

TITLE

Determination of dimethoate and omethoate residues in bare and cropped soil by gas chromatography.

METHOD: 135-005-3

EFFECTIVE DATE OF ORIGINAL METHOD: April 29, 1990
FIRST REVISION DATE: August 7, 1990
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SUMMARY

Dimethoate, omethoate are extracted from soil with aqueous acetone. After concentration on the rotary evaporator the dimethoate and omethoate residues are suspended in acetone. The dimethoate and omethoate residues are analyzed using a gas chromatograph equipped with a flame photometric detector operating in the phosphorus mode. Results are calculated by the direct comparison of peak areas for samples to those of standards.

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I. MATERIALS**A. Instruments**

Balance, Analytical - American Scientific Products
Balance, Top Loading - Sartorius (or equivalent)
Balance, Top Loading - Toledo (or equivalent)
Perkin-Elmer Series 8500 Gas Chromatograph equipped
with a Flame Photometric Detector and AS8300
Autosampler
Column: DB1 25 m, 0.53 mm id, 1.0 um film thickness
- Perkin-Elmer Catalog No. 009-23-64
Multichrom Data Collection Equipment and Software - VG
Instruments
Waterpro Work Station - Labconco

B. Equipment

Beakers
Class A Glassware
Filtering Flasks, 500 mL
Flat Bottom Flasks, 500 mL
Evaporation Flasks, 125 mL and 150 mL
Glass Funnels
Glass Wool, Silane-treated
Graduated Cylinders
Separatory Funnels, 125 mL
Soil Crusher (or equivalent)
Rotary Evaporator
Waring Blenders (or equivalent)
Whatman GF4 Glass-fiber Filter Paper

C. Reagents and Standards**1. Reagents**

Acetone, Optima grade - Fisher Catalog No. A929-4
Water, Type 1
Water, HPLC - Fisher Catalog No. W5-4
Sodium Chloride (crystal), Certified A.C.S. -
Fisher Catalog No. S271-1
Chloroform, Optima grade, Fisher Catalog No. C297-4
Carbowax 400 - Alltech Stock No. 5051

2. Standards

Dimethoate, 99.5% purity, Batch 881010 - Cheminova
Dimethoxon, 98% purity, Batch AC4870-59A - American
Cyanamid

D. Solution Preparation

1. 95/5 (v/v) acetone/water - For every liter prepared, use a graduated cylinder to add 50 mL of Type 1 water to 950 mL of acetone. Cap and mix well.
2. 20% Sodium chloride - For every liter prepared, add 200 (\pm 1)g of sodium chloride to a 1 liter volumetric flask. Bring to volume with Type 1 water.
3. 10% Carbowax 400 - For every 100 mL prepared, add 10 mL of Carbowax 400 to a 100 mL volumetric flask and bring to volume with Type 1 water.

II. STANDARD PREPARATION

Use Class A volumetric glassware for all standard preparation(s).

A. Dimethoate

1. Dimethoate with an approximate concentration of 1000 ug/mL - For every 100 mL prepared add 100 (\pm 2) mg of Dimethoate to a 100 mL volumetric flask. Bring to volume with acetone. Refer to the standard preparation sheet for actual solution concentration.
2. Dimethoate with an approximate concentration of 100 ug/mL, 10 ug/mL, 1 ug/mL, 0.1 ug/mL - Perform a 1:10 serial dilution, or other dilutions as noted in the data, of the 1000 ug/mL standard. The diluent for each standard is acetone. Refer to the standard preparation sheet(s) for actual solution concentration.

B. Omethoate

1. Omethoate with an approximate concentration of 1000 ug/mL - For every 100 mL prepared add 100 (\pm 2) mg of Dimethoxon to a 100 mL volumetric flask. Bring to volume with acetone. Refer to the standard preparation sheet for actual solution concentration.
2. Omethoate with an approximate concentration of 100 ug/mL, 10 ug/mL, 1 ug/mL, 0.1 ug/mL - Perform a 1:10 serial dilution, or other dilutions as noted in the data, of the 1000 ug/mL standard. The diluent for each standard is acetone. Refer to the standard preparation sheet(s) for actual solution concentration.

III. METHOD**A. Sample Identification**

Using the sample code presented in attachment A, all samples will be identified with a unique EPL-BAS sample identification before sample extraction can begin. To

facilitate sample preparation the samples may be ground, as noted in 8.1-3, using the Sponsor identification noted on the sample.

