Attachment: Gas Chromotography Method Validation*

SOP : 4.5 Analysis of Soil and Hydrosoil for Combined Residues of Tralomethrin, Deltamethrin, and trans-Deltamethrin.

1. Principle:

Tralomethrin, deltamethrin, and trans-deltamethrin are extracted from soil and hydrosoil samples with hexanes:-methylene chloride in a 125ml screw cap erlenmeyer flask by vortexing and sonication. The extract is concentrated by rotary evaporation and brought to 5ml with hexanes. The concentrate is analyzed by capillary column electron capture gas chromatography using cool on-column injection and an uncoated deactivated retention gap. The tralomethrin is converted to deltamethrin when subjected to the column environment required for chromatographic separation and trans-deltamethrin is not separated from deltamethrin.

2. Compound Information:

a. Tralomethrin: (1R,3S)3[(1,2,2,2,-tetrabromoethyl)]-2,2-dimethylcyclopropanecarboxylic acid (S)-alpha-cyano-3-phenoxybenzyl ester. Company name RU 25 474.

b. Deltamethrin: (1R,3R)3[(2,2-dibromovinyl)]-cis-2,2-dimethylcyclopropanecarboxylic acid (S)-alpha-cyano-3-phenoxybenzyl ester.

c. trans-Deltamethrin: (1R,3S)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid [(S)-cyano-(3-phenoxyphenyl)-methyl] ester. Company name RU 26 979.

d. Standards: Analytical grade standard of tralomethrin for quantification as supplied by the sponsor.

* Subject: Direct comparison between 30 meter capillary g.c. column and 5 meter megabore g.c. column.

3. Equipment and Reagents

a. Equipment:

1. Balances - Mettler model PM460 top loading (0.001 g) and an AE240 analytical (0.00001 g), or equivalent.

2. Rotary flash evaporator - Buchi model 111B with temperature controlled water bath and refrigerated cooling fluid, or equivalent.

3. Glass volumetric flasks with teflon lined screw caps.

4. Vortex mixer - SMI multi-tube vortexer, or equivalent.

5. Erlenmeyer flasks - 125ml glass with teflon lined screw caps.

6. Sonic water bath cleaner.

7. Pasteur capillary pipets, borosilicate glass, 23cm length.

8. Funnels - Mooney air vent glass funnels, or equivalent.

9. Glass wool - silane treated and rinsed with acetone:hexane (1:1), or equivalent.

10. Evaporating flasks - 250ml glass with 24/40 ground glass joint, or equivalent.

11. Syringes - Hamilton brand gastight, or equivalent.

12. Gas chromatograph - Hewlett-Packard model 5890A with dedicated cool on-column capillary inlet and Ni^{63} electron capture detector or equivalent.

13. Gas chromatographic column - 5 meter x 0.53 mm ID fused silica megabore column with a 2.65 μm film of methylsilicone stationary phase (HP-1 or equivalent).

14. Data Handling - Hewlett-Packard model 9000 series 300 computer and Hewlett-Packard GC Chemstation software, or IBM AT compatible computer with

Nelson Analytical Turbochrom Chromatography software, or equivalent.

15. Teflon beakers (250 ml) and Teflon spatulas.

16. Tube cutter, chisel, and hammer.

17. Aluminum foil.

18. Drying oven.

b. Reagents:

1. Hexanes (ACS) - suitable for pesticide residue analysis (Mallinckrodt nanograde brand or equivalent).

2. Water Saturated Hexanes - Approximately 200 ml Hexanes shaken with one liter of reagent water in a two liter separatory funnel for two minutes.

3. Acetone (ACS) - suitable for pesticide residue analysis (Mallinckrodt nanograde brand or equivalent).

4. Dichloromethane (ACS) - suitable for pesticide residue analysis (Mallinckrodt ChromAR HPLC grade or equivalent).

5. Sodium Sulfate (anhydrous) - Fisher brand certified ACS or equivalent, muffled at 400°C for 4hr.

6. Hydrogen gas - Liquid Carbonics zero grade or equivalent.

7. 90% argon:10% methane gas - Liquid Carbonics zero grade or equivalent.

