DETERMINATION OF METHOMYL IN SOIL BY HPLC
(SLI #91-4-3722)

MORSE LABORATORIES, INC. MODIFICATIONS, 08/27/93

During all analyses, equivalent apparatus, solvents, glassware, or techniques (such as sample concentration) may be substituted for those specified in the method.

Extraction:

Reagents:

Methanol	-	B & J brand high purity solvent
Water	-	Laboratory deionized (DI) water and HPLC grade water, B & J brand high purity solvent.
Acetone	-	EM brand, Omni Solv
Acetonitrile	-	B & J brand high purity solvent
Methomyl	-	Analytical Standard, E. I. DuPont de Nemours

Prepare:

10% acetonitrile in HPLC grade water (v/v).

Methomyl stock solution and spiking standard in acetonitrile.

Instrumentation standards in 15% acetonitrile in HPLC grade water.

Equipment:

Burrell wrist action shaker

250 mL Nalgene bottles

Buchner funnel

Whatman #4 filter paper

Vac-Elut SPS 24 sample process station equipped with an air cadet vacuum pump.

Octadecyl (C_{18}) Bakerbond SPE columns, J.T. Baker #7020-07

SPE column reservoirs, 75 mL, Analytichem International

N-EVAP

Branson 2200 ultrasonic cleaner

Glass wool

Assorted laboratory glassware

1. Weigh 5.0 g of sample into a 250 mL wide mouth Nalgene bottle. Fortify appropriate samples at this time.

2. Add 50 mL of methanol, stopper the bottle securely and shake via a wrist action shaker at high speed for 15 minutes.

3. Suction filter the mixture through 9 cm Whatman #4 filter paper using a Buchner funnel and a 500 mL vacuum flask. Try to keep the solids in the bottle.

4. Add 50 mL of methanol to the solids in the bottle, stopper the bottle and manually shake the sample for 30 seconds.

5. Suction filter the contents (including solids) and collect in the same vacuum flask. Rinse the bottle twice with 15 mL of methanol and pour through the filter.

6. Transfer the combined filtrate to a 250 mL graduated cylinder. Rinse the filter flask twice with 10 mL of methanol and add the rinse to the cylinder. Adjust volume to 150 mL with methanol, mix and take 30 mL aliquot equivalent to 1.0 gm sample. Transfer aliquot to a round-bottom flask. (STOPPING POINT)

7. Evaporate the sample on a rotary evaporator with dry ice condenser at 38-45°C. Vacuum can be increased gradually to 26-30 inches of mercury. Evaporate until condensation of the methanol stops.

   Note: Depending on the moisture content of the soil, 0-5 mL of water will remain in the round-bottom flask.

8. Add 20 mL of DI water to the flask, with contents, and swirl the flask to dissolve the methomyl residues. (STOPPING POINT)

9. Set up octadecyl columns in the Vac-Elut process station. Place small glass wool pad on top of column. Condition each column with two column volumes of methanol followed by two column volumes of DI water. Discard the solvent waste.

   Note: Do not allow the column to elute to dryness until Step #11.

10. Add the sample extract to the column using a disposable pipet. Rinse the container twice with 10 mL of DI water, each time adding the rinse to the column. Sonicate each rinse for 1 minute prior to addition to column. Discard the eluate.

    Note: Flow rate should be adjusted using vacuum control and stopcocks. Flow should not consist of a steady stream but a steady drip such that individual drops can be seen forming at the tip of the column.

11. Allow the column to elute dryness. Increase vacuum to 10 or more inches of mercury and leave column on vacuum for 30 minutes.

    Note: This "drying" procedure is necessary in order to remove all residual water.

12. Turn off vacuum. Volumetrically add 4.0 mL of acetone to the column. Resume vacuum and collect the eluate in a 13 X 100 mm calibrated test tube.

13. Evaporate the eluate to dryness on the N-EVAP with bath temperature less than 45 °C. Once dry, remove samples promptly.

14. Adjust the volume in the tube to 1.0 mL with 10% acetonitrile in HPLC grade water, agitate tube, by hand, to mix. Submit to HPLC for analysis. Final concentration 1.0 mL = 1.0 g.

Instrumentation:

Instrument:	Linear LC-304 Fluorescence Detector with SP8800 Ternary HPLC pump, equipped with Pickering PCX 5000 Post Column Reaction Module.
Column:	25 x 4.6 mm DuPont Zorbax RX-C8 (5 micron particle size).

Mobile Phase: 20% ACN 80% H_2O

Note: Due to matrix interferences, samples may require analysis utilizing gradient elution. An example of a typical gradient is as follows:

Time	% Water	% Acetonitrile
0	95	5
2.5	90	10
7.0	90	10
15	77	23
16	60	40
19	60	40
20	95	5
23	95	5

(Flow rate: 1.0 mL/min.)

Column Temperature: 40 °C

Flow Rate: 1.2 mL/min.

Post Column Conditions:

Reaction Temperature: 100 °C

Hydrolysis Reagent: 0.2N NaOH

Derivatization Reagent: Approximately 500 mg OPA + approximately 2.5 g n,n-dimethyl-2-mercapto-ethylamine-hydrochloride in 1 quart 0.05M sodium borate pH 9.1

Reagent Flow: 0.13 mL/min. each

Detector Settings:

EX Wavelength: 330 nm
EM Wavelength: 466 nm

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