

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Etridiazole

MRID #: 448754-01

Matrix: Water

Analysis: GC/NPD

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Study Title

Analytical Method for Determining Etridiazole and its Metabolites in Water

Data Requirement

Dutch Regulatory Guidelines under Section G2.5
E.U. Commission Directive 96/46EC (16 July 1996)
U.S. EPA OPPTS 850.7100

448754-01

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Study Completion Date

June 21, 1999

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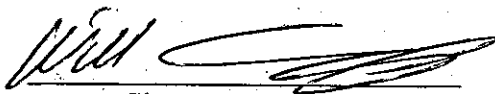
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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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Company: Uniroyal Chemical Company, Inc.

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Study No. 98249

This study was conducted in accordance with EPA Good Laboratory Practice Standards, 40 CFR 160.

However, nonvalidated computer systems were used in the course of this study.

The GC/MS/MS method was not specified in the protocol as a confirmatory technique.

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QUALITY ASSURANCE STATEMENT

The study reported here, Analytical Method for Determining Etridiazole and its Metabolites in Water, (Study No. 98249) was inspected/audited by the Quality Assurance Unit, and the findings were reported to the Study Director and Management on the following dates:

<u>Date(s) Inspected/Audited</u>	<u>Study Phase</u>	<u>Date Reported to Management & Study Director</u>
October 30, 1998	Protocol Review 1	October 30, 1998
November 16, 1998	Protocol Review 2	November 16, 1998
March 29, 1999	0.5 ppb fortification of pond water, day 1	March 30, 1999
June 7, 1999	Raw data/final report audit	June 8, 1999
June 17, 1999	Final Report Audit	June 17, 1999

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CERTIFICATION OF AUTHENTICITY

I, the undersigned, hereby declare that this study was performed under my supervision according to the procedures described herein and that this report provides a true, unaltered, and accurate record of the results obtained.

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APPROVAL

Uniroyal Chemical Company, Inc. Study No. 98249
Crop Protection Research and Development
Registration Chemistry Section

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UPON REQUEST.

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I. SUMMARY

Etridiazole, 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) and its metabolite, 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) are extracted from water using a DVB C-18 SPE disk. The Etridiazole and dichloro-etridiazole are analyzed directly by GC using a nitrogen phosphorous detector. 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) is extracted from the water using a SAX SPE disk. The Etridiazole acid is converted to the Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate (T-28) using 1% sulfuric acid in ethanol. It is then analyzed by GC, using a nitrogen phosphorous detector. Mean recoveries of T-01, T-03, and T-28 from samples fortified at 0.1 ppb and 0.5 ppb are in the 70-110% range. The relative standard deviations (RSD) are $\leq 20\%$. The correlation coefficient (R) is ≥ 0.99 for each analyte. The method meets the criteria for both the EU and EPA.

II. MATERIALS

A. EQUIPMENT

1. Balance, analytical, Model AT 261 Delta, Mettler™
2. Balance, Model 1602 MP8-1, Sartorius
3. Vortex mixer, Fisher Scientific
4. Centrifuge, Model 228, Fisher Scientific
5. Vacuum manifold for SPE disks, 12 port, Supelco
6. Vacuum pump, 1/6 HP, Model 0211-U45N-G8CX, Gast®
7. Analytical Evaporator, Model "N-Evap", Organomation Associates, Inc. The evaporator is connected to house nitrogen line fitted with a filter and a pressure regulator, Balstone Filter Products
8. A pH Meter, Accumet™ 910, Fisher Scientific
9. Magnetic stirrer, Corning™
10. Gas Chromatograph, Hewlett Packard™ 6890+, equipped with a nitrogen phosphorous detector and an autosampler-injector Model HP 7683.
11. Computer with ChemStation HP 3365 on Windows 95 platform controls data capture
12. Finnigan MAT model TSQ 7000 equipped with a Varian™ 3400 gas chromatograph

Note: Equivalent equipment from other sources can be employed.