4. <u>Procedures and Methods:</u>

a. Preparation of Standard Solutions:

All standards for injection into the gas chromatograph are to be in hexanes. The sample introduction technique used requires that the samples and standards be dissolved in solvents of similar boiling points and polarity. In order that the matrix of the standards be as similar as possible to the matrix of the samples, water saturated hexane or hexanes must be used.

Standard solutions will be prepared using analytical grade tralomethrin as received from the study sponsor. A stock standard of tralomethrin is prepared by dissolving approximately 25 mg analytical grade tralomethrin in hexane(s) or acetone in a 50 or 100 ml volumetric flask. Intermediate and working standards are prepared by dilution of these solutions. Working standards are brought to volume with water saturated hexanes. Working standards will be prepared at a minimum of three concentrations, the lowest and highest of which will bracket the sample concentrations. Standards in acetone are used for spiking. Preparation of standards is recorded on standard preparation data sheets.

Standard stock solutions can be stored at -15±5°C for a maximum of three months. Intermediate standards can be stored at -15±5°C for a maximum of six weeks. Working standards can be stored at -15±5°C for a maximum of 7 days. All standards are labeled as described in SOP 15.6. Use of an analytical standard is documented on the "Analytical Standard Log" form as described in SOP 4.8.

b. Preparation and Application of Lab Spikes:

An appropriate volume of an acetone solution of tralomethrin is added to the surface of the sample to be spiked. All spikes will be done on samples from control ponds or the control dosing tank. The tank mix and sediment trap samples are spiked in the 125ml Erlenmeyer flasks into which they are sampled. In each case, two samples will be taken, one for spiking in the field and one for spiking in the laboratory. For the pond sediment spikes, two cores will be taken from the control pond designated for that day. These will be taken at the same time and in the same way as a normal sample. One of these samples is spiked in the field and the other returned to the lab for spiking. The spiking solution is applied to the field spike sample by carefully removing the water from the core and placing the tip of the syringe just at the surface of the sediment. The solution is slowly added so that the solution does not migrate to the walls of the core. The laboratory spike for the pond sediment is added after the core is sectioned and the aliquot for analysis is added to a 125ml Erlenmeyer flask. The tralomethrin levels in the spikes will approximate the level of tralomethrin expected in the samples.

Record the following information on the spiking data sheet.

A. Sample ID spiked.
B. Level spiked.
C. Solution number and Volume of spike.
D. Time and date of spike.
E. Initials of person spiking.

c. Sample Preparation:

1. Sediment Core Samples

a. Three cores are collected from each pond on a sediment core sampling day (excluding lab and field spike cores collected from control ponds) and frozen in the lab until sample analysis.
b. Perform the following steps to remove a one cm section from each of the three frozen cores.
   1. Lay the core on clean aluminum foil.
   2. Measure one cm down from the lowest top point of the sediment. Mark the 1 cm location on the plastic core.
   3. Cut the plastic core with the tube cutter along the 1 cm mark.
   4. Use a chisel and hammer to cut away the plastic core and 1 cm of hydrosoil.
   5. Place the hydrosoil in a 250 ml teflon beaker.
c. Using a teflon spatula, mix the three sections in a teflon beaker. Remove approximately a 10 g subsample and determine the percent dry sediment as described in step 5.f. Also, weigh approximately a 25 g subsample in a tared 125 ml erlenmeyer flask. Proceed with step 4.d. (SOP 4.5).

2. Sediment Core Lab Spike Samples

a. One lab spiking core is collected from the control pond on each sediment core sampling day and frozen in the lab until sample analysis.
b. In the lab, remove a one cm section as described in step 4.c.1.b.
c. Using a teflon spatula, mix the hydrosoil placed in a 250 ml teflon beaker. Remove approximately a 10 g subsample and determine

the percent dry sediment as described in step 5.f. Next, weigh approximately a 25 g subsample in a tared 125 ml erlenmeyer flask. Spike the hydrosoil in the flask as described in step 4.b. (SOP 4.5). Proceed with step 4.d (SOP 4.5).

