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ABSTRACT

An analytical method based on the use of gas chromatography and a electron capture detector (ECD) is described for the trace determination of DPX- 32293 (Asana® Insecticide, MO-70616) in water, sediment, and fish samples.

Water samples - The water sample is extracted and concentrated (eluted through) using an C18 mini-column, the column is dried, and the Asana® Insecticide is eluted from the C18 column with 1% ethyl acetate (EA) in hexane which is concentrated for analysis. The minimum detectable concentration for this method is about 2 pg/gram (2 ppt). For samples containing a large amount of particulate matter the water sample is partitioned with hexane. The hexane is concentrated for cleanup on a Si mini-column. The Si column is eluted with 2% ethyl acetate in hexane which is concentrated for analysis.

Sediment samples - The sediment sample (25 g) is extracted with acetonitrile using a Braunsonic Extractor for 2 minutes at 300 watts. A 5 gram aliquot of the acetonitrile extract is diluted with 170 ml of deionized (DI) water in a separatory funnel. The aqueous solution is eluted through a C18 mini-column, the column is dried, and the Asana® Insecticide is eluted from the C18 column with 1% ethyl acetate in hexane. The EA/hexane is dried over anhydrous sodium sulfate and concentrated to 1.0 ml. This extract is eluted through a Si mini-column and the Asana® Insecticide is eluted with 2% ethyl acetate in hexane. This solution is concentrated for analysis. If the samples are not cleaned up sufficiently, the

samples are placed through a Florisil mini-column before the GLC analysis. The minimum detectable concentration for this method in sediment is about 200 pg/gram (200 ppt).

Fish samples - The fish sample (10 g) is extracted using hexane/isopropanol (IPA) (75%/25%) with a Tissumizer® Extractor for 3 minutes. The IPA is removed by washing the water in a separatory funnel. A 2 gram aliquot of the hexane extract is adjusted to 50 mls with hexane in a 250 ml separatory funnel. This hexane solution is partitioned two times with 50 mls of acetonitrile. The acetonitrile extract is concentrated to about 3-5 mls using a rotary evaporator. One hundred mls of hexane is added and concentrated again to 3-5 mls. Repeat with an additional 100 mls of hexane to completely exchange to hexane. This extract is placed through a Si, Florisil, or combined mini-column cleanup and the Asana® Insecticide is eluted with 2% ethyl acetate in hexane. This sample is concentrated to 2.0 ml (1.0 g/ml) for analysis. The minimum detectable concentration for this method in fish is about 1.0 ng/gram (1 ppb).

INTRODUCTION

The Environmental Protection Agency (EPA) requires field studies to evaluate the potential effects by pesticides on aquatic environments. The mesocosm (mesoponds) approach was designed to evaluate the potential impact of Asana® Insecticide on a series of small managed aquatic ecosystems (ponds).

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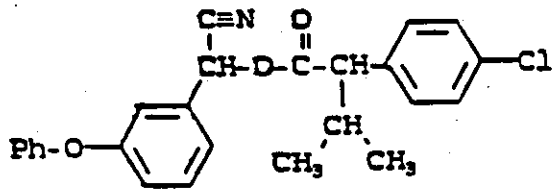
Analytical methodology was needed to assess the levels of Asana® Insecticide in water (1-2 ppt), in sediment samples (100-200 ppt), and in fish samples (1-2 ppb). This paper describes the methods for determining Asana® Insecticide in water, sediment, and fish needed to complete the analyses for the samples taken during the Asana® Insecticide mesopond studies.

STRUCTURE

The structure of DPX-32293 (Asana® Insecticide, MO-70616) is given below.



DPX-32293

EXPERIMENTAL SECTION

Apparatus And Reagents

Gas chromatograph - A Hewlett Packard Model 5880 or a Varian Model 3700 or equivalent instrument equipped with an electron capture detector. See section on Gas Chromatography Conditions for the column and instrument parameters.

Fused silica capillary column, 15-30M X 0.32mm (ID), coated with a cross-linked, non-polar stationary phase, such as DB-1, available from J & W Scientific Inc., Rancho Cordova, California.

Braunsonic extractor/sonicator, No. 1510, B. Braun Instruments, San Francisco, California.

Homogenizer, A STD Tissumizer® equipped with an SDT-182 EN shaft available from Tekmar Company, Cincinnati, Ohio.

Nitrogen Evaporator, N-Evap®, available from Organomation Associates, South Berlin, Massachusetts.

Centrifuge, an IEC Model K floor Centrifuge, International Equipment Co., or equivalent.

Automatic Pipette, Electronic Digital Pipette, Cat No. EDP RCS, 10-1000 Ul, from Rainin Ins. Co., Woburn, Massachusetts.

Common glassware found in a well equipped Residue Lab.

Centrifuge bottles, 250 ml, polypropylene, Nalgene 16195-S from VWR Scientific.

Rotary Vacumm Evaporator, Rotovapor-R from Brinkman Instruments, Westbury, New York.

