

**A HIGH PRESSURE LIQUID CHROMATOGRAPHIC  
METHOD FOR MEASUREMENT OF METHOMYL RESIDUES  
IN FRESHWATER**

**Submitted To:**

**E. I. DuPont de Nemours  
Agricultural Experiment Station  
Building 402, Room 5245  
Wilmington, DE 19898**

**SU Report # 91-4-3717**

**SLI Study # 91.1290.6117.320**

**Author : Timothy Z. Kendall**

**Springborn Laboratories, Inc.  
Environmental Sciences Division  
790 Main Street  
Wareham, Massachusetts 02571**

**4 April 1991**

## INTRODUCTION

A method for determining methomyl [Lannate, N-[[[(Methylamino)carbonyl]oxy]ethanimidothioic acid methyl ester] residue in freshwater is presented. The methodology for detection of methomyl was modeled after a procedure previously described in the literature <sup>1</sup>.

## PRINCIPLE AND APPLICATION

Methomyl is extracted and concentrated from water by solid phase (C<sub>18</sub>) partitioning, the residues eluted from the column with acetone, the eluent evaporated and the residues are dissolved in acetonitrile:water. A high pressure liquid chromatograph equipped for post-column reaction and fluorescence detection (HPLC-PCR) performs the following sequence of operations on the extract: 1) the HPLC column separates methomyl from interfering substances, 2) the mixing tee combines the methomyl eluate with an aqueous solution of potassium hydroxide, ortho-phthalaldehyde and 2-mercaptoethanol, 3) the reaction coil provides thermal energy for the alkaline hydrolysis of methomyl to methylamine which then reacts with the derivatization reagents, 4) the detector measures the fluorescence of the derivatized product at  $\lambda_{exc}$  229 nm/ $\lambda_{em}$  418 nm and 5) the integrator provides a plot of fluorescence versus time. Linear regression analysis of the derivatized product peak areas for samples and reference standards permits calculation of methomyl sample concentration.

## ANALYTICAL METHOD

### Reagents

Acetonitrile, Burdick and Jackson, HPLC grade, UV cutoff @ 188 nm

Water, Barnstead NANOpure, HPLC grade

Acetone, Burdick and Jackson, Analytical grade

---

Springborn Laboratories, Inc.

Methanol, Burdick and Jackson, HPLC grade, UV cutoff @ 203 nm

Potassium hydroxide, MCB, Reagent, A.C.S.

ortho-Phthalaldehyde, Aldrich, 97%

Mercaptoethanol, Aldrich, 98%

Methomyl, Lot No. X1179-379, 98.9% active ingredient, supplied by DuPont

### Preparation of Derivatization Reagent

1. Prepare an aqueous 0.10 M KOH stock solution (solution 1).
2. Accurately weigh 0.0500 grams of o-phthalaldehyde into a 25 mL beaker. To the beaker add 5 mL of reagent grade methanol and swirl to complete dissolution (solution 2).
3. Add 40 mL of solution 1 to approximately 300 mL of HPLC grade water (solution 3). Volumetrically add 1 mL of solution 2 to solution 3 and adjust the volume to 400 mL (solution 4). Degas under vacuum using sonication.
4. To solution 4, volumetrically add 50.0  $\mu$ L of 2-mercaptoethanol. Swirl the solution in order to ensure complete dissolution and homogeneity.

### Equipment

Balance, Ohaus Galaxy 160, four-place analytical balance

Flasks, volumetric, assorted sizes

Pipets, volumetric, assorted sizes

Serum bottles, Wheaton, assorted sizes, with Teflon-lined lids and metal crimp caps