B. SUPPLIES

1. Centrifuge tubes, 15 mL graduated, conical-bottom with screw caps, Fisher Scientific
2. Kolmer evaporation tubes, 10 mL graduated, Fisher Scientific
3. Flasks, 1 L volumetric, Fisher Scientific

4. Filtration suction flask, 1 L, Fisher Scientific
5. DVB C-18 and SAX 50 mm solid phase extraction disks and reservoirs, Bakerbond Speedisk™, J.T. Baker®
6. Assorted volumetric disposable pipettes, Fisher Scientific
7. Customary analytical laboratory glassware and supplies

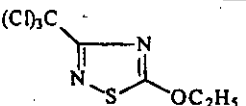
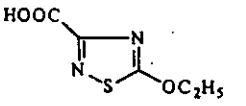
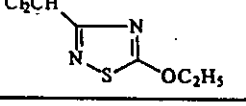
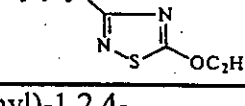
Note: Equivalent supplies from other sources can be employed. It is recommended, however, that SPE disks from J.T. Baker® be used.

C. CHEMICALS AND REAGENTS

1. Hexanes, GC Resolv™ grade, Fisher Scientific
2. Methanol, HPLC grade, Fisher Scientific
3. Methylene Chloride, Optima™ grade, Fisher Scientific
4. Water, HPLC grade, Fisher Scientific
5. Absolute Anhydrous Ethanol, AAPER Alcohol and Chemical Co. and Pharmco
6. Potassium Phosphate, J.T. Baker®
7. Sodium Hydroxide pellets, J.T. Baker®
8. Sulfuric Acid, J.T. Baker®

Note: Chemicals and reagents of equivalent purity from other sources can be employed.

D. STANDARDS-TEST SUBSTANCES

Standard	Uniroyal Chemical Co. Code	Source	CAS No.	Purity %	Molecular Weight	Structure
5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole	T-01	Olin Corp.	2593-15-9	99.6	247.52	
5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid	T-02	Uniroyal Chemical Co.	67472-43-9	100	174.17	
5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole	T-03	Uniroyal Chemical Co.	Not available	99.75	213.08	
Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate	T-28	Uniroyal Chemical Co.	Not available	100	202.2	

Note: All standards are stored in the freezer, except 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole, which is stored, refrigerated. Stability of the standards is expressed as the expiration date as stated on the COA. COA's are in Appendix II.

E. SAFETY AND HEALTH

This method should be performed by trained chemical personnel. Hazards associated with the chemicals used in this analytical method are shown in the MSDS sheets in Appendix III.

III. ANALYTICAL METHOD

A. PRINCIPLE OF THE METHOD

The water sample is passed through a DVB C-18 Speedisk™ under vacuum. The Etridiazole and 3-dichloro metabolite, which are retained on the disk, are eluted with hexane and concentrated under nitrogen. The analysis is by capillary GC with a nitrogen phosphorous detector. The quantitation is by external standard method, using a mixture of 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) and 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) standards to generate the two calibration plots.

The same water sample is then passed through a SAX Speedisk™ under vacuum. The Etridiazole acid (5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid) is eluted with 1% sulfuric acid in ethanol. The Etridiazole acid (T-02) undergoes derivatization in the concentration step under nitrogen. The analysis is by capillary GC with a nitrogen phosphorous detector. The quantitation is by external standard method, using Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate (T-28) to generate a calibration plot. A flow diagram of the analytical procedure is shown in Figure 1.

B. WATER SOURCE AND CHARACTERIZATION

This method should be applicable for water from various types of sources. Pond water from Preston Hill Pond (Uniroyal Chemical Co.), Middlebury, CT was collected for use in this study. The TOC content is 3.3 mg/mL. TOC varies from time to time. Water collected from the same site previously (August 1998), had a TOC of 8.3 mg/mL. Characterization results are located in Appendix IV.

C. PREPARATION OF REAGENTS

C-1. 1% Sulfuric Acid in Ethanol

Fill a 250 mL volumetric flask with ~200 mL of absolute ethanol. Pipette in 2.5 mL of concentrated sulfuric acid. Mix well and bring to the mark with additional absolute ethanol. Cap and store at room temperature.

C-2. Neutralization Solution**C-2a. 2 M NaOH Solution**

Using a top loading balance, weigh 40 g of NaOH and dissolve in 500 mL of HPLC grade water. Cap and store at room temperature.

C-2b. Phosphate Buffer

Slowly add 204 g of KH_2PO_4 to a 2 L beaker containing 400 mL of HPLC grade water, which is being stirred with a magnetic stirrer. Add 350 mL of the 2 M NaOH solution. Continue stirring until all the crystals are dissolved. Titrate to pH 7.0 with the 2 M NaOH solution. This should require the addition of about 100 –120 mL of the 2 M NaOH solution. Bring the final volume to 1 L with HPLC water. Cap and store at room temperature.