3. Sediment Trap Samples

   a. Sediment trap samples are collected in tared 125 ml erlenmeyer flasks as described in step 3 of SOP 4.1 (Use of Sediment Traps for Collection Residue Samples).
   b. In the field, sediment trap field spike samples are spiked as described in step 2 of SOP 4.10 (Field Spiking Procedures).
   c. All samples are returned to the lab and frozen until residue analysis.
   d. To begin analysis in the lab, remove approximately a 2 g subsample and determine the percent dry sediment as described in step 5.f.
   e. Record a weight for the remaining sediment trap sample (approximately 25 g) in each tared flask. Sediment trap lab spike samples are spiked at this time as described in step 4.b. Proceed with step 4.d.

4. Run-Off Tank Mix Samples

   a. Run-off tank mix samples are collected in tared 125 ml erlenmeyer flasks as described in step 2.D.6. of SOP 17.2 (Application of Scout 0.3 EC Insecticide as a Run-off Simulation).
   b. In the field, run-off tank mix field spike samples are spiked according to step 2 of SOP 4.10.
   c. All samples are returned to the lab and frozen until residue analysis.
   d. To begin analysis, remove approximately a 2 gram subsample and determine the percent dry sediment as described in step 5.f.
   e. Record a weight for the remaining run-off tank mix sample (approximately 20g) in each tared flask. Next, run-off tank lab spike samples are spiked as described in step 4.b. Proceed to step 4.d.

d. Liquid-Solid Extraction

1. Before extraction begins, a wet weight is recorded for each soil sample (sediment trap and run-off tank mix) or hydrosoil sample (sediment core) placed in a tared 125 ml erlenmeyer flask with a teflon lined screw cap.

2. The soil is then extracted in the tightly capped flasks by vortexing for four minutes followed by sonication for five minutes with 50 mls of hexane:dichloromethane (85:15).

3. The extract is passed through a funnel into a 250 ml evaporating flask. The funnel contains a plug of silane treated glass wool which has been rinsed with acetone: hexane (1:1) and dried at 100°C.

4. The sample is extracted three more times with 30 ml to 40 ml aliquots of hexane: dichloromethane (85:15). These three extracts are also passed through the glass wool plug to remove fines combined in the 250 ml evaporating flask. The glass wool and funnel apparatus are carefully rinsed into the evaporation flask with hexanes: dichloromethane.

5. The extracts are then concentrated by gentle rotary evaporation (30°C $\pm$ 5°C) and quantitatively transferred to 5 ml volumetric flasks with teflon lined screw caps. These final extracts are then transferred to 5 ml vials with teflon lined screw caps or autosampler vials. These may be stored at -15 $\pm$ 5°C for later analysis by gas chromatography.

e. Lab Tracking of Soil and Hydrosoil Residue Samples.

Lab tracking forms will be completed for all soil and hydrosoil residue samples processed. Record the following on tracking forms:

A. Sample condition upon arrival in laboratory.
B. Date arrived.
C. Sample lab ID number.
D. Sample storage location.
E. Extract storage location.
F. Date extracted.
G. Date gas chromatographic analysis completed.
H. Analytical laboratory supervisor sign-off when analysis is completed.

5. Gas Chromatography:

a. Pre-analysis instrument checks:

Prior to initiation of analysis using the gas chromatograph, a few critical checks of instrument fitness will be performed.

The HP5890 display should indicate 'ready' after run parameters are programmed.

The gas cylinder regulators should indicate enough gas supply to complete the run.

The column head pressure gauges should indicate carrier gas pressure. Also, the flow rate should be checked.

