Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Metalaxyl

MRID #: 422598-04

Matrix: Water/Soil

Analysis: GC/NPD

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APPENDIX IV. METHODS FOR THE DETERMINATION OF METALAXYL AND CGA-62826 RESIDUES IN SOIL/HYDROSOIL, WATER, AND FILTER PAPER

Method for the Determination of Metalaxyl and CGA-62826 Residues in Soil/Hydrosoil

METHOD SUMMARY

The analytical methodology for the measurement of metalaxyl and CGA-62826 residues in soil/hydrosoil is presented below. The average recovery for fortified samples was 88.5% ± 14.6% for metalaxyl and 81.2% ± 15.4% for CGA-62826

Samples were extracted from soil with 50% methanol in water. Samples were centrifuged to separate the solid and liquid layers. The methanol was driven off using a rotary flask evaporator and the compounds removed from the water phase with octadecyl (C-18) solid phase extraction (SPE) column after filtration and acidification. Samples were stripped from the SPE columns with acetone and dried under nitrogen. Triflouroboron in butanol (BF_3-butanol) was added and the samples incubated for 45 minutes. The reaction was stopped with the addition of an aqueous buffer solution and a solvent extraction was performed with hexane. Samples were concentrated and brought to final volume in iso-octane for gas chromatographic nitrogen phosphorous detection.

EQUIPMENT AND REAGENTS

Equipment

1. Balance: four-place analytical balance
2. Separatory funnels: glass, assorted sizes
3. Round bottom flasks: glass, assorted sizes
4. Powder funnels: glass
5. Beakers: glass, assorted sizes
6. Flasks: Volumetric, assorted sizes
7. Instrument: Hewlett Packard 5890 gas chromatograph equipped with a nitrogen phosphorus detector, 7673 autosampler and 3396 integrator
8. Pipets: glass Volumetric, assorted sizes
9. bottles: Wheaton; assorted sizes, 2 mL and 200 µL gc vials with teflon lined lids and metal crimp tops.
10. Syringes: Hamilton, assorted sizes

11. C-18 octadecyl Solid Phase Extraction (SPE) columns: Baker and Analytichem International
12. Temperature controlled water bath
13. Centrifuge tubes; glass, 15 mL glass and 85 mL plastic
14. Glass fiber filters, 7 cm
15. Buchner Funnels: 9 cm
16. Centrifuge
17. Reciprocating Shaker
18. Rotary flask evaporator(s)
19. Nitrogen gas concentrator apparatus
20. Solid Phase Extraction (SPE) apparatus

Reagents

1. Acetone, reagent grade
2. Hexane, high purity solvent
3. 2,2,4 trimethyl pentane, iso-octane: residue grade
4. Sodium sulfate, reagent grade
5. Metalaxyl, lot No. S87-1208, 95.8% active ingredient, supplied by Ciba Geigy Chemical Corporation, Greensboro, North Carolina
6. CGA-62826, lot No. S85-0650, 98% active ingredient, supplied by Ciba Geigy Chemical Corporation, Greensboro, North Carolina
7. Butyl ester of CGA-62826, prepared as 100% active ingredient, synthesized by Springborn Laboratories from CGA-62826.
8. BF_3-Butanol: Supelco, Inc. Bellefonte, Pennsylvania. cat NO. 3-3125M
9. Soil: Agricenter soil shipped to Springborn Laboratory, Inc. Warenam Massachusetts from Agricenter International, Incorporated, Memphis, Tennessee. This soil was used to matrix match method validation and quality control samples.
10. High Purity water: (NANOpure water), glass distilled water passed through a water purification system; Barnsted NANOpure™ water purification system.
11. Sulfuric Acid: 0.5 M (28 mL concentrated per liter)
12. Methanol: residue grade
13. Sodium phosphate, dibasic: reagent grade

PROCEDURE

Preparation of Stock

Approximately (ca.) 0.25 gram (g) of metalaxyl and 0.1 g of CGA-62826 were weighed on a balance in 100-milliliter (mL) volumetric flasks and dissolved in acetone. A metalaxyl primary stock solution was also prepared by weighing approximately 0.1 g of the active ingredient into a 100-mL volumetric flask and dissolved to volume with iso-octane. The metalaxyl and CGA-62826 stock solutions (ca. 1 mg/mL) were stored refrigerated (4 - 10 °C) in 100-mL amber serum vials with a teflon-lined lids. These stocks were used, with appropriate dilution, for quantitation (prepared in iso-octane) and fortification (prepared in acetone). Instrumental standards for CGA-62826 were prepared after derivatization to the butyl ester. Instrumental standards were prepared with both metalaxyl and CGA-62826 in calibration standard solutions.