D. PREPARATION OF THE STANDARD SOLUTIONS**D-1. T-01, T-02, and T-03 Standard Solutions for Fortification****D-1a. T-01, T-02, and T-03 Fortification Stock Solutions**

On an analytical balance, weigh 50 mg of the T-01, T-02, and T-03 standards into a 50 mL volumetric flask and dilute to volume with absolute ethanol. Vortex the flask. The concentration of the stock solution is 1 mg/ mL. Correct the concentration for the purity of the standard (if necessary): $\text{mg/mL} \times (\text{Standard Purity} / 100)$.

D-1b. T-01, T-02, and T-03 Fortification Working Solution A

Pipette 1.0 mL of Fortification Stock Solution, D-1a, into 100 mL volumetric flask and dilute to volume with absolute ethanol. The concentration of the solution is 10,000 ng/mL.

D-1c. T-01, T-02, and T-03 Fortification Working Solution B for 0.1 and 0.5 ppb Recoveries

Pipette 1.0 mL of Fortification Working Solution A, D-1b, into 100 mL volumetric flask and dilute to volume with absolute ethanol. The concentration of the solution is 100 ng/mL.

D-2. Preparation of T-01 and T-03 Calibration Standards**D-2a. T-01 and T-03 Standard Stock Solution**

On an analytical balance, weigh 50 mg of the T-01 and T-03 standard into a 50mL volumetric flask and dilute to volume with hexane. Vortex the flask. The concentration of the stock solution is 1 mg/ mL. Correct the concentration for the purity of the standard (if necessary):
 $\text{mg/mL} \times (\text{Standard Purity} / 100)$.

D-2-b. T-01 and T-03 Standard Working Solution A

Pipette 1.0 mL of Standard Stock Solution, D-2-a, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 10,000 ng /mL.

D-2-c. T-01 and T-03 Standard Working Solution B

Pipette 10.0 mL of Standard Working Solution A, D-2-b, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 1000 ng /mL.

D-2-d. T-01 and T-03 Standard Working Solution C

Pipette 25.0 mL of Standard Working Solution A, D-2-b, into a 50 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 5000 ng /mL.

All standard solutions are kept in the freezer when not in use.

D-3. Dilutions of T-01 and T-03 Calibration Standard**D-3-a. T-01 and T-03 Calibration Standards for the 0.1 ppb level**

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-01 and T-03 Standard Working Solution B (D-2-c). Dilute to volume with hexane. The concentrations of the resulting standard solutions are: 50, 75, 100, 125, and 150 ng /mL, respectively.

D-3-b. T-01 and T-03 Calibration Standards for the 0.5 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-01 and T-03 Standard Working Solution C (D-2-d). Dilute to volume

with hexane. The concentrations of the resulting standard solutions are: 250, 375, 500, 625, and 750 ng/mL, respectively.

D-4. Preparation of T-28 Calibration Standard

D-4-a. T-28 Standard Stock Solution

On an analytical balance, weigh 50 mg of the T-28 standard into a 50 mL volumetric flask and dilute to volume with hexane. Vortex the flask. The concentration of the stock solution is 1 mg/mL. Correct the concentration for the purity of the standard (if necessary):
 $\text{mg/mL} \times (\text{Standard Purity} / 100)$.

D-4-b. T-28 Standard Working Solution A

Pipette 1.0 mL of Standard Stock Solution, D-4-a, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 10,000 ng/mL.

D-4-c. T-28 Standard Working Solution B

Pipette 10.0 mL of Standard Working Solution A, D-4-b, into a 100 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 1000 ng/mL.

D-4-d. T-28 Standard Working Solution C

Pipette 25.0 mL of Standard Working Solution A, D-4-b, into a 50 mL volumetric flask and dilute to volume with hexane. The concentration of the solution is 5000 ng/mL.

All standard solutions are kept in the freezer when not in use.

D-5. Dilution of T-28 Calibration Standard

D-5-a. T-28 Calibration Standards for the 0.1 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-28 Standard Working Solution B (D-4-c). Dilute to volume with hexane. The concentrations of the resulting standard solutions are: 50, 75, 100, 125, and 150 ng/mL, respectively.