Syringes, Hamilton, assorted sizes

Rotary evaporator, Buchii Model R110, with vacuum pump, 40 °C water bath

SPE columns, ODS ( $C_{18}$ ), 1000 mg, Baker

SPE vacuum manifold, Analytichem, VAC-ELUT  
Vacuum pump, 0-25 mm Hg capability

## Apparatus

### HPLC-PCR System

Liquid chromatograph solvent pump, Waters Model M6000A

Pump for PCR reagent, ABI Spectroflow 400

Autosampler, Waters Model 710B Intelligent Sample Processor

Detector, Kratos Model 980 programmable fluorescence detector

Integrator, Hewlett-Packard Model 3396 A

Pulse Dampers (2), SSI Model LP - 21

Heater Controller, Fiatron Model TC-55

Column Heater, Fiatron Model CH-30

Purge Valve, SSI three-way valve

Mixing Tee, Beckman, High Efficiency Mixer, No. 596809

Reaction Coil, 3 m x 0.5 mm ID knitted teflon delay tubes, two in series, Supelco

Single bead string reactor, Supelco

Heat Sink, 1.0 m x 0.50 mm ID PFTE tubing

Back Pressure Loop, 4 m x 0.50 mm ID PFTE tubing

Back Pressure Regulator, Rainin, 15-60 psi

Chromatographic Column, Phenomenex Ultremex ODS, 5 $\mu$ m, 250 mm x  
4.6 mm I.D.

## Detailed Procedure

### I. Preparation of Stock Solution

#### A. Methomyl (1 mg/mL)

1. Weigh 100 milligrams (A.I.) of methomyl on an analytical balance.
2. Transfer the methomyl to a 100-mL volumetric flask and dissolve to the mark with acetonitrile.
3. Transfer the stock solution to a 100-mL amber serum vial and seal with a Teflon-lined crimp cap.
4. Store this stock solution in a refrigerator maintained at 4 °C.

### II. Control Sample Fortification

- A. Rinse all glassware with HPLC grade water prior to fortification.
- B. To each container (beaker or volumetric flask) add 50.0 mL of water. The samples representing 0.100 ppb were prepared by fortification of 75.0 mL of water.
- C. Fortify each sample with methomyl by volumetric addition of dilutions of the primary stock solutions. It is important to keep the fortification volume as low as possible so that the polarity of the sample remains constant.

NOTE: The fortification levels produced in the control water samples for the method validation/recovery were 25.0, 5.00, 1.00, 0.200 and 0.100 ppb (three replicates at each level). An additional three water samples were left unfortified and utilized as control samples.

### III. Extraction

#### A. Control Water

1. Condition each SPE column with 2-3 column volumes of methanol followed by 2-3 column volumes of HPLC-grade water. NOTE: Ensure that the column does not elute to dryness until step 5.
2. At a vacuum pressure of 10 mm Hg, add the sample to the column.
3. Rinse the sample container with 10-15 mL of HPLC-grade water and add to the column.
4. Repeat step 3.
5. Allow the column to elute to dryness. Increase the vacuum to 25 mm Hg and leave column on full vacuum for 25-30 minutes. This "drying" procedure is necessary in order to remove all residual water.
6. Turn off the vacuum. Place collection tubes in vacuum manifold. Volumetrically add 4.0 mL of acetone to the column. Resume vacuum at 5-10 mm Hg and collect eluent.
7. Evaporate the eluent to dryness under a gentle stream of nitrogen.
8. Volumetrically pipet the requisite volume of 10% acetonitrile:90% HPLC grade water into the collection tube and vortex or mix well in order to ensure complete dissolution of the methomyl residues.

NOTE: a) For the 0.100 ppb recovery samples, a final volume of 0.750 mL would yield a concentration of 10.0  $\mu\text{g/L}$ .

b) The volume added for samples of unknown concentration would be such that the final concentration falls in the midrange of the standard curve.