Other instrumental problems will be indicated by lack of performance during the analysis. Corrective action may be taken as indicated in SOP 16.2. [Use and Maintenance of Gas Chromatograph (Hewlett Packard 5890A)]. The operator of the gas chromatograph is responsible for recording routine and nonroutine maintenance events in the gas chromatograph maintenance log book as described in step 3.b of SOP 16.2.

b. Instrumental Setpoints

The chromatographic setpoint values may be changed only by the supervisor of the analytical lab and only if proper record of the change is made in the gas chromatograph run log. The critical setpoints are listed in the gas chromatograph run log.

c. Gases

The carrier gas used is Hydrogen. The Electron Capture make-up gas is 90% argon and 10% methane. Gas flow rates will be checked at least once a week or whenever a change in the flows is suspected. Flow checks will be recorded on the G.C. run log forms under the maintenance section.

A molecular sieve moisture trap and an oxygen trap should be installed in line for each of the gasses used on the gas chromatograph.

d. Calibration of Gas Chromatographic Detector

A series of standard solutions plus a solvent blank will be analyzed immediately prior to sample analyses. Additionally, selected standards will be run at intervals of no more than 6 injections to check column detector stability. After the sample injections, standards will be run that bracket the sample concentrations. Extracts with concentration above that of the highest standard may be diluted to adjust the concentration to within the range of standards used.

e. Analysis of extracts

The extracts and standards from section 4 are analyzed by high resolution gas chromatography using a 5 meter X 0.53mm fused silica capillary column. The non-polar methylsilicone stationary phase is 2.65µm thick. On-Column injection is used with injection volumes of 0.5µl to 5µl. The injections are made using the auto-injector on the HP5890 gas chromatograph. Manual injections may be made up to 10µl. In all cases, the injection volume of standards and samples must be identical. The oven is rapidly temperature programmed to a temperature which allows the pyrethroid to migrate through the analytical column. The chromatograms are stored in computer readable form and on printouts generated at the end of each separation. An arrow is drawn on the printed chromatogram indicating the analyte peak location. The analyst will initial all printed chromatograms.

f. Correction to dry weight basis:

Water content of sediment samples is determined by drying an aliquot in a tared aluminum drying pan to constant weight in a drying oven at 105°C ±5°C. The dry weight of the sample is determined from the following calculations:

Aliquot Wet Weight(g)=[Wet Sediment + Pan(g)] - Pan Wt.(g)

Aliquot Dry Weight(g)=[Dry Wt.(Pan + Sed.)(g)]-Pan Wt.(g)

$$\text{Percent Dry Sediment} = \frac{\text{Final Dry Aliquot Wt.(g)}}{\text{Initial Wet Aliquot Wt.(g)}} \times 100$$

Dry Sample Mass = Wet Sample Mass X Percent Dry Sediment(÷100)

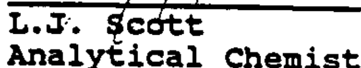
g. Calculations:

Calculation of tralomethrin concentration in the samples, based on the calibration of section 4-d of this SOP, will be by linear regression analysis. This may be carried out by calculator or computer using peak areas or automatically by the data acquisition computer. After comparison of the peak areas for samples to the peak areas for standards using linear regression, the analyte concentration in the original sample in ng/g (ppb) may be calculated as follows:

$$\text{Tralomethrin concentration in dry sample (ng/g)} = \frac{\text{extract conc. (pg/}\mu\text{l) X extract vol. (}\mu\text{l)}}{\text{1000 (pg/ng) X dry sample mass (g)}}$$

Validation Report for Standard Operating Procedures 4.3 and 4.5 (Issues No. 2)

Subject: Direct Comparison Between 30 meter Capillary G.C. Column and 5 meter Megabore G.C. Column

Prepared By: 
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Date: 8 May 89

During the completion of the analytical portion of this project, it seemed to be more efficient from an instrument maintenance point of view if a megabore GC column could be substituted for a capillary GC column. Particularly in view of the fact that most of low level samples eg water/sediment had already been assayed and that higher level samples remained. To implement this change a comparison of standards and samples were made to document the equivalence of GC columns. As might be expected, the area response in millivolts was different with both columns; but as Table 1 shows the pg/ul tralomethrin results were very comparable. The standard curves do not represent a straight line since the response was determined over a 1,000 fold range of concentrations. Only linear portions of curves were used as appropriate for the sample assays.

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