D-5-b. T-28 Calibration Standards for the 0.5 ppb level

Into 10 mL volumetric flasks, pipette 0.5, 0.75, 1.0, 1.25, and 1.5 mL of T-28 Standard Working Solution C (D-4-d). Dilute to volume with hexane. The concentrations of the resulting standard solutions are: 250, 375, 500, 625, and 750 ng/mL, respectively.

E. FORTIFICATION OF WATER SAMPLES**E-1. Fortification with T-01, T-02, and T-03 at the 0.1 ppb level**

Fill a 1 L volumetric flask with ~975 mL of water. Pipette 1 mL of Fortification Working Solution B (D-1-c) into the flask. Bring to the mark with additional pond water. Mix well, shaking by hand.

E-2. Fortification with T-01, T-02, and T-03 at the 0.5 ppb level

Fill a 1 L volumetric flask with ~975 mL of water. Pipette 5 mL of Fortification Working Solution B (D-1-c) into the flask. Bring to the mark with additional pond water. Mix well, shaking by hand.

F. EXTRACTION AND CLEANUP PROCEDURE**F-1. Extraction and cleanup of T-01 and T-03**

1. Mount a DVB C-18 Speedisk™ to a SPE manifold. Wash with 20 mL of methylene chloride. Allow the disk to soak for at least 2 minutes. Apply a low house vacuum to dry the disk. Add hexane (2 X 15 mL) and dry the disk again.
2. Condition the disk with 10 mL of methanol. Allow disk to soak for 1 minute. Pull through most of the remaining methanol, leaving 3-5 mm of methanol above the surface of the disk. From this point to the end of sample addition, do not allow the disk to run dry.
3. Repeat step 2 using 10 mL of HPLC grade water.
4. Transfer the disk to a 1 L suction flask connected to a vacuum pump. Attach the reservoir/adaptor to the disk housing. Add water sample under vacuum at about 15 inches of Hg. (Allow 3-5 minutes for 1 L sample.) Transfer disk back to SPE manifold and dry under full vacuum (~20 inches of Hg) for at least 30 minutes. Transfer the eluted water back to the 1 L flask to be saved overnight, at ambient temperature for the extraction of T-02.
5. Using gravity, elute the 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) and 5-Ethoxy-3-dichloromethyl-1,2,4-thiadiazole (T-03) with 10 mL of hexane

6. into a 10 mL Kolmer evaporation tube. Apply gentle vacuum, < 5 inches of Hg., to elute any remaining analyte.
7. Concentrate the extract under a gentle stream of nitrogen gas at ambient temperature to less than 1 mL. Bring to the 1 mL mark with hexane. Vortex gently and transfer to autosampler vial for GC-NPD analysis.

F-2. Extraction and cleanup of T-02.

1. Mount a SAX Speedisk™ to a SPE manifold. Wash with 20 mL of 1 % H₂SO₄ in ethanol. Allow the disk to soak for at least 2 minutes. Apply a low house vacuum to dry the disk.
2. Condition the disk with 10 mL of methanol. Allow disk to soak for 1 minute. Pull through most of the remaining methanol, leaving 3-5 mm of methanol above the surface of the disk. From this point to the end of sample addition, do not allow the disk to run dry.
3. Repeat step 2 using 10 mL of HPLC grade water.
4. Transfer the disk to a 1 L suction flask connected to a vacuum pump. Attach the reservoir/adaptor to the disk housing. Add water sample from F-1, step 4 (pg. 17) under vacuum at 15 inches of Hg. (Allow 3-5 minutes for 1 L sample.) Transfer disk back to SPE manifold and dry under full vacuum (~20 inches of Hg) for at least 30 minutes. Discard the eluted water.
5. Using gravity, elute 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) with 10 mL of 1 % H₂SO₄ in ethanol into a 15 mL graduated conical centrifuge tube. Apply gentle vacuum, < 5 inches of Hg., to elute any remaining analyte.