Sample Fortification

Method validation/recovery samples were prepared in Agricenter soil. The soil samples were fortified with dilutions of the metalaxyl and CGA-62826 primary stock solutions and resulted in soil concentrations of 100, 40 and 10 ug/kg. Additionally, four samples (25 grams) were left unfortified and used as matrix blanks. Recovery experiments were performed on two separate days.

Extraction

Recovery samples were prepared in approximately 25 grams of Agricenter soil. Each sample was spiked with metalaxyl and its acid metabolite, CGA-62826. After mixing with 35 mL of 50% methanol in water on a reciprocating shaker for 5 minutes, samples were centrifuged at approximately 3000 rpms for 30 minutes. The liquid portion was poured into a roundbottom flask. This extraction procedure was performed a total of three times with all the liquid fraction being added to the roundbottom flask. Upon completion of the solid liquid extraction, the residual soil was discarded.

The liquid phase was placed on a rotary evaporation apparatus in a water bath set at approximately 50 °C. Samples were evaporated until condensation stopped. The water fraction was poured into a centrifuge tube and the sample centrifuged for another 30 minutes. Finally, the pH was adjusted to approximately 2 with dilute sulfuric acid.

The test materials were removed from the acidified water using octadecyl bonded silica (C-18) solid phase extraction (SPE) columns. The columns were activated with methanol and water according to the manufacturers instructions prior to sample addition. Samples were applied under vacuum, sufficient to cause approximately 5-10 mL flow through a plastic reservoir with a filter disc, onto the C-18 cartridge. The water passing through the column was discarded.

Once all solution had been added to the column, the column was rinsed with two volumes of pH adjusted water (acidified to approximately pH 2, approximately 10 mL) and two column volumes of 15% methanol in water. The vacuum was increased and the column allowed to air dry. Vacuum was reduced and test materials removed with two 2.5 mL rinses of acetone into a collection vessel.

Derivatization of CGA-62826

Acetone residues from the SPE were brought to dryness under a gentle stream of nitrogen gas. Triflouroboron in butanol (BF_3-butanol) was utilized to convert CGA-62826 to its butyl ester for gas chromatographic detection.

The esterification reaction was carried out in a water bath set to 85 °C for at least 45 minutes. Samples were allowed to cool to room temperature and the reaction terminated with the addition of dilute sodium sulfate (10 mL 7% w/v) and sodium phosphate (1 mL 0.1 M).

Secondary Liquid Liquid Partition

The sodium sulfate, sodium phosphate solution from the derivatization was extracted with 10 mL hexane three times to remove the metalaxyl and butylated CGA-62826 from the water phase. The hexane extract was dried through anhydrous sodium sulfate to remove traces of water and collected in a roundbottom flask. The hexane was evaporated to dryness with rotary flask evaporators and the residues dissolved in iso-octane. Samples prepared at or near the screening level of 10 µg/kg were brought to a final volume of 2 mL, while samples prepared at higher levels were brought to a larger final volume. The final extract was analyzed by gas chromatography.

Gas Chromatography

Gas Chromatographic (GC) analysis was conducted utilizing the following equipment and conditions:

Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen phosphorous detector, Model 7673 autosampler and Model 3396 Integrator

Column: RT_x-35; 30m (length) x 0.53 mm (ID)

Flows (mL/min)

carrier gas (He): approximately 20
makeup (He): approximately 10
Air: approximately 100
Hydrogen: approximately 3.5

Injection Volume: 4 µL

Analysis

The metalaxyl and CGA-62826 stock was diluted with iso-octane in order to prepare appropriate GC calibration standards of approximately 50, 100, 400 and 800 µg/L. Standard solutions contained both metalaxyl and CGA-62826. Analyses of the samples and standards were performed by programmed injection. A standard curve was constructed plotting the peak response observed versus the concentration (µg/L) of the standard injected for both compounds. The equation constructed from a Linear regression analysis was used to determine the concentration of metalaxyl and CGA-62826 in the test sample.

