M-1912
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Recommended Method of Analysis

Terbufos (CL 92,100): GC Method for the Separate Determination of CL 92,100 and Related Compounds (CL 94,301 and CL 94,320) in Soil

A. Principle

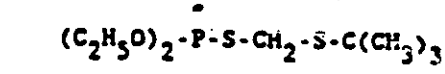
Residues of CL 92,100 and related compounds are extracted from soil by shaking with 10% aqueous acetone. CL 92,100 and related compounds are partitioned into methylene chloride after dilution of the extract with water and addition of sodium chloride to avoid emulsion formation. The methylene chloride is evaporated and the residue dissolved in a measured volume of acetone. Quantitation is accomplished by gas chromatography with an instrument equipped with a flame photometric detector in the phosphorus mode. The results are calculated by comparison of the individual peak heights to those in an external standard. The validated sensitivity of the method is 0.05 ppm for each compound.

B. Reagents

1. Analytical Standards: Obtained from American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540

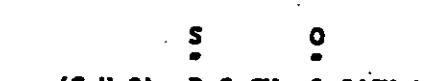
Note: This method is virtually identical to Methods M-1638 and M-1684 except that the extractant has been changed from 10% aqueous methanol to 10% aqueous acetone, isothermal GC has been replaced by temperature-programmed GC and several minor changes in sample handling have been incorporated.

a. Terbufos, (CL 92,100)



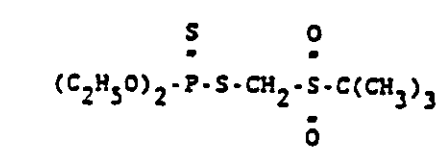
Phosphorodithioic acid, S-(tert-butylthio) methyl O,O-diethyl ester

b. Terbufos sulfoxide, (CL 94,301)



Phosphorodithioic acid, S-(tert-butylsulfinyl) methyl O,O-diethyl ester

c. Terbufos sulfone, (CL 94,320)



Phosphorodithioic acid, S-(tert-butylsulfonyl) methyl O,O-diethyl ester

2. Solvents: B & J Brand High Purity Solvents, Baxter Burdick and Jackson, or equivalent.

   a. Methylene Chloride

   b. Acetone

   c. Acetonitrile (Non-Spectro)

3. Reagents: "Baker Analyzed" Reagents, J. T. Baker Chemical Company.

   a. Sodium Sulfate, Anhydrous, Granular

   b. Sodium Chloride, Crystal

4. Solutions:

   a. 10% Water in Acetone (Extraction Solvent): Add 100 mL of distilled water to a 1-L volumetric flask, dilute to the mark with acetone, and mix well.

   b. 25% Sodium Chloride in Water: Add 250 g of sodium chloride to a 1-L volumetric flask, dissolve in distilled water and dilute to the mark.

5. GLC Packing: 1% EGSS-X on 80/100 mesh Supelcoport, Supelco special order GC packing, Supelco, Inc.

C. Apparatus

1. Gas Chromatograph: Tracor Model 540 or equivalent instrument equipped with a flame photometric detector (phosphorus mode).

2. Gas Chromatographic Column: 92 cm x 2 mm i.d. borosilicate glass column packed, using vacuum, with 1% EGSS-X on 80/100 mesh Supelcoport. Condition the column overnight with column temperature set at 185°C and with carrier flow of 25 mL/min.

3. Glass Wool, Silane Treated: Catalog No. 2-0411, Supelco, Inc.

4. Microliter Syringe: 10-mcL Capacity, Series No. 700, Hamilton Company.

5. Rotary Evaporator: Buchler Instruments or equivalent, equipped with a glass evaporator trap (No K-570200, Kontes Glass Company) between the concentration flask and the glass shaft of the evaporator. During evaporation, warm the flask in a water bath maintained at approximately 35°C.

6. Recorder: Spectra-Physics, SP 4290 recording integrator, or equivalent.

7. Balance, Analytical: Sartorius, precision of ± 0.05 mg.

8. Balance, Pan: Sartorius Model 2254, precision of ± 5 mg.

9. Glassware: Assorted laboratory.

10. Filter Paper: Whatman, Inc.

a. Glass Microfibre Filter, 934-AH, 9.0 cm.

b. Qualitative, No. 1, 18.5 cm.

11. Aquatest IV: Karl Fisher moisture titrator, Photovolt Corporation, Indianapolis, Indiana.

12. Horizontal Reciprocating Shaker: A. H. Thomas Co., No. 8291-S10.

13. Nipple Type Laboratory Bulb: Baxter Scientific Products R5002-1.

14. Disposable Pasteur Pipet: Baxter Scientific Products P5215-1.

D. Preparation of Standard Solutions: (These solutions are stable for at least one month if stored in amber glass under refrigeration).