G. DERIVATIZATION OF T-02 WITH 1 % H₂SO₄ IN ETHANOL

1. Concentrate and derivatize the sample from F-2, step 5, by immersing the conical centrifuge tube to the 10 mL mark in a 50°C (±1°C) water bath and evaporating the solvent under a gentle stream of nitrogen gas to a final volume of 2 mL. This takes about 30-45 minutes.
2. Cool the sample to room temperature and add 5 mL of neutralization solution (phosphate buffer). Vortex at high speed for 30 seconds.
3. Add 5 mL of hexane to sample and vortex for 1 minute at high speed.
4. Centrifuge the sample for 2 minutes using a single speed centrifuge at about 3300 rpm.
5. Transfer 4.5 mL of the hexane layer to a 10 mL Kolmer evaporation tube.
6. Repeat steps 3 and 4.
7. Transfer the second 4.5 mL of the hexane layer to the 10 mL Kolmer evaporation tube. The final volume of hexane extract should be exactly 9 mL. The extract contains Ethyl 5-ethoxy-1,2,4-thiadiazole-3-carboxylate (T-28).

8. Concentrate the extract under a gentle stream of nitrogen gas at ambient temperature to less than 1 mL. Bring to 1 mL mark with hexane. Vortex gently and transfer to autosampler vial for GC-NPD analysis.

H. GC-INSTRUMENTATION

H-1. Description

Gas Chromatograph, Hewlett Packard, Model HP 6890+, equipped with a nitrogen phosphorous detector and an autosampler-injector Model HP 7683.

Instrument parameter settings and a computer with ChemStation HP 3365 on Windows 95 platform controls data capture.

H-2. Conditions

Column Type: J & W DB-17, 30m, 0.25 μ m, 0.25 Film Thickness Model #: 122-1732				
NPD Detector *: Hydrogen: 3.0 mL/min Air: 60 mL/min Constant column & Makeup 10 mL/min Makeup gas Helium Adjust offset 35 Temp. 325°C				
Injector: Splitless Temp: 210°C Pressure: 25 psi Purge Time: 2.00 min Purge flow: 60.0 mL/min Liner: Cyclosplitter Gooseneck				
Carrier gas: Helium, at 2.7 mL/min				
Oven Temp Program:	Initial temperature: 50°C Initial Time: 2.00 min			
	Ramp #	Rate °C/min	Final Temperature °C	Final Time min
	1	30.0	100	0
	2	10.0	180	0.5
	3	15.0	220	0
	4	50.0	270	7
Final Run Time 22.83 min				

**Note: NPD beads have a very short life.*

H-3. Calibration and Injection Sequence

1. Start the sequence by injecting the solvent blank and then the standards, starting with the lowest concentration.
2. Inject a solvent blank and then a reagent blank followed by the control samples.
3. Inject fortified controls and then the samples.
4. Complete the sequence by injecting the standards, starting with the lowest concentration.

I. POTENTIAL INTERFERENCES

This method could have potential interference from other halogenated pesticides that might elute with similar retention times.

Contamination from SPE disks can cause interference, therefore washing the disk as described in Section F-1 and F-2 is very important. A confirmatory technique should be used as required by the guidelines, if a problem is suspected (see section J).

J. CONFIRMATORY TECHNIQUE

The method for the identification of T-01, T-03, and T-02 as T-28 was by GC/MS/MS with chemical ionization(CI). Confirmation of the identity of T-01, T-03, and T-02 as T-28 was made by the comparison of the mass spectra of the peaks in the extract isolated from the 0.1 ppb fortified pond water with the spectra of the standards. The molecular ions for T-01, T-03, and T-28 are 247 and 213, and 202, respectively. The daughter ions of m/z 247 are 218 and 182 m/z. The daughter ions of m/z 213 are 184 and 148 m/z. The daughter ions of m/z 202 are 174, 146, and 128 m/z. Chromatograms and spectra are in Appendix V.

K. TIME REQUIRED FOR ANALYSIS

The time required to complete the extraction and concentration of T-01 and T-03 is about five hours. The time required to complete the extraction, derivatization and concentration of T-28 is about six hours. The GC-NPD analysis is usually carried out overnight, using an autosampler.

L. MODIFICATIONS OF POTENTIAL PROBLEMS

None.