9. Remove an aliquot of each solution and proceed to Section IV, High Pressure Liquid Chromatography.

#### IV. High Pressure Liquid Chromatography

A. **Method:** The acetonitrile-water mobile phase is pumped through a pulse damper, auto-sampler and chromatographic column to a mixing tee. With a second pump, a derivatizing reagent composed of potassium hydroxide, o-phthalaldehyde and 2-mercaptoethanol is pumped through a pulse damper and three-way valve (used for washing the pump) into the opposing port of the mixing tee. The combined eluents then pass through (2) 3-meter teflon capillary delay tubes heated to 125 °C. It is in this coil that methomyl is hydrolyzed to methylamine which subsequently is derivatized. The reaction tube is connected to a single string bead reactor which in turn is connected to a length of PTFE tubing, which serves as a heat sink. The eluent then passes through and is detected by the fluorescence detector. To the effluent end of the detector is connected a coil of PTFE tubing which terminates with a submerged back pressure regulator which creates a 3-4 bar back pressure and ensures that the mixture in the coil does not boil (see Figure 1).

B. **Instrumental Conditions:** Waters Model M6000A liquid chromatograph solvent pump equipped with Waters Intelligent Sample Processor Model 710 B, Fiatron Model TC-55 Column Heater, Kratos Model 980 fluorescence detector and Hewlett-Packard Model 3396A integrator.

Column: Phenomenex Ultremex 5 $\mu$  ODS, 250 mm (length) x 4.6 mm ID

Mobile Phase: 25% acetonitrile:75% HPLC grade water

Mobile Phase Flow rate: 1.4 mL/minute

KOH Concentration: 0.01 M

KOH Flow rate: 0.6 mL/minute

Reaction Coil Temperature: 125°C

Pressure: 2500 psi

Chartspeed: 0.3 cm/minute

Injection Volume: 100  $\mu$ L

Excitation wavelength: 229 nm

Emission wavelength: 418 nm

PMT signal: 0.1 V

Sensitivity: 0.050 AUFS

Rise Time: 2.0 seconds

Attenuation: 2<sup>3</sup>

Threshold: 3

Peak Width: 0.20 seconds

### C. Analysis

1. Prepare standard solutions containing methomyl. Standard solution concentrations used for the recovery study were 10.0, 25.0, 50.0, 75.0 and 100  $\mu\text{g/L}$ .
2. Inject 100  $\mu\text{L}$  of the 10.0  $\mu\text{g/L}$  standard solution. Adjust the attenuation so that the peak signal results in at least a fifteen percent deflection from the baseline.
3. Inject 100  $\mu\text{L}$  of each of the standards, document the peak areas, and determine the correlation coefficient of the line. Proceed to step 4 if the correlation coefficient is greater than or equal to 0.985.
4. Inject 100  $\mu\text{L}$  of several samples.
5. Identify the methomyl peak by its retention time and document the peak area.
6. After each set of samples, reinject 100  $\mu\text{L}$  of each of the standards and document the peak areas.
7. Construct a standard curve for each analyte (using all standard results) by plotting peak area observed versus the concentration ( $\mu\text{g/L}$ ) of the standard injected.
8. The standard linear regression analysis for methomyl is used to determine the concentration in each sample.
9. In order to determine the analytical result for each sample, the following equation is used:



Analytical Result ( $\mu\text{g/L}$ ) = A X D.F.

