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Residue Analytical Method
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**General Method for the Analysis of
Ethephon Residues in a
Variety of Substrates**

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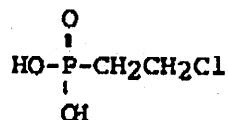
General Method for the Analysis of Ethephon Residues in a Variety of Substrates

Scope

Applications of ethephon crop growth regulator to agricultural crops may result in residues in/on harvested crops. Metabolism studies have shown that parent ethephon is the only residue of biological concern. In this analytical method, ethephon is degraded to ethylene which is then quantified.

Common Name: Ethephon

Structure:



Chemical Name: (2-Chloroethyl) Phosphonic Acid

Molecular Weight: 144.49

CAS Registry Number: 16672-87-0

Summary

Indigenous ethylene is removed from the sample while any ethephon is protected in an acidic medium of aqueous tartaric acid. The solution is then made basic through the addition of trisodium phosphate to decompose any ethephon to yield ethylene. The derived ethylene is then quantified by gas chromatography. All reactions are carried out in sealed glass pressure bottles with provision for syringe sampling of the headspace.

Equipment

Pressure reaction bottles. 250 mL. Lab Glass, Inc., Vineland, NJ, Cat. No. LG-3921, or equivalent (See Note 1).

Crown caps with neoprene gaskets, Lab Glass, Inc., Vineland, NJ, Cat. No. LG-3922, or equivalent. These metal caps have two small holes to allow sampling by piercing the gasket with a syringe needle. Gaskets may be used repeatedly if 23 gauge needles are used.

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Bottle capper to apply crown caps.

Water bath capable of maintaining a temperature of 60° to 65 °C, and holding a number of 250 mL pressure bottles.

Reciprocating shaker, Atlab Shaker, Thomas Scientific or equivalent.

Hypodermic needles, -23 gauge.

Gas-tight syringe capable of delivering a two mL gas chromatographic sample.

Polyethylene or polypropylene pipets, 1 mL graduated, 5 mL graduated, and 10 mL.

Polyethylene or polypropylene volumetric flasks.

Gas chromatograph equipped with a flame detector.

Reagents

Water, distilled or otherwise purified

Tartaric acid, Fisher A-314, or equivalent

Igepal® CA-720, Rhône-Poulenc, Cranbury, NJ, or an equivalent ethoxylated octylphenol surfactant, CAS: 9002-93-1.

Stabilizing solution - Tartaric acid, 0.5M, plus 0.4% Igepal® CA-720; dissolve 75 grams of tartaric acid in water, add four grams of Igepal® CA-720, and dilute to one liter.

Sodium phosphate, tribasic, dodecahydrate, EM Science SX0730-2, or equivalent (See Note 2).

Hydrochloric acid, Mallinckrodt 2612-1, or equivalent.

10% aqueous hydrochloric acid, dilute 10 mL of concentrated acid to 100 mL with water.

Standard Ethephon Solution:

Weigh 100.0 to 102.0 mg of ethephon (corrected for purity) into a 100 mL polyethylene or polypropylene volumetric flask, dissolve in

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purified water, add about one-half mL of 10% aqueous HCl, and dilute to volume. Then add one mL of water for each mg of ethephon in excess of 100 mg weighed into the flask. Thus, if 101.6 mg of ethephon was weighed, add an additional 1.6 mL of water to the flask to give a total of 101.6 mL of solution. This solution now contains 1 mg of ethephon per mL.

Make three serial 10 mL to 100 mL dilutions in purified water (add about 0.5 mL of 10% aqueous HCl to each) to give solutions containing 0.1 mg/mL, 0.01 mg/mL and 0.001 mg/mL. Other dilutions may be made as required, but about 0.5 mL of 10% aqueous HCl should be added per 100 mL of solution to protect the ethephon from degradation. These solutions can be used for spiking and for the preparation of GC standards.

