INTRODUCTION

This method was developed to analyze soil samples in conjunction with terrestrial field dissipation studies. Carbaryl residues are extracted from soil samples with an acetone/water/phosphoric acid mixture. After filtration, the carbaryl is partitioned into dichloromethane. The majority of interfering substances (optional) are then eliminated from the extract utilizing a Florisil Sep-pak®. The solvent is evaporated and the residue quantified by high pressure liquid chromatography with fluorescence detection of carbaryl as 1-naphthol after post-column hydrolysis with sodium hydroxide.

SAFETY

Please refer to the Rhône-Poulenc Safety and Health Program Manual for guidelines concerning the handling of hazardous material, solvents, waste disposal and other related safety issues.

EQUIPMENT

1. A high performance liquid chromatograph, HP Model 1084-B, HP Model 1090, HP 1050 Series, or equivalent.
2. Fluorescence detector, Hewlett-Packard 1046B, or equivalent:

   Optimum wavelengths: Excitation - 330 nm.
   Emission - 465 nm.

3. Reverse-phase HPLC column, 15 cm x 4.6 mm, 5 µm particle size, DuPont Zorbax or equivalent.
4. Lab-Line model 3520 Junior Orbital Shaker(Lab-Line Instruments, Inc., Melrose Park, Illinois), or equivalent.
5. Thelco model 186 or equivalent water bath capable of maintaining a 35°C temperature.
6. Polyethylene bottles, 250 ml, Nalgene, cat #B-7532-8(American Scientific Products) or equivalent.
7. Separatory funnels, 500 ml.
8. Buchner funnels, 7.0 cm.
9. Plastic funnels, 100 mm top diameter.
10. Erlenmeyer flasks, 500 ml.
11. Side-arm filter flasks, 250 ml.
12. Glass fiber filter discs, 7.0 cm, grade 394(Baxter Scientific), or equivalent
13. ~~Filter units, 0.45 µm membrane, Millex HV(Millipore)~~.
16. Plastic disposable syringes, 10 ml. or 20 ml
17. ~~Disposable aluminum weighing dishes, 57 mm-diameter (Fisher)~~
    glass jars 45 cm in diameter

18. Post-column hydrolysis system(Figure 1):

1. Eldex model A-30-S or equivalent metering pump, or equivalent.
2. Precision GCA model, or equivalent, water bath. Fill with silicone oil and set temperature to 100°C.
3. Reagent bottle, Omnifit 3-valve, 1000 ml, with fittings(Rainin).
4. Pressure regulator, 0-30 psig with 1/8" fittings(Supelco). Maintain helium pressure in bottle at 5-10 psig, or equivalent.
5. Pump inlet filter, 2 µm, for 1/8" tubing(Rainin), or equivalent.
6. Mixing tee, Valco zero dead volume, ss with 1/16" fittings, 0.25 mm bore, or equivalent.
7. Teflon tubing, 1/16" od x 0.25 mm id, with ss fittings. Use three meters of tubing to construct the hydrolysis coil, or equivalent.
8. Teflon tubing, 1/8" od, with Omnifit fittings and grippers(Rainin), or equivalent.
9. ~~...~~ pressure regulator at end of outlet.

MATERIALS AND REAGENTS

1. Acetone, HPLC grade
2. Carbaryl, analytical standard
3. Dichloromethane
4. Diethylene glycol
6. Helium UHP
7. Hyflo Super Cel or equivalent
8. Methanol, HPLC grade
9. Sodium hydroxide, reagent grade.
   Prepare a 0.5 N solution in water for the post-column hydrolysis.
10. Sodium sulfate, anhydrous granular.
11. Water, purified by Milli-Q system.
14. Extraction Solvent: 50/50 acetone/water(v/v) with 2 ml H_3PO_4 per liter of solution.
15. Drierite® calcium sulfate dessicant.
16. Phosphoric acid, 85%, reagent grade.
17. Waters Florisil Sep-Pak® Plus or equivalent solid phase extraction cartridges.

STANDARD SOLUTIONS

1. Stock Solution:

Weigh 100.0 mg of carbaryl into a 100 ml volumetric flask. Dissolve in methanol and dilute to the mark to obtain a 1.00 mg/ml solution. ~~Add one drop of phosphoric acid and mix.~~ Check pH of solution and ~~record~~.

