher ACS
 - d) H₂O - Nanopure HPLC in-house
 - e) Potassium hydroxide - Bake Adamson

V. EQUIPMENT

- 1) Whatman 22 mM x 80 mM Thimbles - Fisher
- 2) Freeze-dryer — in-house
- 3) 15 mL Polypropylene Centrifuge tubes - VWR
- 4) 100 mL Polypropylene - Nalgene
- 5) 10 - 1 mL Polypropylene pipettes - Nalgene
- 6) Goldfish Fat/Oil Extractor - Labconco
- 7) Glass stoppered graduated cylinder (50 mL)
- 8) 0.5 mL glass pipette
- 9) Table top centrifuge
- 10) Nitrogen blow down w/Heated base
- 11) Aldrich - Diazald Kit
- 12) 10 µg/mL syringe

VI. INSTRUMENTATION AND PARAMETERS

- 1) Tracor 222 Gas Chromatograph
- 2) Tracor Flame Photometric Detector, Phosphorus filter
- 3) Column 8% QF - 1 + 3% OV-17 gas chrom Q, 80/100, 6' x 1/8" (#127)
- 4) Spectrum Filter (electronics)
- 5) Fisher chart recorder

Parameters

Oven: 150°C
Inlet: 230°C
FPD: 240°C
Carrier: Nitrogen Roto 3
Air: Roto 150
Hydrogen: Roto 100
Input Atten: 10³
Bucking Range: 5
Filter: Gain 1
c.o.f. 0.01
Atten: 4
Chart speed: 1 cm/min

VII. PROCEDURE

A. Sample preparation and storage:

- 1) Samples were weighed wet, freeze dried, and weighed again before being stored.

B. Extraction:

- 1) One gram of dry weight were placed in thimble and covered w/cotton.
- 2) Extract w/50 mL of methanol for 4 hours.
- 3) Add 0.5 mL of 10% methanolic HCl to the methanol extract.
- 4) Transfer extract to a 50 mL graduated cylinder, rinse w/MeOH and bring up to 50 mL.
- 5) Transfer 1/5 of Extract (10 mL) to a 15 mL P.P. graduated centrifuge tube.

- 6) Concentrate to about 1.5 mL, using gentle stream of dry nitrogen in a 30-35°C water bath.
- 7) Add 0.5 mL 10% methanolic HCl.
- 8) Add 8 mL ethyl ether, mix well and let stand for about 10 min., shake again and then centrifuge for about 10 min.
- 9) Decant supernatant to a clean P.P. graduated centrifuge tube.
- 10) Rinse residue with 2 - 1 mL ethyl ether and add rinsings to extract.
- 11) Concentrate to 1 - 1.2 mL using a gentle stream of dry nitrogen at 30-35°C.
- 12) In a fume hood add about 5 mL diazomethane.
- 13) Cap tightly and let stand for 15 minutes.
- 14) Concentrate to about 2 mL using nitrogen.
- 15) Bring up to 5 mL w/Acetone, ready for G.C.

Quantitation:

Sample Calculations

4.0 cm \approx 0.5 μ g/mL (from std. curve)

0.5 μ g/mL x 5 mL Total Volume = 2.5 μ g

2.5 μ g \div 0.5g Aliquot = 5.0 ppm based on Freeze Dry Weight (determined for each sample)

5.0 x 0.1433 (moisture factor) = 0.72 ppm Ethephon based on Wet Weight

VIII. SENSITIVITY

Using at least 3 points prepare a standard curve based on peak heights. Calculate ppm and use moisture factor to obtain ppm based on wet weight.

A. Points on Standard Curve:
0.020, 0.050, 0.10, 0.20, 0.50 μ g/mL (as Ethephon)

B. Lowest concentration detected:
1.2 ppm based on dry weight
0.15 ppm based on wet weight

Limit of Detection:
0.5 ppm based on dry weight

Highest concentration detected:
6.0 ppm based on dry weight
0.76 ppm based on wet weight

X. RECOVERIES

A. Spikes: (1g)	<u>Ave.</u>	Spikes: (2g)	<u>Ave.</u> (Based on dry weight)
100 ppm	108%	2.0 ppm	90%
10 ppm	92%	1.0 ppm	75%
5.0 ppm	101%		
1.0 ppm	62%		
2.0 ppm	90%		

NOTE: Samples were spiked just before 4 hr extraction with MeOH.

B. Fortifications:	<u>Ave.</u> (Based on a wet weight of 20g)
0.2 ppm	70%
1.0 ppm	72%

XI. COMMENTS

Treatment of storage fortification

The full 20g of wet weight sample of cranberry which was about 2.5g dry weight was placed in a thimble. Extraction took place for 4 hr. with 50 mL of MeOH after which 1/5 was removed for analysis. The aliquot was then treated the same as all other samples.

CORNELL ANALYTICAL LABORATORIES—RESIDUE METHOD SERIES
CORNELL UNIVERSITY
GENEVA, NEW YORK 14456

I. TITLE

Residue Analyses of Ethephon by GC/FPD-Phosphorus Mode. Version: #3

Prepared by: A. Roloson Date: 4/6/93

Verified by: G. Helfman Date: 6/16/93

Commodity(s) in this version: Peach (Freeze Dried)

II. REFERENCES

Detailed Method of Analysis for Residues of (2-chloroethyl) Phosphonic Acid (Ethephon) in a Variety of Sample Types. Rhone-Poulenc Ag Company, SOP-90070, Issue 1.3, May 13, 1990.

