Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Thiram

MRID #: 452286-02

Matrix: Water

Analysis: HPLC/ELCD

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ANALYTICAL METHOD FOR DETERMINATION OF THIRAM IN POND WATER

PRINCIPLE OF THE METHOD

The water sample is passed through a SPE C-18 cartridge, under vacuum. The Thiram is retained on the cartridge and eluted with acetonitrile. The analysis is by LC with an electrochemical detector. The quantitation is by external standard method, using a solution of Thiram to generate the calibration plot. The LOQ for the method is 0.1 ppb.

MATERIALS

A. EQUIPMENT

1. Analytical Evaporator, Model "N-Evap", Organomation Associates, Inc. The evaporator is connected to a house nitrogen line fitted with a filter and a pressure regulator, Balstone Filter Products
2. Balance, top load, Model Mettler PL 1200
3. Balance, analytical, Model 1602MP, Sartorius
4. Vortex mixer, Fisher Scientifc
5. Vacuum manifold for SPE cartridges, 12 port, Supelco
6. House vacuum system
7. Water purification system, Model Milli-Q, Millipore
9. pH meter, Orion, model 520A
9. Automatic pipette, 250 µL, Model 2 dpa, Rainin

Note: Equivalent equipment from other sources can be employed.

B. SUPPLIES

1. Assorted volumetric disposable pipettes, Fisher Scientific
2. Customary analytical laboratory glassware and supplies
3. Filtration suction flask, 1L, Fisher Scientific
4. 5 mL volumetric flasks
5. 2g, C-18 SPE cartridges, Waters
6. 0.2µm Nylon, 47mm Filter Membrane, Lida Man. Corp.

Note: Equivalent supplies from other sources can be employed. It is recommended, however, that Waters SPE cartridges be used.

C. CHEMICALS AND REAGENTS

1. Acetonitrile, Fisher Scientific, HPLC grade
2. Water, Milli-Q purified
3. Ammonium acetate, Fisher Scientific, reagent grade
4. Acetic acid, glacial, Fisher Scientific, Trace Metal grade

Note: solvents and chemicals of equivalent purity from other sources can be used.

D. WATER SOURCE AND CHARACTERIZATION

This method should be applicable for water from various sources. Pond water from Preston Hill Pond, Middlebury, CT was collected for use in this study. The pond water is characterized by its dissolved organic carbon, pH, total hardness and silt content.

E. PREPARATION OF THE STANDARD SOLUTIONS

Prepare two liters of HPLC grade, (Milli Q) water at pH ~ 3 (2.7 – 3.1), by adding about 80 µL of acetic acid/L. This is Milli Q water pH 3.

E-1. Thiram Stock Solution

On an analytical balance, accurately weigh approximately 50 mg of Thiram standard into a 5 mL volumetric flask and dilute to volume with acetonitrile. Sonicate the flask The concentration of the stock solution is 10 mg/mL. Correct the concentration for the purity of the standard: mg/mL x (percent purity /100).

E-2. Thiram Standard Working Solution

Pipette 25 µL of Thiram Stock solution B-1 into a 25 mL volumetric flask and dilute to volume with Milli Q water, pH3. The concentration of the solution is 10 µg/mL (10ppm).

E-3. Thiram Fortification Solution for Recoveries and for Calibration Plot

Pipette 1.0 mL of Thiram working solution B-2 into a 10 mL volumetric flask and dilute to volume with Milli Q water pH 3. The concentration of the solution is 1.0 µg/mL (1ppm).

E-4a. Calibration Plot for Thiram in the 0.1 ppb Range

For diluting standard solutions for the calibration curve use solvent which is prepared by mixing 70% of acetonitrile with 30% HPLC water pH 3. Mix and equilibrate the solvent. This is solvent I.

Into three 5 mL volumetric flasks, pipette 80, 100 and 120 µL of Thiram E-3 (1ppm) solution. Dilute to volume with solvent I. The concentration of the resulting standard solutions are 16, 20, and 24 µg/mL.

E-4b. Calibration Plot for THIRAM in the 1 ppb Range

Into three 5 mL volumetric flasks, pipette160, 200 and 240 µL of Thiram E-3 (1ppm) solution. Dilute to volume with solvent I. The concentration of the resulting standard solutions are 32, 40, and 48 µg/mL.

F. FORTIFICATION OF WATER SAMPLES

Filter required volume of pond water through the 0.2µm nylon filter membrane. To prevent breakdown of the Thiram, before fortification, the pond water has to have maintained pH ~ 2.7 - 3, (~200 µL of acetic acid / L of water, mix).