Other weighings and dilutions may be made and recorded. Solutions will be stored in a refrigerator at all times when not in use. Solutions should be allowed to warm to room temperature prior to use.

As an example of spiking, 0.5 mL of the 0.01 mg/mL solution (5 µg of ethephon) added to 25 grams of sample will give a 0.20 ppm spike which is recommended in normal circumstances for recovery data. Other spiking levels may be used.

Use polyethylene or polypropylene pipets and flasks for all dilutions and transfers (See Note 3).

Standards for Gas Chromatography

Since this procedure uses headspace samples for GC analysis, fresh standards should be prepared with each batch of samples analyzed. Preparation is similar to that of the samples except that the crop material is replaced by an equal volume of water, and the preliminary cleanup is not required. Note that standards are prepared by the alkaline degradation of known amounts of ethephon, and are prepared on a parts-per-million basis. A 0.20 ppm standard for use with 25 grams of crop samples is prepared as follows:

1. Weigh 25 grams of the crop substrate being analyzed into a 250 mL graduated mixing cylinder, add 100 mL of water, mix well and allow the water to soak into the crop substrate if necessary (dry crops such as straw may require a short waiting time). Swirl to wash substrate from the wall of the cylinder if necessary. Read the volume of substrate plus water and subtract 100 mLs to give the volume of 25 grams of substrate.

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For more dense crops, a 100 mL mixing cylinder and 50 mL of water may be used. An organic solvent may be used in place of water in the volume determination step if the crop resists wetting by water.

2. Pipet 0.5 mL of 0.01 mg/mL ethephon into a clean pressure bottle, and add any water necessary to account for bottle volume variations, as described in Note 1, plus a volume of water equal to that of 25 grams of crop substrate as determined above.
3. Add 100 mL (125 mL if cottonseed - see Step 4 in Procedure) of tartaric acid stabilizing solution, and proceed as in Step 11 of the analytical Procedure (See Note 4).

Standards should be prepared in several concentrations to bracket the levels expected to be found in the samples. Also, standards should be prepared in duplicate or in sufficient number to demonstrate that adequate cap seals have been obtained.

Procedure

1. Grind or prepare the crop substrate for analysis as required by the appropriate SOP.
2. Weigh the appropriate amount of substrate (Table 1) into a clean (See Note 5) 250 mL pressure bottle. Some substrates listed in Table 1 show a limit of quantification above 0.02 ppm. This is because some substrates contain indigenous matter that decomposes in mild base to give ethylene.
3. Add water to pressure bottles to adjust for bottle volume variations (See Note 1).
4. Add 100 mL (125 mL for cottonseed) of tartaric acid stabilizing solution, and cap bottle.
5. Place bottle in 60 to 65 °C water bath for about one hour. Shake bottles well every 10 to 15 minutes during this time. If numerous samples are being analyzed at one time and shaker availability is limited (see Steps 7 and 9), the time may be increased a bit. The time increase will normally not exceed five to ten minutes.
6. While bottle contents are hot, release pressure in bottle by inserting a -23 gauge hypodermic needle through the cap. The bottles may be stood up in the water bath for this step.

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7. As soon as pressure has been released, remove needle and shake samples on a mechanical reciprocating shaker for 5 minutes.
8. Return sample bottle to the heated water bath for an additional 30 minutes. Shake the bottles about every 10 minutes. Again, if numerous samples are being analyzed at one time and shaker availability is limited (see Steps 7 and 9), the time may be increased.
9. Shake hot sample on a reciprocating shaker for 5 minutes.
10. Remove cap, flush bottle with a gentle stream of nitrogen for about five seconds to remove any residual ethylene, and cool to room temperature.
11. Add 30 grams of tribasic sodium phosphate, cap bottle immediately, and place in 60 to 65 °C water bath for one hour. Shake samples well every 10 to 15 minutes. Previously prepared standards enter the analysis procedure at this point. Again, if numerous samples are being analyzed at one time and shaker availability is limited, this time may be increased.
12. Remove samples (and standards) from water bath, and shake hot samples (and standards) on a flat bed reciprocating shaker for 5 minutes.
13. Cool samples and standards to room temperature (overnight is recommended to allow for fairly complete equilibration).
14. Analyze a 2.0 mL portion (See Note 6) of headspace gas on a chromatograph equipped with a flame detector. Operating parameters, which may be adjusted as necessary for the equipment being used, in our laboratory are as follows:

Instrument	Varian 3740
Column	6' x 2 mm id glass column packed with 80/100 mesh Porapak® T
Column Temperature	45 to 50 °C
Injector Temperature	50 °C
Detector Temperature	150 °C
Carrier Gas	Helium -35 mL/ min.
Hydrogen	-30 mL/min.
Air	-220 mL/min.

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With many samples, the chromatogram normally contains only the air peak upset and the ethylene peak. The chromatograms of some substrates contain an ethane peak which is very close after the ethylene peak, and a few contain a peak which, judging from the retention time of about 12 minutes, is very likely to be propane. The presence of this peak in the chromatogram increases the time of each gas chromatographic run well above the usual four minutes. If possible, parameters should be such that a 0.01 ppm standard will give an easily measured peak at an attenuation with low noise. This will allow the determination of very low residues. The attenuation may be increased as necessary for greater concentrations of analyte. Ethylene retention time should be one to two minutes.

15. Quantify the sample concentration of ethephon by comparing peak area or height of standards of known concentration. A very acceptable method is using least squares regression of the response versus the amount injected as illustrated.

$$\text{Height (or Area)} = \text{Intercept} + \text{Slope} \times \text{ppm}$$

$$(y = b + mx)$$

This rearranges to:

$$\text{ppm found} = \frac{\text{Height (or Area)} - \text{Intercept}}{\text{Slope}}$$

An example calculation is as follows:

Peak Size (can be area or height) from sample
= 6237 integrator units
Intercept = 87.4 (calculated from standards)
Slope = 29440.4 (calculated from standards)

Then:

$$\text{ppm} = \frac{6237 - 87.4}{29440.4}$$

$$\text{ppm} = 0.209$$

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Spike recoveries are calculated according to the following formula:

$$r = \frac{pf - pc}{pr} \times 100$$

Where:

r = percent recovery
pf = ppm found in spiked sample
pc = ppm found in unspiked sample
pr = ppm of fortification

Net Recovery = ppm Found - ppm in Unspiked Control

$$\text{Percent Recovery} = \frac{\text{Net Recovery}}{\text{Spike Level}} \times 100$$

Quantification using recorded integrator data is recommended if peak areas are used since minor variations between injections are overcome in these area measurements. Variations in peak heights due to injection variations tend to be magnified when retention times are short, and ethylene retention time is less than two minutes in these analyses.

If residues cover a very wide range, it might be advisable to use two calibration curves. One curve for the lower concentrations, and a second for higher concentrations. When a single curve covers a large range of concentrations, the response from the higher concentration standards tends to dominate the slope and intercept. This could give rise to serious error when samples with low residues are being analyzed.

Notes

1. The advertised volume of the pressure bottles used in the development of this method was 250 mL. Measurements, however, showed the volumes to extend over a wide range. Since the samples injected into the chromatograph are drawn from the headspace volume after the addition of substrate and reagents, this variation is substantial when compared to headspace volume available. This is overcome by adding sufficient water to each bottle so that the volume available for the analytical procedure is equal in all bottles. That is, 17 mL of water would be added to a bottle with a total volume of 267 mL, etc., after the addition of the sample and/or standard. This 'calibration' volume should be adjusted for the volume of ethephon standard solution added when spiked samples or calibration standards are

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prepared. Accurate analysis demands that the headspace volumes of all samples and standards be essentially equal.