Calculations

The following equation was utilized in calculating the analytical results:

Analytical Result (µg/L) = A x D.F.

where:

Analytical Result = concentration of metalaxyl and CGA-62826 (µg/kg

A = concentration (µg/L) of extracted sample from the regression analysis

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

RESULTS

Analytical results for the recovery of metalaxyl and CGA-62826 from soil/hydrosoil are presented in Table 19 and Table 20. The average recovery for metalaxyl was 88.5% ± 14.6% and CGA-62826 was 81.2 ± 15.4%. The method limit of quantification for parent and metabolite was 10.0 µg/kg.

Method for the Determination of Metalaxyl and CGA-62826 Residues in Rice Paddy Water

METHOD SUMMARY

The analytical methodology for the measurement of metalaxyl and CGA-62826 residues in rice paddy water is presented below. The average recovery for fortified samples w: 94.7% ± 12.6% for metalaxyl and 104% ± 17.1% for CGA-62826

All samples were extracted using octadecyl (C-18) solid phase extraction (SPE) column after filtration and acidification. Samples were stripped from the SPE columns with acetone and dried under nitrogen. Triflouroboron in butanol (BF_3-butanol) was added and the samples incubated for 45 minutes. The reaction was stopped with the addition of an aqueous buffer solution and a solvent extraction was performed with hexane. Samples are concentrated and brought to final volume in iso-octane for gas chromatographic nitrogen phosphorous detection.

EQUIPMENT AND REAGENTS

Equipment

1. Balance: four-place analytical balance
2. Separatory funnels: glass, assorted sizes
3. Round bottom flasks: glass, assorted sizes
4. Powder funnels: glass, assorted sizes
5. Beakers: glass, assorted sizes
6. Flasks: glass, Volumetric, assorted sizes
7. Instrument: Hewlett Packard 5890 gas chromatograph equipped with a nitrogen phosphorus detector, 7673 autosampler and 3396 integrator
8. Pipets: glass, Volumetric, assorted sizes
9. Sample Storage Bottles: Wheaton, assorted sizes with teflon lined lids and metal crimp tops.
10. Syringes: Hamilton, assorted sizes
11. C-18 octadecyl Solid Phase Extraction columns: Baker
12. Temperature controlled water bath:
13. Centrifuge tubes, 15 mL
14. Glass fiber filters, 11 cm
15. Buchner Funnels: 11 cm
16. Rotary Flask Evaporator(s)
17. Nitrogen gas concentrator apparatus

18. Solid Phase Extraction (SPE) apparatus

Reagents

1. Acetone, reagent grade
2. Hexane, high purity solvent
3. Methanol: residue grade
4. 2,2,4 trimethyl pentane, Iso-octane: residue grade
5. Sodium sulfate, reagent grade, Anhydrous and 0.7% (70 grams per liter)
6. Metalaxyl, lot No. S87-1208, 95.8% active ingredient, supplied by Ciba Geigy Chemical Corporation, Greensboro, North Carolina
7. CGA-62826, lot No. S85-0650, 98% active ingredient, supplied by Ciba Geigy Chemical Corporation, Greensboro, North Carolina
8. Butyl ester of CGA-62826, prepared as 100% active ingredient, synthesized by Springborn Laboratories from CGA-62826.
9. BF_3-Butanol: Supelco, Inc. Bellefonte, Pennsylvania, cat NO. 3-3125M
10. Pond water: Agricenter pond water shipped to Springborn Laboratories, Inc. Wareham, Massachusetts from Agricenter International, Incorporated, Memphis, Tennessee. This water was used to matrix match method validation and quality control samples.
11. High Purity water: glass distilled water passed through high efficiency water purification system: Barnsted NANOpure™ water purification system
12. Sulfuric Acid: 0.5 M (28 mL concentrated per liter)
13. Sodium Phosphate dibasic: 0.1 M (14.2 grams per liter)

PROCEDURE

Preparation of Stock

Approximately (ca.) 0.25 gram (g) of metalaxyl and 0.1 g of CGA-62826 were weighed on a balance in 100-milliliter (mL) volumetric flasks and dissolved in acetone. The metalaxyl and CGA-62826 stock solutions (ca. 1 mg/mL) were stored refrigerated (4 - 10 °C) in 100-mL amber serum vials with teflon-lined lids. These stocks were used, with appropriate dilution, for quantitation (prepared in iso-octane) and fortification (prepared in acetone). Instrumental standards for CGA-62826 are prepared after derivatization to the butyl ester. Instrumental standards were prepared with both metalaxyl and CGA-62826 in calibration standard solutions.