III. STANDARDS

A. C.R.M.:

#209 Ethephon, Chem Service, 98.0% pure

B. Stock Solutions:

#209 Ethephon, 1000 µg/mL in MeOH from which the 100 µg/mL, 10 µg/mL and 1.0 µg/mL standards were prepared.

C. Working Standards:

#209 Ethephon was converted to Dimethyl Ethephon by taking 1000 µg/mL and adding diazomethane. After the reaction the solution was brought up to 100 mL for a final concentration of 10.0 µg/mL (as Ethephon). From this solution the working standards consisting of 1.0, 0.5, 0.2, 0.1, 0.02 µg/mL were created. All dilutions were done with Acetone.

IV. REAGENTS

- 1) MeOH - Fisher - HPLC
- 2) Ethyl Ether - ACS (Redistilled) but not necessary
- 3) Acetone - (Redistilled in-house)
- 4) HCL - Fisher ACS
- 5) Diazomethane -- Prepared in-house
 - a) Diazald - Aldrich
 - b) 2-(2-ethoxyethoxy)ethanol - Aldrich
 - c) Ethyl Ether - Fisher ACS
 - d) H₂O - Nanopure HPLC in-house
 - e) Potassium hydroxide - Bake Adamson

V. EQUIPMENT

- 1) Whatman 22 mM x 80 mM Thimbles - Fisher
- 2) Freeze-dryer — in-house
- 3) 15 mL Polypropylene Centrifuge tubes - VWR
- 4) 100 mL Polypropylene - Nalgene
- 5) 10 - 1 mL Polypropylene pipettes - Nalgene
- 6) Goldfish Fat/Oil Extractor - Labconco
- 7) Glass stoppered graduated cylinder (50 mL)
- 8) 0.5 mL glass pipette
- 9) Table top centrifuge
- 10) Nitrogen blow down w/Heated base.
- 11) Aldrich - Diazald Kit
- 12) 10 μ L syringe

VI. INSTRUMENTATION AND PARAMETERS

- 1) Tracor 222 Gas Chromatograph
- 2) Tracor Flame Photometric Detector, Phosphorus filter
- 3) Column 8% QF - 1 + 3% OV-17 gas chrom Q, 80/100, 6' x 1/8" (#127)
- 4) Spectrum Filter (electronics)
- 5) Fisher chart recorder

Parameters

Oven: 150°C
Inlet: 230°C
FPD: 240°C
Carrier: Nitrogen Roto 3
Air: Roto 150
Hydrogen: Roto 100
Input Atten: 10³
Bucking Range: 5
Filter: Gain 1
c.o.f. 0.01
Attn: 4
Chart Speed: 1 cM/min.

VII. PROCEDURE

A. Sample preparation and storage:

- 1) Samples were weighed wet, freeze dried, and weighed again before being stored.

B. Extraction:

- 1) One gram of dry weight were placed in thimble and covered w/cotton.
- 2) Extract w/50 mL of methanol for 4 hours.
- 3) Add 0.5 mL of 10% methanolic HCl to the methanol extract.
- 4) Transfer extract to a 50 mL graduated cylinder, rinse w/MeOH and bring up to 50 mL.
- 5) Transfer 1/5 of Extract (10 mL) to a 15 mL P.P. graduated centrifuge tube.

- 6) Concentrate to about 1.5 mL, using gentle stream of dry nitrogen in a 30-35°C water bath.
- 7) Add 0.5 mL 10% methanolic HCl.
- 8) Add 8 mL ethyl ether, mix well and let stand for about 10 min., shake again and then centrifuge for about 10 min.
- 9) Decant supernatant to a clean P.P. graduated centrifuge tube.
- 10) Rinse residue with 2 - 1 mL ethyl ether and add rinsings to extract.
- 11) Concentrate to 1 - 1.2 mL using a gentle stream of dry nitrogen at 30-35°C.
- 12) In a fume hood add about 5 mL diazomethane.
- 13) Cap tightly and let stand for 15 minutes.
- 14) Concentrate to about 2 mL using nitrogen.
- 15) Bring up to 5 mL w/Acetone, ready for G.C.

C. Quantitation: Sample Calculation.

3.8 cm \approx 0.4 μ g/mL (from std. curve)
 0.4 μ g/mL x 5 mL Total Volume = 2.0 μ g
 2.0 μ g \div 0.2g Aliquot = 10.0 ppm based on Freeze Dry Weight (determined for each sample)
 10.0 ppm x 0.8300 (moisture factor) = 8.3 ppm Ethephon based on Wet Weight

VIII. SENSITIVITY

Using at least 3 points prepare a standard curve based on peak heights. Calculate ppm and use moisture factor to obtain ppm based on wet weight.

A. Points on Standard Curve:
 0.020, 0.10, 0.20, 0.50 mL (as Ethephon)

B. Lowest concentration detected:
 All below 1.0 ppm dry weight

Limit of Detection:
 1.0 ppm based on dry weight

Highest concentration detected:
 All below 1.0 ppm dry weight

X. RECOVERIES

A. Spikes:	<u>Ave.</u>	
5.0 ppm	89%	
2.0 ppm	82%	Based on 1 gram dry weight
10 ppm	77%	
2.5 ppm	60%	Based on 2 gram dry weight

NOTE: Samples were spiked just before the 4 hr extraction with MeOH.

B. Fortifications: (Based on a wet weight of 20g)

1.0 ppm	55%
0.20 ppm	60%
(below detection)	

XI. COMMENTS

Treatment of storage fortification

The full 20g of wet weight sample of peach which was about 2.5g dry weight was placed in a thimble. Extraction took place for 4 hr. with 50 mL of MeOH after which 1/5 was removed for analysis. The aliquot was then treated the same as all other samples.