M. METHODS OF CALCULATION

M-1. Calibration Plot

Peak area counts of the standard (T-01, T-03, or T-28) is the dependent variable and the concentration of the standard solution, expressed as $\eta\text{g/mL}$, is the independent variable which are employed to generate a linear regression equation to determine the intercept, the slope and the linearity of detector response (coefficient of determination, R^2).

$$Pk.area = Intercept + Slope \times (\eta\text{g/mL})_{Std.}$$

M-2. Calculate the amount of T-01 or T-03 in the Hexane Extract

Using the peak area of T-01 or T-03 found in the extract, the concentration is determined using the following equation:

$$T-01(\eta\text{g/mL}) = \frac{Pk.area_{sample} - Intercept}{Slope}$$

If peaks are found in the control samples. A corrected peak area value is determined using the following formula:

$$Peak\ area_{sample\ corrected} = Peak\ area_{sample} - Peak\ area_{control}$$

M-3. Calculate the amount of T-02 Equivalents in the Hexane Extract

Using the peak area of T-28 in the analyte calculate the concentration of T-02 equivalents in the hexane extract and multiply this by the ratio of molecular weights of the two standards (T-02/T-28) and also multiply by the volume correction (10/9mL, see Section G, steps 5, 6 & 7).

$$T-02(\eta\text{g/mL}) = \frac{Pk.area_{sample} - Intercept}{Slope} \times \frac{174}{202} \times \frac{10}{9}$$

M-4. Calculate the amount (ppb) of T-01, T-02, or T-03 found in the sample

Divide the amount of analyte calculated in Section M-2 or M-3 by the sample volume, expressed in mL.

$$ppb = \frac{\eta g / mL}{mL}$$

M-5. Calculate % Recovery of T-01, T-02, or T-03 from Fortified Control

Divide the value calculated in Section M-2 or M-3, which is the amount of analyte found in the extract, by the amount of analyte added to the control sample and multiply this value by 100.

$$\% Recovery = \frac{\eta g Found}{\eta g Added} \times 100$$

M-6. Calculate the 95% Confidence Limits

$$CL = \frac{t \times SD}{\sqrt{n}}$$

Where SD = standard deviation

n = the number of observations

t = the value t for n-1 degrees of freedom at 95% confidence as taken from the table C.3, page 267 of Quality Assurance of Chemical Measurements, John K. Taylor, Lewis Publishers Inc. 1987.

N. CHROMATOGRAMS

Copies of representative chromatograms of the solvent blank, standard, reagent blank, control and fortified control for the 0.1 ppb level of T-01 and T-03 are shown in Figures 2 - 6. Copies of representative chromatograms of the solvent blank, standard,

reagent blank, control and fortified control for the 0.1 ppb level of T-02 as T-28 are shown in Figures 7-11. Copies of representative chromatograms of the solvent blank, standard, reagent blank, control and fortified control for the 0.5 ppb level of T-01 and T-03 are shown in Figures 12-16. Copies of representative chromatograms of the solvent blank, standard, reagent blank, control and fortified control for the 0.5 ppb level of T-02 as T-28 are shown in Figures 17-21.

III. RESULTS AND DISCUSSION

A. METHOD VALIDATION RESULTS

The analytical method for determining Etridiazole and its metabolites was validated at 0.1 ppb and 0.5 ppb for accuracy (recovery), precision, linearity and specificity. Four samples of Preston Hill Pond water were fortified at each level, extracted and analyzed by GC-NPD. The experiment was repeated on a subsequent day. Mean recoveries were all within the 70-110% range, the relative standard deviations (RSD) were $\leq 20\%$, and the correlation coefficient (R) was ≥ 0.99 for each analyte. Linearity data was determined for each level using a five point calibration curve to obtain a correlation coefficient (R) for T-01, T-03, and T-28 (Table I). The correlation coefficient (R) for T-01 ranged from 0.9984 – 0.9998. The correlation coefficient (R) for T-03 ranged from 0.9971 – 0.9998. The correlation coefficient (R) for T-28 ranged from 0.9934 – 0.9960. Results reported in tables are rounded up values from the spreadsheets.

B. ACCURACY/RECOVERY

Mean recoveries for 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) at the 0.1 ppb fortification level were 97.8 % for Day 1 and 86.0 % for Day 2. The RSD for Day 1 was ± 2.61 and ± 11.6 for Day 2. The confidence level at 95% was ± 2.13 and ± 8.30 , respectively. A summary of the results are found in Table II. Mean recoveries for 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) at the 0.1 ppb fortification level were 103 % for Day 1 and 79.2 % for Day 2. The RSD for Day 1 was ± 8.59 and ± 12.8 for Day 2. The confidence level at 95% was ± 9.98 and ± 5.17 , respectively. A summary of the results are found in Table III. Mean recoveries for 5-Ethoxy-3-(dichloromethyl)-1,2,4-thiadiazole (T-03) at the 0.1 ppb fortification level were 108 % for Day 1 and 89.3 % for Day 2. The RSD for Day 1 was ± 5.09 and ± 6.90 for Day 2. The confidence level at 95% was ± 4.59 and ± 5.15 , respectively. A summary of the results are found in Table IV.

