ANALYTICAL METHOD FOR RESIDUES OF THIOPHANATE-METHYL, METHYL-2-BENZIMIDAZOLE CARBAMATE, AND ALLOPHANATE IN SOIL USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

TCM-004-1B (Hughson Location)

October 5, 1987

In this method, thiophanate-methyl (T-M), methyl-2-benzimidazole carbamate (MBC), and allophanate are extracted from soil (Hughson location) with aqueous methanol. After evaporating the solvent, the analytes are extracted into chloroform. The chloroform is then dried and evaporated and the residue reconstituted in methanol and quantitated by HPLC.

I. REAGENTS

A. All reagents should be AR grade or better.

B. All water should be distilled or deionized.

C. HPLC grade solvents

D. The following special reagents should be prepared:

1. 1 N HCl - dilute 83 mLs concentrated HCl to 1 liter with water.

2. 4% ethanol in chloroform - dilute 4 mLs 95% (190 proof) ethanol to 100 mLs with chloroform (Omnisolv, Catalog No. 1054, or equivalent). Prepare daily as needed.

3. Citric acid/phosphate buffer - dissolve 4.9 grams citric acid monohydrate and 4.2 grams sodium phosphate (dibasic) heptahydrate in water (sonication speeds dissolution) and dilute to one liter with water. Prepare daily - buffer should be discarded if algae growth is observed.

II. EQUIPMENT

A. Class A volumetric glassware should be used for all steps requiring volumetric measurement.

B. The following special equipment is recommended:

1. Wrist action shaker
2. Rotary evaporator (Buchler PTFE-1GN or equivalent)
3. HPLC with UV detector
4. Syringe filters (Gelman ACRO LC13 ,No. 4452, or equivalent)
5. Vortex mixer (Scientific Industries Inc. Model K850-C or equivalent)
6. Ultrasonic cleaner

III. METHODOLOGY

A. Sample Preparation

Thaw samples sufficiently to allow thorough mixing and sub-sampling.

B. Sample Extraction

1. Soil

a. Weigh 100 gram sub-sample into a 500 mL Erlenmeyer flask.

b. Add 150 mLs methanol and 50 mLs water to the flask.

c. Stopper and shake at maximum speed on a wrist action shaker for 15 minutes.

d. Transfer the sample to 250 mL centrifuge bottles without rinses and centrifuge at 2,000 rpm for 15 to 20 minutes.

e. Decant the aqueous methanol into a 250 mL graduated cylinder and record the volume. Discard the pellet.

f. Transfer the solution to a clean one liter flat bottom flask with three approximately 10mL rinses of methanol, and evaporate the alcohol on a rotary evaporator (bath temperature no more than 40°C).

C. Isolation of T-M, MBC, and Allophanate Residues

1. Add 100 mg sodium acetate to aqueous as a buffer. Adjust the pH of the aqueous solution to 6.8 ± 0.1 using 1 N HCl and 1 N ammonium hydroxide.

2. Add 25 mLs 4% ethanol in chloroform to the flask. Stopper and shake ten minutes on a wrist action shaker at maximum setting. Transfer to a 500 mL separatory funnel.

3. Allow the layers to separate (if an emulsion forms add an additional 10 mLs of the 4% ethanol in chloroform solution and stir well with a stick). Collect the chloroform (bottom layer) in a 500 mL flat bottom flask equipped with a glass funnel containing a plug of silanized glass wool and about 4 grams (½ a 5 mL beaker full) of anhydrous sodium sulfate.

4. Repeat Steps 2 and 3 until a total of three extractions have been completed.

5. Rinse the glass funnel with about 10 mLs of the 4% ethanol in chloroform solution.

6. Discard the aqueous layer.

D. Analysis of Residues

1. Evaporate the dry chloroform extracts down to about 4 to 5 mLs on a rotary evaporator (water bath <40°C). Add about 30 mLs methanol to the flask, swirl to mix, and evaporate to about 4 to 5 mLs on a rotary evaporator again.

2. Transfer the flask contents quantitatively with two approximately 2mL methanol rinses to a graduated centrifuge tube and evaporate below final volume of 1 mL with a gentle stream of nitrogen in a <40°C water bath.

3. Adjust the final volume to 1.0 mL with methanol, stopper, vortex to mix, and pass through a 0.45 micron syringe filter.

4. Inject an appropriate aliquot into the HPLC and quantitate with external standards taking into consideration the volume recovered in Step III.B.1.e.