F-1. Fortification with Thiram 0.1 ppb level

Measure 1000 mL of pond water using a 1000 mL graduated cylinder. Decant the sample into a 1 L glass bottle. Using a 250 µL syringe, add 100 µL of fortification solution E-3, (100ng/1000mL). Mix very well.

F-2 Fortification with Thiram at the 1 ppb level

Measure 200 mL of pond water using a 250 mL graduated cylinder. Decant the sample into a .5 L glass bottle. Using a 250 µL syringe, add 200 µL of fortification solution B-3 (200ng/200mL).). Mix very well.

G. EXTRACTION PROCEDURE

Prepare about 0.5L of solvent II by mixing 40% of acetonitrile with 60% HPLC water pH 3. Mix well and equilibrate. This is solvent II.

Condition 2g C-18 SPE cartridges with 5mL ACN and 10mL Milli Q water pH3, in sequence.

1. Place a C-18 SPE cartridge onto 1L vacuum flask attached to a house vacuum. Pass fortified pond water at the rate of about 1L / 20 min.
2. Remove cartridge and Assemble on a vacuum manifold. Wash with 10mL of solvent II.
3. Without removing the cartridge from the manifold, pass 1mL of acetonitrile to the waste.
4. Substitute the waste container with a 5mL volumetric flask and elute the analyte with 5mL of acetonitrile, exactly to the meniscus (if necessary, concentrate under nitrogen). Mix and transfer to an autosampler vial for LC-ECD analysis.

H. LC-ECD CHROMATOGRAPHY OPERATING CONDITIONS

H-1. LC Opration Parameters

Column: Phenomenex, Luna 250 x 4.60 mm, 5µ, ODS (2), with guard cartridge, Brownlee New Guard RP-18
Mobile phase: isocratic, 50:50 (v:v) acetonitrile and 0.01M ammonium acetate @pH 3.5, (to 0.01M amm. Acetate add ≈3mL of acetic acid, mix)
Injection volume: 25µL
Flow: 1mL/min
Run time: 20min

H-2. ECD Opration Parameters

Mode DC
Glassy carbon electrode, potential 950mV
Range, 50nA
Time constant, 1sec.

I. CALCULATIONS

I-1 Calibration Plot

Peak areas of the standard Thiram are the dependent y variables and the concentrations of the standard solutions, expressed as ng/mL, are the independent x variables. They are used to generate a linear regression equation to determine the intercept, the slope, and the linearity of the detector response (coefficient of determination) R^2.

$$Peak\ Area = Intercept + Slope\ x\ (ng/mL)_{std}$$

I-2. Calculate the amount of Thiram in the extract

Using the peak area of Thiram found in the extract **y'**, determine the concentration, expressed as ng/mL, **x'**, using the following equation:

$$x',\ Thiram,\ (ng/mL) = \frac{(Peak\ Area_{y'} - Intercept)}{Slope}$$

I-3. Calculate the amount (ng) of Thiram found in the sample

ng = ng/mL x Volume of the sample (mL)

I-4. Calculate the % Recovery of Thiram from fortified pond water

Divide the value calculated in equation I-2, which is the amount of analyte found in the extract, by the amount of analyte added to the fortified pond water, multiply this value by 100, and adjust by purity of standard.

$$\%Recovery = \frac{ng\ Found}{ng\ Added} x100x\%purity$$

Example of the calculation method:

A water sample was fortified at the 0.1 ppb.level with Thiram and analyzed on 2/7/00. Sample #1, (Appendix B).

100 ng Thiram were added to a 1000 mL water sample. The volume of an extracted sample is 5mL, and it the same as the volume for calibration plot. The concentration for the calibration curve is expressed as ng/mL (16ng/mL, rather then 80ng/5mL, etc.). The peak area in the extract was 4204.94.

The concentration, ***x'***, ng/mL of Thiram in the extracted sample was calculated using the equation:

$$x',\ (ng/mL),\ Thiram = \frac{(Peak\ Area_{y'} - Intercept)}{Slope}$$

{4204.94-(-217.19)}/249.19=.17.75 ng/mL

The *Intercept* and the *Slope* are calculated from calibration plot.

% Recovery:

$$\frac{ng\ found}{ng\ added} x\ 100 x\%purity = \%\mathrm{Rec.}$$

(17.75 / 20) x 100 x 99.8% = 88.5 % Recovery

J. INJECTION SEQUENCE

Inject the standards, starting with the lowest concentration, five extracts, and set of standards again. Usually, at the beginning of the sequence the controls and reagent blank are injected. This completes the injection sequence.
Note 1: Reagent blank: depending on the level analyzed, either 200mL or 1000mL of Milli Q water pH 3 is brought through the entire extraction and concentration procedure identically to the samples.