DETERMINATION OF PYRIDABEN (BAS 300 I) IN SOIL

References:

1. BASF Method Number D9201, entitled "GC METHOD FOR RESIDUE DETERMINATIONS OF PYRIDABEN (BAS 300 I), IN POND SEDIMENT."

Principle:

Pyridaben is extracted from soil using methanol followed by a cleanup consisting of a florisil column. Detection and quantitation is achieved by use of a gas chromatograph equipped with an electron capture detector. The limit of quantitation is 0.01 μg/g.

During all analyses, equivalent apparatus, solvents, glassware, or techniques (such as sample concentration) may be substituted for those specified in the method. In the event an equivalent piece of equipment or an equivalent technique is used, its use will be documented in the study records.

Apparatus and Equipment:

Gas Chromatograph: Hewlett-Packard (HP) gas chromatograph Model 5890A equipped with a HP Model 7673 autosampler, a HP Model G1223A electron capture detector (Nickel-63) and a 3365 II ChemStation.

Gas Chromatographic Columns:

Primary: Fused silica megabore chromatographic column, 30 m x 0.53 mm i.d., 1.5 μm film thickness, crosslinked DB-5 (J&W Scientific, Folsom, CA).

Alternate: Fused silica megabore chromatographic column, 30 m x 0.53 mm i.d., 1.5 μm film thickness, crosslinked DB-1 (J&W Scientific, Folsom, CA).

Orbital shaker, Model 3518: (Lab-Line Instruments, Melrose Park, IL).

Ultrasonic cleaner: Branson Model 2200 (Branson Ultrasonic Corporation, Danbury, CT).

Vortex mixer: (Scientific Industries, Inc., Bohemia, NY).

Rotary evaporator with Dewar condenser and waterbath: (Buchler Instruments, Lenexa, KS).

Vacuum Pump: Sargent-Welch Model 8890

Gravity convection oven: VWR Model 1300U. (Sheldon Manufacturing, Cornelius, OR).

Liquid chromatography column: Glass column equipped with a Teflon stopcock and 250 mL solvent reservoir, 10 mm i.d. x 30 cm long

Balances:

Analytical: Capable of weighing 0.0000 g for weighing analytical standards

Top Loading: Capable of weighing 0.00 g for weighing sodium chloride

Erlenmeyer flasks with ground glass stoppers: 250 mL, 500 mL

Evaporation flasks, flat-bottom: 125 mL

Filter flasks: 500 mL

Graduated glass mixing cylinders: 250 mL, 500 mL

Buchner funnels: 9 cm i.d.

Glass funnels: assorted sizes

Glass wool: Pyrex

No. 4 Whatman Filter paper: 9 cm

Autosampler vials: with crimp caps (Hewlett-Packard Co., Wilmington, DE) ✓

Glass culture tubes, screw cap, disposable: 13 x 100 mm

Volumetric, glass, class A pipets: 4 mL, 5 mL, 10 mL and other assorted sizes

Graduated, glass, class A pipets: assorted sizes

Volumetric flasks, class A: assorted sizes

Assorted laboratory glassware

Reagents and Materials:

Acetonitrile:	B & J Brand High Purity Solvent, Baxter Healthcare Corp., Burdick and Jackson Division.
Alcohol, Anhydrous Reagent (Ethanol):	BAKER ANALYZED® Reagent, J. T. Baker Chemical Company
Methanol:	B & J Brand High Purity Solvent, Baxter Healthcare Corp., Burdick and Jackson Division.
Diethyl ether, without preservative:	B & J Brand High Purity Solvent, Baxter Healthcare Corp., Burdick and Jackson Division.
Hexane:	ULTRA RESI-ANALYZED, J. T. Baker Chemical Company.
Pyridaben:	Analytical grade, BASF Corporation
Toluene:	OmniSolv® glass distilled, EM Science, A Division of EM Industries, Inc.
Water:	B & J Brand High Purity Solvent, Baxter Healthcare Corp., Burdick and Jackson Division.
Florisil:	PR grade, 60-100 mesh, U.S. Silica
Sodium chloride:	Crystal, Certified ACS, Fisher Scientific
Sodium Sulfate:	Anhydrous, granular, Analytical reagent grade, Mallinckrodt

Reagents and Materials to be Prepared:

1. Activated Florisil: Activate by placing in oven at 130 °C ± 10 °C for at least 24 hours.

2. 5% deactivated florisil: deactivate prior to each use by adding 5% water (w/w) to cooled activated florisil in a tightly stoppered Erlenmeyer flask. Shake until no lumps are present. Allow to equilibrate for 2-3 hours but use within 4 hours of preparation.