5. HPLC conditions are as follows:

   a. Column - µBondapack C18 300 x 3.9 mm or equivalent

   b. Mobile Phase - Linear gradient from 15% acetonitrile, 85% citrate/phosphate buffer to 50% acetonitrile, 50% buffer over 20 minutes

   c. Flow - 1.5 mL/min

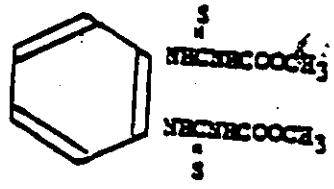
   d. Detector - UV - 254 nm

   e. Temperature - Ambient

E. Structural Formulas and Molecular Weights

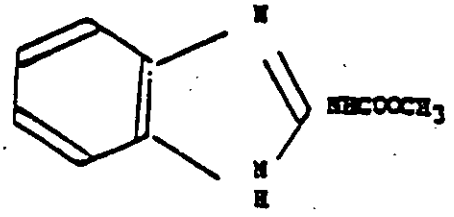
1. T-M - M.W. 342.4

T-M = Thiophanate-methyl (TOPSIN M) = Dimethyl [(1,2-phenylene)bis(iminocarbonothioyl)]bis[carbamate]



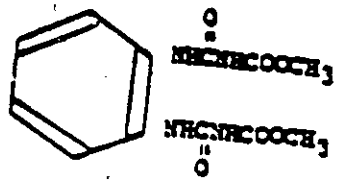
2. MBC - M.W. 191.2

MBC = Methyl-2-benzimidazole carbamate (2-Methoxycarbonylamino-benzimidazole) - stable transformation product (major transformation product)



3. Allophanate - M.W. 310.4

FH-432 = Dimethyl-4,4'-o-phenylenebis(allophanate) - relatively stable transformation product



DEVIATIONS FROM THE METHOD

The following deviations did not affect the integrity of the study:

- Due to lack of sufficient sample, only 50 g of soil were analyzed per assay instead of the 100 g specified in the Method.
- To validate Fisher methanol, the 0.1 ppm recovery for Analysis Set 10H was extracted with 150 mL of Fisher methanol instead of 150 mL of B&J methanol.
- To stop the breakdown of T-M to MBC, acidified methanol was substituted for neutral methanol at the point of solvent exchange with chloroform/ethanol extract. Samples were brought to final volume with acidified methanol. This deviation was started from set 15H.
- To achieve better sensitivity, the following program was used on the Kratos 783 detector (from set 16H on):

	Minutes	Wavelength	Range
Step 00 time:	0.0	281	0.100
Step 01 time:	8.0	254	0.050
Step 02 time:	26.0	281	0.050
Step 03 time:	27.0	281	0.050

CALCULATIONS

To Calculate ppm of T-M, MBC or Allophanate

$$\frac{\frac{Y - B}{M} \times \frac{V_T}{V_R} \times V_F \times \frac{1}{D}}{W}$$

Where:

X = concentration of analyte in ppm

Y = peak height obtained from the chromatogram (mm)

B = y-intercept obtained from the linear regression of the standard curve

M = slope obtained from the linear regression of the standard curve

V_T = total volume of solution added to the sample for extraction (mL)

V_R = volume recovered after centrifuging the sample (mL)

V_F = final volume (mL)

D = dilution factor as a fraction

W = sample weight (g)

Example: 50 g of soil sample was weighed for analysis

$$X = \frac{\left(\frac{5.5 - (-1.36)}{1.02}\right) \times \left(\frac{200 \text{ mLs}}{181 \text{ mLs}}\right) \times 2 \text{ mLs} \times \frac{1}{\frac{1}{2}}}{50 \text{ g}} = 0.569 \text{ ppm}$$

To calculate percent recovery:

$$\text{Percent recovery} = 100\% \times \frac{\text{ppm found}}{\text{ppm added}}$$

Example: The control soil (50g) was weighed and fortified with 1 mL of 2.5 ug/mL standard (0.050 ppm)

Amount found = 0.048 ppm

$$\text{Percent recovery} = \frac{0.048 \text{ ppm}}{0.050 \text{ ppm}} \times 100\% = 96.0\%$$

To calculate percent moisture:

$$\text{Percent moisture} = 100\% - \frac{[\text{dry sample + container wt. (g)}] - \text{container wt. (g)}}{[\text{wet sample + container wt. (g)}] - \text{container wt. (g)}} \times 100\%$$

$$\% \text{ solids} = 100\% - \% \text{ moisture}$$

$$\frac{(\% \text{ solids} + \% \text{ solids})}{2} = \text{Average \% solids}$$

$$\text{Dry sample weight (g)} = \frac{\text{average \% solids} \times \text{sample weight (g)}}{100\%}$$

Calculations of dry lot concentrations:

$$X' = \frac{X \times W}{\text{dry sample weight}}$$

Where:

X' = Concentration of analyte in ppm (dry weight basis)
X = Concentration of analyte in ppm (weight received basis)
W = Initial weight of the sample in grams = 50g