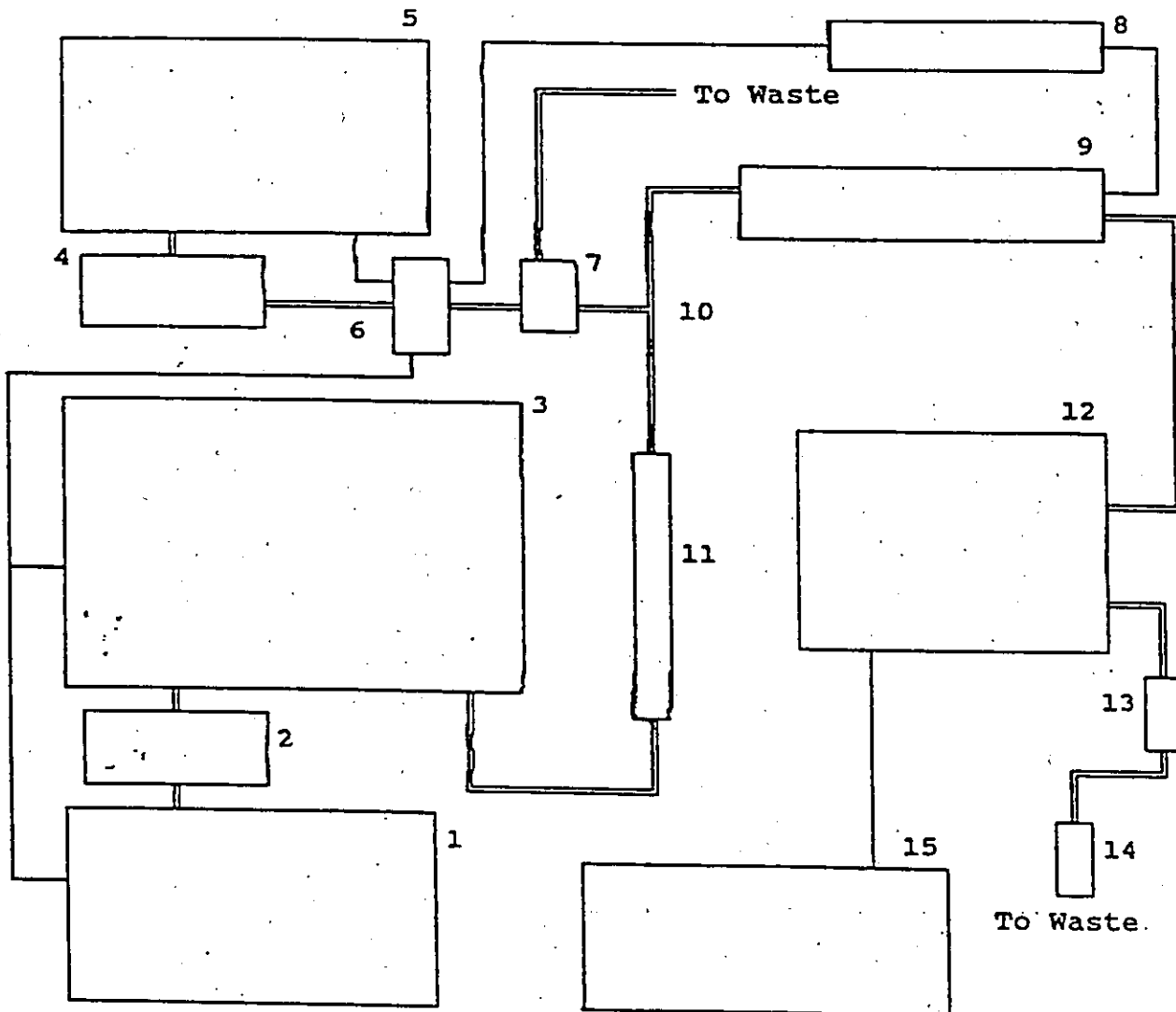
where:

Analytical Result = concentration of methomyl

A = concentration ( $\mu\text{g/L}$ ) of sample from the regression analysis

D.F. = dilution factor, ratio of the final volume (mL) of the sample to the initial volume (mL) of sample used

Figure 1. Schematic of HPLC-PCR system utilizing OPA derivatization and fluorescence detection of methomyl.



- |                            |                             |
|----------------------------|-----------------------------|
| 1. Mobile Phase Pump       | 9. Post Column Reactor      |
| 2. Pulse Damper            | 10. Mixing Tee              |
| 3. Autosampler             | 11. Analytical Column       |
| 4. Pulse Damper            | 12. Fluorescence Detector   |
| 5. Derivatization Pump     | 13. Back Pressure Coil      |
| 6. Low Pressure Transducer | 14. Back Pressure Regulator |
| 7. Purge Valve             | 15. Integrator/Computer     |
| 8. Heater Controller       |                             |

Springborn Laboratories, Inc.