3. Ethanolic diethyl ether: 2% ethanol in diethyl ether (v/v). Prepare daily in a tightly stoppered container.

4. Florisil column elution mixture: 20% ethanolic diethyl ether in hexane (v/v). Prepare daily in a tightly stoppered container.

Standard Preparation:

Stock solutions: Correcting for purity, prepare a 1000 µg/mL stock solution of Pyridaben in acetonitrile. This solution is stable for three months when stored at 1 to 8 °C in amber glass bottles with Teflon-lined screw caps.

Fortification solutions: Serially dilute the stock solution with acetonitrile to make 100 µg/mL, 10 µg/mL and 1.0 µg/mL solutions. Other concentrations are similarly prepared as appropriate. These solutions are stable for three months when stored at 1 to 8 °C in amber glass bottles with Teflon-lined screw caps.

Instrumentation solutions: Prepare in toluene and store in amber glass bottles with Teflon-lined screw caps. Suggested concentrations are: 0.03 µg/mL, 0.02 µg/mL, 0.01 µg/mL, and 0.002 µg/mL.

Sample Preparation:

1. Allow frozen soil samples to completely thaw. Shake sample containers to thoroughly mix samples prior to weighing sub sample for extraction.

Extraction:

The extraction approach presented here has been devised to accommodate two different initial sample sizes, either 20 or 50 grams. The 20 gram approach is used for the analysis of field samples, whereas, the 50 gram approach is used for the analysis of field-fortified samples (where the entire sample submitted must be analyzed).

20 gram samples:

1. Weigh 20 g of soil sample into a 250 mL Erlenmeyer flask with ground glass stopper. Fortify appropriate samples at this time.

2. Add 60 mL of methanol to the extraction flask.

3. Place flask onto an Orbital shaker.

4. Extract sample for 20 minutes on shaker at approximately 200 RPM.

5. After shaking, remove flask from shaker and let sit for approximately a minute to allow settling of the soil.

6. Decant extraction solvent into a 9 cm Buchner funnel with No. 4 Whatman filter paper under vacuum and collect in a 500 mL filter flask.

7. Add an additional 60 mL of methanol to the extraction flask.

8. Repeat Steps 3 and 4.

9. Filter sample through same funnel used for the initial filtration and combine filtrates.

10. Rinse extraction flask twice with 10 mL portions of methanol and pour through filter.

11. Pour combined methanol extracts into a 250 mL graduated mixing cylinder and bring to a final volume of 140 mL by addition of methanol. Record as extraction solvent volume on worksheet.

12. Cap mixing cylinder and invert to mix contents.

13. Using a 10 mL and 4 mL volumetric pipet, transfer a 14.0 mL aliquot of the methanol extract (representing 2.0 g of sample) to a 125 mL evaporation flask.

14. Concentrate the methanol extract aliquot to dryness using a rotary evaporator at 40-45 °C.

15. Dissolve the residue in 3.0 mL of Florisil column elution mixture. Proceed with Florisil column cleanup.

50 gram samples:

1. Transfer soil sample into a 250 mL Erlenmeyer flask with ground glass stopper. Fortify appropriate samples at this time.

2. Add 150 mL of methanol to the sample container, cap and shake, and add to the extraction flask.

3. Place flask onto an Orbital shaker.

4. Extract sample for 20 minutes on shaker at approximately 200 RPM.

5. After shaking, remove flask from shaker and let sit for approximately a minute to allow settling of the soil.

6. Decant extraction solvent into a 9 cm Buchner funnel with No. 4 Whatman filter paper under vacuum and collect in a 500 mL filter flask.

7. Add an additional 150 mL of methanol to the extraction flask.

8. Repeat Steps 3 and 4.

9. Filter sample through same funnel used for the initial filtration and combine filtrates.

10. Rinse extraction flask twice with 25 mL portions of methanol and pour through filter.

11. Pour combined methanol extracts into a 500 mL graduated mixing cylinder and bring to a final volume of 350 mL by addition of methanol. Record as extraction solvent volume on worksheet.

12. Cap mixing cylinder and invert to mix contents.

13. Using a 10 mL and 4 mL volumetric pipet, transfer a 14.0 mL aliquot of the methanol extract (representing 2.0 g of sample) to a 125 mL evaporation flask.

14. Concentrate the methanol extract aliquot to dryness using a rotary evaporator at 40-45 °C.

15. Dissolve the residue in 3.0 mL of Florisil column elution mixture. Proceed with Florisil column cleanup.

Note 1: Gram sample to mL solvent extraction ratios are equivalent using either 20 g or 50 g approach.

Note 2: Glassware employed with sample extractions steps should be rinsed with methanol before use.