2. A very granular trisodium phosphate is recommended since dry reagent is added to the pressure bottle. Powders tend to be difficult to transfer rapidly.
3. In the previous development of methodology to determine residues of ethephon in treated crops, it was demonstrated that used glassware (presumably because it has been etched by repeated cleaning in an alkaline detergent) adsorbs ethephon very strongly in absence of crop material. It was also demonstrated that new glassware, which has not been so etched, and polyethylene/polypropylene labware do not adsorb ethephon. Even though it is unlikely that ethephon in water solutions would adsorb onto glassware at the concentrations of these standards, it has not been so proven. Therefore it is recommended that polyethylene/polypropylene labware be used to prepare standards and fortify samples.
4. Tartaric acid is added to the standards even though the rest of the cleanup procedure is omitted so that the liquid phases of both samples and standards, and ethylene solubility therein, will be more nearly equal.
5. The bottles should be completely dry as far as any light organic solvents are concerned. Even traces of light organic solvents such as methanol that may be present from glassware rinsing will cause chromatographic problems as they elute from the GC column in extremely broad peaks. If solvents are used in glassware cleaning, the bottles may be placed in an oven at about 100 °C until dry.
6. Smaller portions of headspace may be analyzed if very large ethephon residues are found which are beyond the linear range of the system used. Also, smaller aliquots of crop substrate may be used in the sample workup in the unlikely event that large residues are found and the sample has to be reanalyzed. If smaller sample aliquots are used, appropriate adjustments must be made in the preparation of standards.

Method Recovery

Net spike recoveries, the ppm found in the analysis of a spiked sample less the ppm found in the analysis of the same unspiked substrate, should range from 70% to 120% of the added spike level.

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.Safety

Appropriate Material Safety Data Sheets should be available. Normal laboratory procedures should be used when handling the standards and solutions. Sealed pressure bottles are heated in this analysis, so care must be taken to assure that the bottles show no cracks which might lead to failure under pressure. Also, when the internal pressure of the bottles is released in Step 6, be sure that the needle is pointing away from the operator and away from any personnel in the area.

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Table 1
Recommended Sample Weights

Substrate weights along with minimum detectable levels (MDL) and limits of quantification (LOQ) that have been determined are as follows:

Substrate	Sample Weight	MDL ppm	LOQ ppm
Apples	25 grams	0.01	0.02
Barley Grain	25 grams	0.08	0.16
Barley Straw	10 grams ¹	0.3	0.8
Blackberries	25 grams ²	0.02	0.02
Cantaloupe	25 grams	0.01	0.02
Cherries	25 grams	0.01	0.02
Cottonseed	10 grams ³	0.05	0.07
Cucumbers	25 grams	0.01	0.02
Grapes	25 grams	0.01	0.02
Macadamia Nuts	25 grams	0.01	0.02
Peppers	25 grams ²	0.02	0.03
Pineapple	25 grams	0.01	0.02
Tobacco	5 grams ⁴	0.2	0.3
Tomatoes	25 grams	0.01	0.02
Sugarcane	25 grams	0.01	0.02
Walnuts	25 grams	0.01	0.02
Wheat Grain	25 grams	0.06	0.11
Wheat Straw	10 grams ¹	0.5	1.0

¹Ten gram aliquots are required for straw samples because of the large volume occupied by the straw. If larger aliquots are used, there is insufficient room in the pressure bottle to allow for adequate mixing.

²Blackberries and peppers have large established tolerance levels (30 ppm). Sample size may be decreased to 5 grams to prevent detector overloading during chromatography when high residues are suspected.

³Cottonseed requires a maximum sample size of 10 grams. If larger sample aliquots are used, the resulting sample in the pressure bottle is too thick to allow for adequate mixing and ethylene evolution. Also, a 125 mL aliquot of the tartaric acid mixture is required to give a solution that will allow mixing and ethylene evolution.

⁴Treated tobacco has shown very ethephon residues so only a 5 gram sample aliquot has been used.