B. Sample Preparation

If necessary, when a group of samples is to be prepared the following order of grinding should be followed, if possible:

1. For every replicate, grind all control samples before any treated samples. Also grind the lowest depths before proceeding to the higher depths. This will minimize any possibility of contamination between samples. The grinding apparatus should be cleaned, as described in the operator's manual, between replicates.
2. Remove the soil from the core. Obtain the total weight of the sample by placing the entire sample onto a balance. Record the weight of the entire sample.
3. Grind sample along with dry ice using a soil crusher (or equivalent). Transfer the sample to a container labelled with the EPL-BAS sample identification and/or the Sponsor code of the sample, and place in the appropriate freezer for storage. Allow dry ice to sublimate before using the soils for analysis.

C. Moisture Determination

This section applies only to soil samples and does not include lab spiked samples, field spiked samples, or freezer stability samples.

1. Record the weight of an aluminum weigh boat. (Do not tare). Add 5.0 (\pm 0.1) g of the ground soil to the weigh boat, record the weight. Place the weigh boat in a 120 (\pm 5) $^{\circ}$ C oven overnight.
2. Remove the samples from the oven and allow to cool. Record the weight of the aluminum weigh boat plus the dried soil.

D. Extraction

This section refers to soil, filter paper, and irrigation/dilution water samples analyzed for Dimethoate and Omethoate, and does not apply to tank mix or formulated product samples.

1. Weigh 25.0 (\pm 0.05) g of sample in to a tared container (for example a beaker, weigh boat, or bottle). If samples are not extracted on the day of weighing, return the samples to the freezer until extraction can be started.

NOTE: filter paper, water samples and tank mixes will not be weighed. Filter papers will be extracted as stated in steps 2 and 4 through 6. Water samples and tank mixes will be dilutions and will be recorded on sample dilution sheets.

2. Transfer the sample to a blender jar.
3. Prepare fortification samples by pipeting the appropriate standard solution onto the designated samples. Approximate fortification levels should include 0.01, 0.10, and/or 0.50 ppm.
4. Using a graduated cylinder, add 250 mL of 95/5 acetone/water solution to the blender jar and blend for approximately 3-5 minutes.

Note: For filter paper extractions rinse the petrie dish with the 250 mL of 95/5 acetone/water adding all rinses to the blender jar along with the filter paper.

5. Vacuum filter the slurry through a glass-fiber filter paper and collect the filtrate. Discard the pellet.
6. Using a graduated cylinder measure 200 mL of the filtrate and transfer it to a 500 mL flat bottom flask. Rotovap the samples to approximately 25 mL at 30-35°C.

E. Chloroform Partition

1. Transfer the sample to a 125 mL separatory funnel. Using a graduated cylinder or a repeater pipet, add 25 mL of a 20% sodium chloride solution to the rotovap flask, swirl, and add to the sample in the separatory funnel.
2. Using a graduated cylinder or a repeater pipet, add 50 mL of chloroform to the sample, shake briefly and allow the layers to separate.
3. Filter the lower (organic) layer through a glass funnel, fitted with a small plug of glass wool and topped with about 2 inches of anhydrous sodium sulfate, into an evaporation flask.
4. Add another 50 mL of chloroform to the separatory funnel, shake briefly, and filter as described in step 3 above. Collect into the same flask. Rinse the sodium sulfate with about 10 mL of chloroform and collect in the same flask. Discard the aqueous phase.
5. Add about 6 drops of 10% Carbowax 400 solution and rotary evaporate the organic phase to just dryness at 30 - 35°C.

Note: The temperature of the evaporation flask must not exceed 35°C and should not remain on the rotary evaporator after the solvent been removed.

6. Using a Class A volumetric pipet, add 2.0 mL of acetone to the rotovap flask. Swirl the acetone around the flask and quantitatively transfer the acetone to a culture tube. Store refrigerated until analysis can be performed.

F. Extraction for o-desmethyl dimethoate.

Steps for extraction of o-desmethyl dimethoate will be described once a method for this extraction has been validated.

G. Chromatography

1. Gas Chromatograph

Perkin-Elmer 8500 gas chromatograph equipped with a Flame Photometric Detector operating in the phosphorus mode, Perkin-Elmer AS 8300 autosampler. The injection type is flash vaporization. The volume injected is 5 µL.
Column: DB1 25 m, 0.53 mm id, 1.0 µm film thickness
- Perkin-Elmer Catalog No. 009-23-64

Recommended Operating Conditions for GC analysis

Column temperature	165°C hold 4 min then increase at 20°C per min to 300°C then hold for 2 minutes
Injector temperature	250°C
Detector temperature	350°C
Carrier gas (Helium)	20 mL/min

Analysis data will be collected using Multichrom, a computer program designed for data collection.