Florisil column cleanup:

1. Activate the florisil in an oven maintained at 130 °C ± 10 °C for at least 24 hours prior to use. Remove the florisil and allow to cool in a tightly stoppered container. Prepare 5% (w/w) deactivated florisil by weighing 47.5 g of activated florisil into a glass stoppered Erlenmeyer flask and adding 2.5 g of water. Stopper the flask and shake to distribute the water until no lumps are observed. The florisil should be deactivated prior to each use. (Allow to equilibrate 2-3 hours but no longer than 4 hours prior to use.)

2. Tamp a plug of glass wool into the bottom of a 10 mm i.d. x 30 cm long glass column. Add 20 mL of florisil column elution mixture. Gently tap the column (to remove air and promote packing) while adding 5.0 g of 5% deactivated florisil. Pour 1.0 ± 0.1 g of anhydrous sodium sulfate on top of the florisil packing. Drain off the excess solvent until the level falls to within approximately one mm of the top of the anhydrous sodium sulfate.

3. Transfer the sample extract (from Step 15 above) to the column, drain and discard.

4. Rinse the flask with 5 mL of florisil column elution mixture, transfer to the column, drain and discard.

5. Add 60 mL florisil column elution mixture to the flask and swirl to rinse.

6. Elute pyridaben with the 60 mL of florisil column elution mixture from Step 5 and collect in a 125 mL flat bottom flask.

7. Concentrate the eluate using a rotary evaporator at 40-45 °C to approximately 1-2 mL. Blow the sample to dryness using N_2.

8. Using a 5 mL volumetric pipet, add 5.0 mL of toluene to the flask. Sonicate for 5 minutes to dislodge any residue adhering to the walls of the flask.

9. Using a disposable Pasteur pipet, transfer entire sample from Step 8 to a 13 × 100 mm culture tube and submit to GC for analysis. The final concentration is 1 mL = 0.4 g.

Note 1: If sample extracts must be stored overnight prior to instrumental analysis, **[they must be sonicated for at least 5 minutes]** prior to injection into GC or transfer to autosampler vials.

Note 2: Any further dilutions will be conducted by instrumentation personnel, using toluene, if necessary.

Instrumentation:

Operating conditions: Follow the manufacturer's instructions and the laboratory SOP for the operation of the gas chromatograph and electron capture detector.

Gas Chromatography:

Any of the following columns and conditions may be used for this analysis. It is recommended, however, that consistency be employed during the course of the study (unforeseen circumstances excluded).

Primary:

Column:	Crosslinked, fused silica megabore, 30 m x 0.53 mm i.d., 1.5 µm film thickness, DB-5 (J&W Scientific)
Injection port:	Packed port
Injection port liner:	2 mm i.d. glass inserts lightly packed with fused silica wool (Hewlett-Packard)
Carrier gas:	Helium at 10 mL/min.
Detector make-up gas:	Nitrogen at 50 mL/min.
Temperatures:	Detector: 300 °C Injector: 250 °C Column: Initial = 255 °C hold 11 minutes Rate = 10 °C/min. Final = 280 °C hold 11.5 minutes
Injection volume:	2.0 µL
Retention time:	8.2 minutes

Alternate:

Column:	Crosslinked, fused silica megabore, 30 m x 0.53 mm i.d., 1.5 μm film thickness, DB-1 (J&W Scientific).
Injection port:	Packed Port
Injection port liner:	Silanized Flash Vaporization Megabore Liner (J&W Scientific)
Carrier gas:	Helium at 12 mL/min.
Detector make-up gas:	Nitrogen at 50 mL/min.
Temperatures:	Detector = 300 °C Injector = 250 °C Column: = 250 °C Isothermal
Injection volume:	2 μL
Retention time:	7.4 minutes

Note: The column and conditions stated in the method have been satisfactory for the matrix being analyzed. The specific column packing, carrier gas, column temperature and flow rate listed are typical conditions for this analysis. Specific conditions used will be noted on each chromatographic run and will not otherwise be documented.

Calibration:

Calibrate the gas chromatograph by injecting standard solutions containing 0.002, 0.01, 0.02, and 0.03 μg/mL. If necessary, the last three concentrations can be adjusted to permit use of the entire range of the recorder or integrator scale. However, the lowest concentration of 0.002 μg/mL must always be employed. Prepare a calibration curve by plotting response versus standard concentration. The calibration curve is derived from all standards injected (including curve check standards) with an analysis set using least squares fit. This calibration curve also serves as the standard curve used for calculation purposes.