2. Chromatography/Spiking Solutions:

Prepare solutions in methanol from the stock solution to obtain solutions of 0.10, 0.50, 1.00 and 5.00, 10.0, and 100 µg/ml carbaryl. Use methanol as a 0.00 µg/ml standard. Additional standards in the range of 0.0-10.0 µg/ml may be prepared and used for chromatography standards or spiking solutions if appropriate.

Store standard solutions between -5°C and -20°C. Warm to room temperature before use. Discard standard solutions after 2 months.

Appearance of a 1-naphthol peak on the front side of the carbaryl peak indicates probable decomposition of carbaryl.

PROCEDURE

1. Weigh approximately 50 grams of soil and approximately 10 grams of Hyflo Super Cel into a 250 ml plastic bottle. Also weigh an appropriate amount of soil for moisture determination at this time. Refer to SOP 87430 for the soil moisture determination procedure to be used. The amount of soil weighed for the analysis and moisture determination should be determined to the nearest tenth of a gram and recorded.

2. Fortification of samples for determination of recoveries should be done at this time.

3. Add 125 ml(approximately) of extraction solvent to the plastic bottle and shake on the orbital shaker at approximately 300 rpm for approximately 10 minutes.

4. Centrifuge briefly (or allow sufficient time for settling) and filter the liquid by suction through a Buchner funnel on a 7.0 cm glass fiber filter. Leave as much soil as possible in the bottle.

5. Add 75 ml(approximately) of extraction solvent to the bottle. Shake the bottle for one minute and filter. Transfer the soil to the filter funnel. Rinse the bottle and filter funnel with 2 x 25 ml(approximately) extraction solvent.

6. Using a suitable size separatory funnel, partition carbaryl residues from the combined filtrate into three 50 ml(approximately) portions of dichloromethane.

7. Pass the dichloromethane extracts through a glass wool stoppered plastic funnel containing approximately 100 grams anhydrous sodium sulfate. Collect the combined extracts into an appropriate sized Erlenmeyer flask.

STEPS 8 and 9 are OPTIONAL and should be employed for sample clean-up if necessary to prevent interferences in the chromatography. The clean-up step is typically employed with soils samples containing concentrations of carbaryl below 0.100 ppm.

8. Evaporate the solvent to an approximate volume of 5 milliliters. if sitting overnight in freeze add 1 drop diethyleneglycol.

9. Add the sample extract to the Florisil Sep-Pak® and collect all eluate in an appropriate sized Erlenmeyer flask. Rinse the Sep-Pak® with an additional 15 mL dichloromethane. Add 1 drop diethylene glycol.

10. Evaporate the dichloromethane eluate JUST to dryness.

11. Allow the flask to come to room temperature and add an appropriate amount of methanol. Use 2 ml for expected amounts of carbaryl less than 3 µg. For larger amounts, dilute so that the final concentration of carbaryl does not exceed 5 µg/ml.

12. Inject 20 µl of the extract into the HPLC. Filtration of the sample prior to analysis may or may not be indicated.

13. Liquid Chromatographic Conditions:

    Mobile Phase: Methanol/Water (typically 62:38 60:40)
    Flow Rate: 1.5 ml/min
    Approximate Run Time: 6.0 min Approximate t_r carbaryl = 4.3 minutes

QUANTIFICATION OF CARBARYL IN SOIL:

Determine the calibration line for carbaryl by the linear least squares method using peak area or height versus standard concentration (in µg/ml).

Concentration of carbaryl (µg/ml) in the injected sample (A) = $\frac{\text{(Peak area or height)} - \text{Intercept}}{\text{Slope}}$

This equation is valid only if the volume of the sample extract injected into the HPLC equals the volume of carbaryl standard injected.

Calculate the concentration of carbaryl (ppm) in the original soil sample on a dry weight basis using the following equation:

$$\text{ppm (corrected) Carbaryl} = \frac{100\ AV(M+1)}{RS}$$

where:
V = Sample dilution volume in ml
R = Percent Recovery
S = Weight of soil taken for analysis
M = Soil moisture correction factor

Determination of Percent Recovery

Fortification of untreated control samples should be made at level approximating those expected in samples. If any carbaryl is present in the untreated control sample (UTC), that amount must also be considered in the calculation. If multiple recoveries are performed in a series of samples on the same day, the recovery results are averaged to get a single percent recovery for all of the samples in the series. Acceptable recoveries should typically be between 75-125%.

$$\text{Percent Recovery (R)} = \frac{100(\mu g \text{ found in Recovery} - \mu g \text{ found in UTC})}{\mu g \text{ spiked in UTC Sample}}$$

FIGURE 1. HPLC Post Column Hydrolysis/Detection System