H. Freezer Stability

For each analyte and at each time frame, two freezer stability samples will be fortified. One will be fortified at approximately 0.05 ppm and the second fortified at about 0.1 ppm. Control samples which will be analyzed as controls and which will also be used for lab spike preparation will also be set up for freezer stability. This is equivalent to 20 samples per time frame.

1. Remove an aliquot of the 0-6", 6-12", 12-18", 18-24",

24-36" and 36-48" soil samples from the control day -1 interval for bareground and set these aliquots aside for sample analysis.

2. Composite the remaining soil. Use this soil to prepare the freezer stability samples.

3. Weigh 25.0 (\pm 0.05) g of sample in to a tared bottle.

4. Spike appropriate samples as follows:

0.05 ppm Dimethoate - Use a class A volumetric pipet to add 2.0 mL of 0.6972 ug/mL Dimethoate standard to the sample. Allow the solvent to evaporate then cap and shake sample briefly. Store frozen.

0.05 ppm Omethoate - Use a class A volumetric pipet to add 2.0 mL of 0.7000 ug/mL Omethoate standard to the sample. Allow the solvent to evaporate then cap and shake sample briefly. Store frozen.

0.10 ppm Dimethoate - Use a class A volumetric pipet to add 2.0 mL of 1.295 ug/mL Dimethoate standard to the sample. Allow the solvent to evaporate then cap and shake sample briefly. Store frozen.

0.10 ppm Omethoate - Use a class A volumetric pipet to add 2.0 mL of 1.300 ug/mL Omethoate standard to the sample. Allow the solvent to evaporate then cap and shake briefly. Store frozen.

5. At the appropriate time intervals remove the corresponding freezer stability samples and extract as described in Section D. Extraction. For each analysis set prepare two lab spikes for each analyte, one at about 0.05 ppm and the second at about 0.10 ppm.

I. Tank Mix and Formulated Product Analysis

1. Bring the formulated product and tank mix samples to room temperature.
2. While the suspension is being stirred, transfer a 100 μ L aliquot of the tank mix sample to a 500 mL volumetric flask. Bring to volume with acetone. Prepare this dilution in duplicate. Alternate dilutions may be required based on analytical results.
3. For formulated product analyses: weigh the formulated product into a volumetric flask and bring to volume with acetone. Prepare in duplicate. If the resulting solution concentrations are within the analytical standard range (0.08 to 7.9 ug/mL), no further dilutions are required. Otherwise dilute the samples appropriately using acetone as the solvent.

4. No further processing of the samples is required.
 Analyze samples using the GC conditions noted above.

J. Calculations

1. Moisture Determination:

$$\text{Moisture content} = \frac{\text{Combined Fresh Wt} - \text{Combined Dry Wt}}{\text{Combined Dry Wt} - \text{Container Wt}}$$

2. Concentration of Residue(s) in Sample:

For each set of samples analyzed a standard curve is developed. From this curve the concentration of the analyte (in ug/mL) will be determined. Determination of the sample concentration in ppm will be made using the following calculation(s):

$$\text{Concn (ug) in sample} = \frac{\text{Concn (ug/mL)} \times \text{Final Vol} \times \text{Extract Vol} \times \text{Diln Factor}}{\text{Aliquot of Extract Used}}$$

3. Recovery for Fortified Samples:

$$\text{Recovery} = \frac{\text{Concn (ug)}}{\text{ug added to sample}}$$

4. Concentration of the analyte, based on the dry weight of the soil:

$$\text{Concn (ppm) in sample} = \frac{\text{Concn (ug)}}{\text{Sample Wt Extracted} \times (1 - \text{Moisture Content})}$$

For example:

Concn (ug/mL)	determined from Multichrom
Final Vol	2.0 mL (Step III E.6)
Extract Vol	250 mL (Step III D.4)
Sample Wt Extracted	25.0 g (Step III D.1)
Aliquot of Extract Used	200 mL (Step III D.6)
Moisture Content	9.9 (Step III C.2)

Calculations not noted above may also be found on "Sample Calculation" spreadsheets, which are presented along with the data.

Analytical Method Flow Chart