1. Stock Solutions: (Equivalent for each compound).

Accurately weigh about 50 mg of the appropriate analytical standard of known purity and transfer to a 100-mL volumetric flask. This may be accomplished by attaching a nipple-type laboratory bulb to a disposable Pasteur pipet and drawing up 5 to 10 drops of standard. Weigh the pipet and standard using an analytical balance, transfer 5 drops of standard to the flask and reweigh. Dilute to the mark with acetone, mix well and calculate the concentration in mcg/mL. The three solutions prepared in this way are the stock standards which should be prepared at monthly intervals.

2. Standard Fortification Solutions

a. Prepare a combination standard solution containing 100 mcg/mL of each of the three compounds of interest by calculating the volume of each stock standard required to give this concentration when diluted to 100 mL.

$$\text{mL stock standard to be transferred} = \frac{10{,}000}{\text{concentration of stock standard in mcg/mL}}$$

After transferring the appropriate volume of each of the stock standards to the 100-mL flask, dilute to the mark with acetone and mix well. Designate as Fortification Standard A.

b. Prepare a combination standard containing 10 mcg/mL of each compound by transferring 10 mL of Fortification Standard A to a 100-mL volumetric flask, diluting to the mark with acetone and mixing well. Designate as Fortification Solution B.

3. Gas Chromatographic Standards

Pipet 4-, 2-, 1- and 0.5- mL aliquots of Fortification Standard A (100 mcg of each compound/mL) into separate 100-mL volumetric flasks, dilute to the respective marks with acetone and mix well. These solutions contain 4-, 2-, 1 and 0.5 mcg/mL of each compound and will be used as GC standards to show linearity of response. The 1 mcg/mL standard will be the working external standard against which all apparent residues in samples will be calculated.

E. Gas Chromatographic Conditions

1. Instrument: Tracor Model 540

2. Detector: Flame photometric detector (phosphorus mode).

3. Column: 92 cm x 2 mm i.d. glass, packed with 1% SGSS-X on 80/100 mesh Supelcoport.

4. Instrument Conditions*:

Initial:	125°C	Final:	170°C
Hold Time:	0 min.	Hold Time:	10 min.
Temp. Ramp:	4°C/minute		

Inlet Temperature	250°C
Detector Temperature	230°C
Helium Flow Rate	25 mL/min.
Hydrogen Flow Rate	150 mL/min.
Air Flow Rate	125 mL/min.

5. Recording Integrator: 0.5 cm/min chart speed.

6. Sensitivity: Electrometer sensitivity set so that a 5-ng injection of the working standards (Section D.3) gives a peak height of approximately 40-60% FSD (full scale deflection) for CL 92,100 and CL 94,320. A somewhat smaller response will be obtained for CL 94,301.

7. Retention Times (approximate):

CL 92,100:	2.2 min.
CL 94,301:	10.6 min.
CL 94,320:	12.3 min.

F. Sample Preparation

1. Frozen soil samples should be allowed to thaw for several hours before processing. (Do not allow soil samples to remain at room temperature for any great length of time).

2. Thawed soil should be mixed thoroughly, removing large stones and vegetation, to obtain a homogeneous sample. A sieve should be used to help mix large (greater than 2 pounds) samples.

3. After mixing, samples are returned to the freezer if analysis is not performed immediately.

4. Prior to analysis the moisture content of all soil should be determined

*Depending on the instrument and detector used and the condition of the GC column, these conditions may need to be adjusted to obtain equivalent response and resolution as shown in Figure M-1912.A.

G. Determination of Moisture

Before using the Aquatest IV, it should be checked for accuracy using a standard water solution.

1. Inject three 50-mcL aliquots of methanol (B & J Brand or equivalent) into the Aquatest unit to determine the water content of the methanol. For a good bottle of methanol, the water content should be less than 10 mcg/50 mcL.

2. Using a pan balance, weigh out 1.0 g of soil from a well-mixed sample onto a piece of weighing paper.

3. Transfer the sample to a liquid scintillation vial and add 10.0 mL of methanol.

4. Cap the vial and shake vigorously for 1 minute.

5. Allow the sample to settle for at least 10 minutes.

6. Inject duplicate 50-mcL aliquots of the methanol extract into the Aquatest unit. If duplicate values are not within ± 10% of each other make two more injections.

