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


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) Goldfisch 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 μL 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 Attenuation: 10^3
- Bucking Range: 5
- Filter: Gain 1, c.o.f. 0.01
- Attenuation: 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 = 0.4 µg/mL (from std. curve)
   0.4 µg/mL x 5 mL Total Volume = 2.0 µg
   2.0 µg ÷ 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 µg/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: | Ave. |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>1.0 ppm</td>
<td>82%</td>
</tr>
<tr>
<td>0.20 ppm</td>
<td>100%</td>
</tr>
<tr>
<td>Based on 20g sample (wet weight)</td>
<td></td>
</tr>
<tr>
<td>0.20 ppm</td>
<td>&lt;0.5 ppm</td>
</tr>
<tr>
<td>Based on 10g sample (wet weight)</td>
<td></td>
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<tr>
<td>(below limit of detection)</td>
<td></td>
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</tbody>
</table>

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.
I. **TITLE**

Residue Analyses of Ethephon 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**


III. **STANDARDS**

A. CRM:
   #209 Ethephon, Chem Service, 98.0% pure

B. Stock Solutions:
   #209 Ethephon, 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 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 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 = 0.5 μg/mL (from std. curve)
0.5 μg/mL x 5 mL Total Volume = 2.5 μg
2.5 μg + 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 μg/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)     Ave.     Spikes: (2g)     Ave. (Based on dry weight)
   100 ppm         108%     2.0 ppm         90%
   10 ppm          92%      1.0 ppm         75%
   5.0 ppm         101%     0.5 ppm         70%
   1.0 ppm          62%     0.2 ppm         40%
   2.0 ppm          90%

NOTE: Samples were spiked just before 4 hr extraction with MeOH.
B. Fortifications: *Ave.* (Based on a wet weight of 20g)

<table>
<thead>
<tr>
<th>ppm</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>70%</td>
</tr>
<tr>
<td>1.0</td>
<td>72%</td>
</tr>
</tbody>
</table>

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.
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**


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

(p1) Ethephon by GC/FPD-P    Version  #3
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) Goldfisch 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 µL syringe

VI. INSTRUMENTATION AND PARAMETERS

1) Tracer 222 Gas Chromatograph
2) Tracer 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: $10^3$
- 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/MethOH 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 = 0.4 µg/mL (from std. curve)
0.4 µg/mL x 5 mL Total Volume = 2.0 µg
2.0 µg + 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:

<table>
<thead>
<tr>
<th>PPM (as Ethephon)</th>
<th>Ave.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 ppm</td>
<td>89%</td>
<td></td>
</tr>
<tr>
<td>2.0 ppm</td>
<td>82%</td>
<td></td>
</tr>
<tr>
<td>10 ppm</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>2.5 ppm</td>
<td>60%</td>
<td></td>
</tr>
</tbody>
</table>

Based on 1 gram dry weight

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)

<table>
<thead>
<tr>
<th>PPM</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 ppm</td>
<td>55%</td>
</tr>
<tr>
<td>0.20 ppm</td>
<td>60%</td>
</tr>
</tbody>
</table>

(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.