Mean recoveries for 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01) at the 0.5 ppb fortification level were 101 % for Day 1 and 91.0 % for Day 2. The RSD for Day 1 was ± 1.71 and ± 7.34 for Day 2. The confidence level at 95% was ± 1.45 and ± 5.59 , respectively. A summary of the results are found in Table V. Mean recoveries for 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) at the 0.5 ppb fortification level were 72.2 % for Day 1 and 72.8 % for Day 2. The RSD for Day 1 was ± 20.0 and ± 16.4 for Day 2. The confidence level at 95% was ± 3.02 and ± 3.70 , respectively. A summary of the results are found in Table VI. Mean recoveries for 5-Ethoxy-3-(dichloromethyl)-1,2,4-thiadiazole (T-03) at the 0.5 ppb fortification level were 91.9 % for Day 1 and 85.6 % for Day 2. The RSD for Day 1 was ± 1.17 and ± 7.76 for Day 2. The confidence level at 95% was ± 0.898 and ± 5.55 , respectively. A summary of the results are found in Table VII.

C. PRECISION

The analytical method precision was determined from the fortified pond water samples. It is expressed as the relative standard deviation (RSD). A sample was injected six times on two different days for the 0.1 ppb and 0.5 ppb levels. The average results for T-01, T-02 as T-28 and T-03 at the 0.1 ppb level were 3.22, 3.88, and 1.73 %, respectively (Table VIII). The average results for T-01, T-02 as T-28 and T-03 at the 0.5 ppb level were 1.55, 1.99, and 1.16 %, respectively (Table IX).

D. LIMIT OF DETECTION (LOD)

The LOD for most studies is defined as 3 times the baseline noise level. Due to interference peaks found in the chromatograms of some of the extracts in this study, the LOD is defined as $\frac{1}{2}$ the peak area of the lowest quantification standard, 0.05 ppb. The LOD, therefore, is about 0.025 ppb.

E. LIMIT OF QUANTITATION (LOQ)

The LOQ for the purpose of this study was targeted at 0.1 ppb which was the lower fortification level employed. At this level, mean recoveries were within the acceptable range of 70 and 110 % with the $RSD \leq 20\%$.

F. RUGGEDNESS

No ruggedness testing was done.

G. SELECTIVITY and SPECIFICITY

The analytical method using gas chromatography with nitrogen phosphorous detection shows that there is excellent selectivity (separation) of Etridiazole from its metabolites.

The specificity of each analyte was determined by capillary GC/MS/MS with CI using a Finnigan MAT model TSQ 7000 equipped with a Varian 3400 gas chromatograph. Extracts of water samples fortified at the 0.1 ppb level were used for confirmation. The samples were concentrated to 100 μ L to enhance sensitivity. The reconstructed ion chromatogram of the sample containing T-01 and T-03 show the molecular ions 247 and 213, respectively, corresponding to the standard chromatograms and spectra. The daughter ions of molecular ion 247 were 218 and 182 m/z for the sample and standard, confirming the structure. The daughter ions of molecular ion 213 were 184 and 148 m/z for the sample and standard, confirming the structure. The reconstructed ion chromatogram of the sample containing T-28 shows the molecular ion 202 corresponding to the standard chromatograms and spectra. The daughter ions of molecular ion 202 were 174, 146, and 128 m/z for the sample and standard, confirming the structure. Chromatograms and spectra showing these confirmations are in Appendix V.

H. LIMITATIONS

None are known.

IV. CONCLUSION

This analytical method is applicable for the analysis of Etridiazole (5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (T-01)), 5-Ethoxy-1,2,4-thiadiazole-3-carboxylic acid (T-02) and 5-Ethoxy-3-(dichloromethyl)-1,2,4-thiadiazole (T-03) in water from any source at the 0.1 ppb level. Interferences can occur from improperly washed SPE disks. The expected recoveries and relative standard deviations are excellent and within the USA and EU regulatory guidelines.

V. REFERENCES

Taylor, John Keenan, Quality Assurance of Chemical Measurements, Lewis Publishers, 1987.

Dutch Regulatory Guidelines under Section G2.5

E.U. Commission Directive 96/46EC (16 July 1996)

U.S. EPA OPPTS 850.7100