7. Inject smaller aliquots if the titration time is greater than two minutes.

8. Calculate the percent of water in the sample by using the following equation.

$$\% \text{ water} = [\text{Average mcg}_s - \text{Average mcg}_b] \times 0.02 \times \frac{50}{A}$$

Where:

Average mcg_s = Average micrograms titrated for sample.

Average mcg_b = Average micrograms titrated for methanol blank (same volume as sample).

A = Microliters of sample injected.

9. If an Aquatest IV unit is not available, dry a 50-g subsample of soil in a hood or oven to constant weight to determine the moisture content.

H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. A fortified sample should also be processed with each daily set of samples analyzed.

1. Weigh a 100-g subsample of control soil into a 32 oz. narrow-mouth bottle.

2. Add by pipet a volume of standard fortification solution appropriate to the fortification level to be tested.

3. Add the solution dropwise and mix the sample well before adding the extraction solvent.

4. Continue with the extraction and cleanup steps as described in the following sections.

I. Analysis of Soil

1. Weigh a 100-gram subsample of soil into a 32-oz. narrow-mouth jar.

2. Add 400 mL of 10% water in acetone and cap with a polyseal cap.

3. Shake for 60 min. on a horizontal shaker at high speed.

4. Filter using a Buchner funnel, with the aid of vacuum and 2 glass-fiber filter papers, into a filter flask.

5. Wash the extraction bottle and soil marc with one 90-mL aliquot of the extracting solvent.

6. Transfer the filtrate to a 500-mL mixing graduated cylinder, dilute to 500 mL with the extraction solvent, and mix thoroughly.

7. Transfer a 250-mL aliquot of the filtrate into a 1,000-mL separatory funnel.

8. Add 300 mL of water and 20 mL of 25% sodium chloride solution.

9. Add 100 mL of methylene chloride.

10. Shake vigorously for 45 seconds.

11. Place a Whatman No. 1 qualitative filter paper in a powder funnel and add 50 grams of granular, anhydrous, sodium sulfate.

12. Percolate the lower, methylene chloride, layer in the separatory funnel through the sodium sulfate into a 500-mL round bottom flask.

13. Add an additional 100 mL of methylene chloride to the separatory funnel.

14. Shake vigorously for 45 seconds.

15. Drain the lower layer through the sodium sulfate.

16. Wash the sodium sulfate with an additional 50 mL of methylene chloride.

17. Evaporate the combined extracts to near dryness using a rotary evaporator.

18. Add 30 mL of acetonitrile and evaporate to dryness.

19. Dissolve the residue in 5 mL of acetone for quantitation by gas chromatography.

J. Gas Chromatographic Analysis

1. Adjust the GC conditions to obtain similar retention times and responses to those shown in the attached figure.

2. For temperature programmed analysis, inject the 1.0 mcg/mL combination standard at 125°C. Raise the column temperature at a rate of 4°/min to 170°C where an isothermal hold of 10 minutes is maintained until recycling back to 125°C. The recordering integrator attenuation is lowered by one-half at 4 minutes into the run to yield satisfactory peak height for the later eluting compounds.

3. A 5-mcL injection of standard is made followed by two sample injections, and another standard injection.

4. An average of the standards injected before and after two samples is used to quantitate the samples bracketed by the standards.

5. If a sample peak goes off-scale, dilute an aliquot to an appropriate volume and reinject to obtain a peak size close to the standard.

K. Calculations

For each compound, use the sample peak height and the average peak height measurement of the external standard obtained before and after the sample injection as follows:

$$ppm = \frac{R(SAMP) \times (V1) \times (V3) \times (V5) \times C(STD) \times DF}{R(STD) \times (V2) \times (V4) \times (Wt)}$$

Where:

R(SAMP) = Peak height (mm) for sample solution

R(STD) = Average peak height (mm) for standard solution

W = Weight of sample taken for analysis in grams on a dry basis*

V1 = Volume in mL of extracting solvent

V2 = Volume in mL of extract taken for analysis

V3 = Volume in mL of final solution used for GC analysis

V4 = Volume in mcL of sample solution injected

C = Concentration in mcg/mL of standard solution

V5 = Volume in mcL of standard solution injected

DF = Dilution Factor

Figure M-1912.A presents typical chromatograms for the analysis of soil by temperature-programmed gas chromatography.

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*To determine the dry weight of soil used for analysis, calculate as follows:

$$\text{Dry Sample Weight (W)} = \text{Initial Sample Weight (g)} \times \frac{100 - \% \text{ moisture}}{100}$$