Sample Fortification

Method validation/recovery samples were prepared in Agricenter pond water. Samples were fortified with dilutions of metalaxyl and CGA-62826 primary stock solutions and resulted in water concentrations of 100, 50, 10, 5, 2 and 1 µg/L (two replicates at each level). Additionally four samples (200 mL) were left unfortified and served as matrix blanks. Recovery experiments were performed on two separate days. The first set of samples were prepared in duplicate at 5, 2 and 1 µg/L. The second set of samples were prepared at 100, 50 and 10 µg/L. The lower sample set had unfortified samples processed at the screening level (1.00 µg/L, 200 mL water brought to a final volume of 2.00 mL for analysis).

Extraction

Recovery samples were prepared using Agricenter pond water. Samples were spiked with metalaxyl and its acid metabolite, CGA-62826. Recovery samples were extracted from the pond water after filtration and acidification using octadecyl bonded silica (C-18) solid phase extraction (SPE) columns. The columns were activated with methanol and water according to the manufacturers instructions prior to sample addition. Samples were applied under vacuum sufficient to cause approximately 5-10 mL flow through the C-18 cartridge. The water passing through the column was discarded.

Once all 200 mL of sample had been added to the column, the column was rinsed. Two column volumes of water and two column volumes of 15% methanol in water were added as rinses to wash the column of unbound materials. The vacuum was increased and the column allowed to air dry. The vacuum was reduced and the test materials removed with 5 mL of acetone. The effluent from the column was collected in a receiving vessel.

Derivatization of CGA-62826

Acetone residues from the SPE were brought to dryness under a gentle stream of nitrogen gas. Triflouroboron in butanol (BF_3-butanol, 1 mL) was added to each sample to convert CGA-62826 to its butyl ester for gas chromatographic detection.

The esterification reaction was carried out in a water bath set to 85 °C for at approximately 45 minutes. Samples were allowed to cool to room temperature and the reaction terminated with the accition of dilute sodium sulfate (10 mL 7% w/v) and sodium phosphate (1 mL 0.1 M).

Secondary Liquid Liquid Partition

The sodium sulfate, sodium phosphate solution from the derivatization was extracted with 10 mL hexane three times to remove the metalaxyl and butylated CGA-62826 from the buffered water phase. The hexane was dried through anhydrous sodium sulfate to remove traces of water and collected in a roundbottom flask. The hexane was evaporated to dryness with rotary flask evaporators and the residues dissolved in 2.00 mL iso-octane. The final extract was analyzed by gas chromatography.

Gas Chromatography

Gas Chromatographic (GC) analysis was conducted utilizing the following equipment and conditions:

Hewlett-Packard 5890 gas chromatograph equipped with a nitrogen phosphorous detector, Model 7673 autosampler and Model 3396 Integrator

Column: RT_x-35; 30m (length) x 0.53 mm (ID)

Flows (mL/min)

- carrier gas (He): approximately 20
- makeup (He): approximately 10
- Air: approximately 100
- Hydrogen: approximately 3.5

Injection Volume: 4 µL

Analysis

The metalaxyl and CGA-62826 stock was diluted with iso-octane in order to prepare appropriate GC calibration standards of approximately 50, 100, 400 and 800 µg/L. Standard solutions contained both metalaxyl and CGA-62826. Analyses of the samples and standards were performed by programmed injection. A standard curve was constructed plotting the peak response observed versus the concentration (µg/L) of the standard injected for both compounds. The equation obtained from a Linear regression analysis was used to determine the concentration of metalaxyl and CGA-62826 in the test sample.

Calculations

The following equation was utilized in calculating the analytical results:

Analytical Result (µg/L) = A x D.F.

where:

Analytical Result = concentration of metalaxyl or CGA-62826 (µg/L)

A = concentration (μg/L) of extracted sample from the appropriate regression analysis
D.F. = dilution factor, ratio of final volume (mL) of the sample to volume (mL) of sample extracted

RESULTS

Analytical results for the recovery of metalaxyl and CGA-62826 from rice paddy water are presented in Table 21 and Table 22. The average recovery for metalaxyl was 94.7% ± 12.6% and CGA-62826 was 104 ± 17.1%. The method limit of quantification for parent and metabolite was 1.00 μg/L.