Calculations:

Concentration of sample extract (in g/mL) submitted to GC:

20 g sample:

$$\frac{20\ g}{140\ mL} \times \frac{14.0\ mL\ aliquot}{5.0\ mL\ final\ volume} = 0.4\ g/mL$$

50 g sample:

$$\frac{50\ g}{350\ mL} \times \frac{14.0\ mL\ aliquot}{5.0\ mL\ final\ volume} = 0.4\ g/mL$$

Concentration of the analyte in the sample using external standard method:

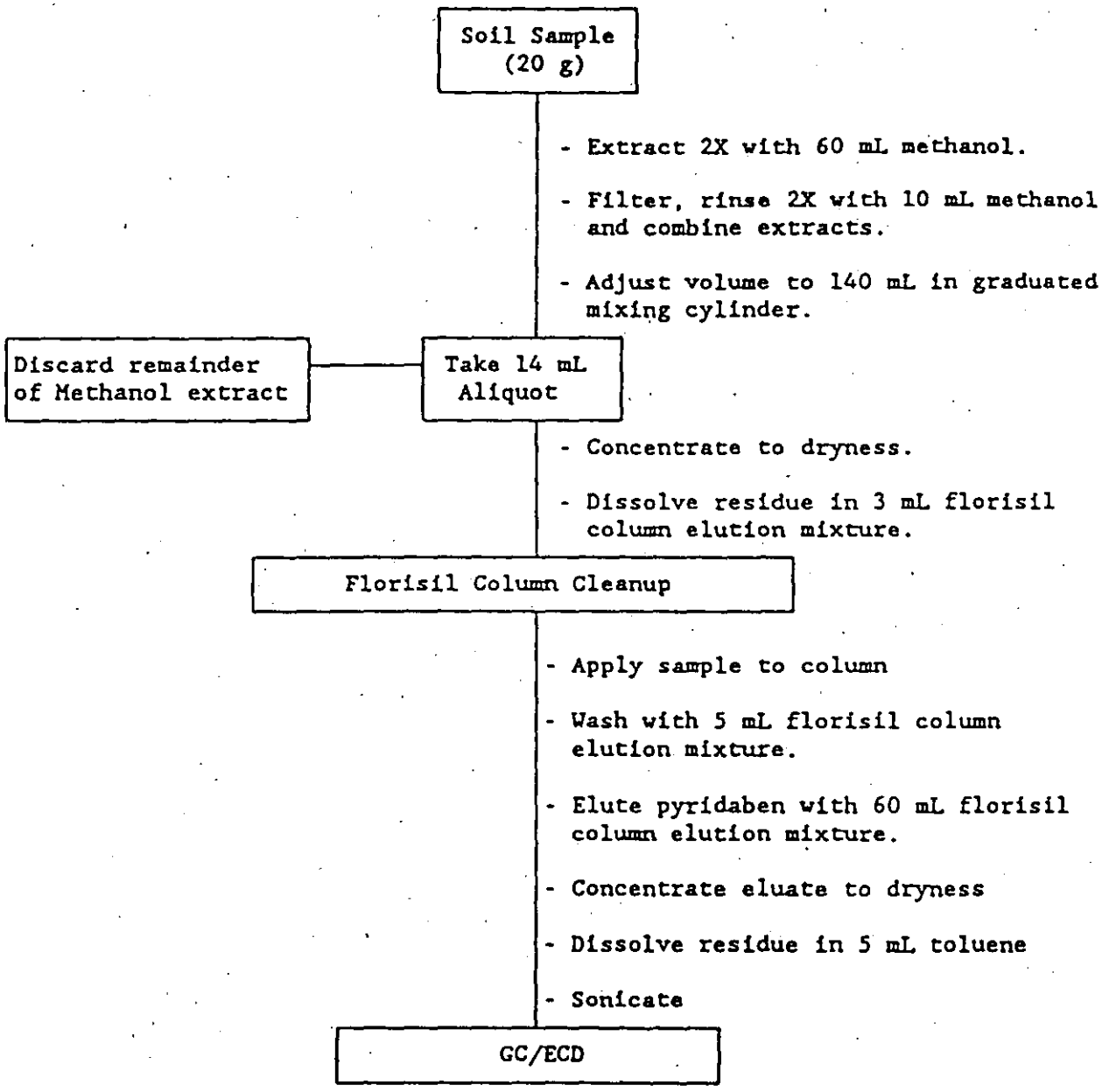
$$\mu g/g = \frac{\mu g\ analyte/mL \times dilution\ factor}{g\ sample/mL}$$

where:

µg/g	=	Total micrograms of analyte present per gram of soil.
µg/mL	=	Concentration of analyte derived from the standard curve based on peak height of response from the sample injected.
g/mL	=	Concentration of sample extract submitted to GC.
dilution factor	=	Dilution of sample extract required to produce an analyte response bracketed by standards.

FLOW CHART NO. 1

Analytical Procedure for Treated Soil Samples



FLOW CHART NO. 2

Analytical Procedure for Field Fortified Soil Samples