Bond Elut® Cartridge mini-columns, C18, Si, 500 mg, and Vacuum Box, adapters, and Bond Elut® accessories available from

Analytichem International, Harbor City, California.

Florisil Mini-columns, 1000 mg, from J.T. Baker Chem. Co. Phillipsburg, New Jersey.

Solvents - Ethyl acetate, hexane, acetone, 2-propanol, and acetonitrile. Pesticide Grade from Fisher Scientific, Fair Lawn, New Jersey or from American Burdick and Jackson, Muskegan, Michigan.

Analytical Standard, Asana® Insecticide (MO-70616, code 2-3-0-0, 99%) available from E. I. Du Pont de Nemours & Co., Experimental Station, Wilmington, Delaware.

SAMPLE PREPARATION AND EXTRACTION

Water - For Solid Phase Extraction

Remove the samples from storage and equilibrate to room temperature. Weigh each sample, bottle and contents. Decant each sample (200-250 mls) into a separate 500 ml separatory funnel. Weigh the empty bottle and calculate the weight of each sample and record. Add 20 ml of acetonitrile to each empty bottle and shake. Add this rinse to the separatory funnel containing the sample. Repeat with another 20 ml acetonitrile wash and a 10 ml acetone wash. Shake the separatory funnel thoroughly for about 1 minute. Save solutions for the solid phase extraction step.

Water - For Hexane Extraction

Remove the samples from storage and equilibrate to room temperature. Weigh each sample, bottle, and contents. Decant each

sample (250 ml) into separate 500 ml separatory funnels. Weigh the empty bottle and calculate the weight of each sample and record. Add 10 ml of acetone to each empty bottle and shake. Add this rinse to the separatory funnel containing the sample. Add 50 ml of hexane to the separatory funnel and shake for 1 minute. Let the phases separate. This sometimes takes about 30 minutes or in severe emulsion cases may take centrifuging. Drain the lower aqueous phase to waste. If hexane is cloudy add anhydrous sodium sulfate to dry. Quantitatively transfer the hexane into a 50 ml tube and concentrate to 1.0 ml (250 g/ml) for analysis. If interferences are encountered, proceed to the Liquid/Solid Chromatography Cleanup step below.

Sediment

Remove the samples from storage and let thaw to a workable consistency. Take the sediment layer as sampled from the field and push the sample from the bottom of the sampling tube up through the tube until about 1 cm is showing above the top lip of sampling tube. Carefully cut this layer with a knife or spatula and carefully weigh approximately 25 g (to 0.1 g) into a weighed 250 ml Nalgene jar. Add 150 ml acetonitrile to the jar and extract on the Braunsonic Extractor for 2 minutes at 300 watts. Cap the sample and centrifuge at 2000 rpm for 5 minutes. Decant the acetonitrile extract into a 250 ml volumetric flask and save. Add another 100 ml of acetonitrile to the sample and extract on the Braunsonic Extractor again for 2 minutes at 300 watts. Again centrifuge and decant the acetonitrile into the 250 ml volumetric flask with the

first extract. Make up to the 250 ml mark with acetonitrile and mix thoroughly. This extract is now at 0.1 g/ml sediment to solvent ratio if 25 g of sample was used. Measure a 2-5 gram aliquot (20-50 ml if 25 gram used) of the extract into a clean 250 ml separatory funnel. Add 170 ml of DI water and shake. Save this solution for the solid phase extraction step. This procedure does not correct for dry weight basis for the sediment samples.

Fish

Remove the samples from storage and let thaw to a workable consistency. Macerate a representative fish sample in a blender or meat grinder to a homogeneous sample. Carefully weigh 10 grams (to 0.1 g) into a weighed 250 ml Nalgene jar. Add 160 mls of hexane/isopropanol (75%/25%) to the jar. Extract using the Tekmar Extractor for 3 minutes. Centrifuge the samples for about 5 minutes. Decant the extracts into 250 ml separatory funnels. Add about 100 mls of DI water to the extract and gently shake for about 1 minute. Allow the phases to separate and drain off the lower aqueous phase and discard. Wash the hexane extract with two additional 100 ml volumes of DI water. Place the hexane extract into screw capped bottles with teflon or foil lined caps. Add about 1 grams of anhydrous sodium sulfate to the bottle cap and shake. This extract is equivalent to 10 g in 120 mls of hexane (2.0 g/24 mls). Proceed to the Liquid/Liquid Partition Cleanup section.

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SOLID PHASE EXTRACTION CLEANUP

Condition enough Bond Elut® C18 columns for the water or sediment samples prepared above. Using a vacuum box apparatus and reservoirs that mount above the C18 columns, condition with 12 mls of acetonitrile followed by 12 mls of DI water.

Place the sample solutions from above through the C18 columns, adjusting the vacuum for a flow of about 2-4 ml per minute. The extracted solutions can be taken away to waste and discarded. Save the separatory funnels for a later rinse with hexane/EA. After complete elution of the sample, increase the vacuum to maximum for five minutes. After drying, turn the vacuum off and place labelled 15 ml collecting tubes in the vacuum box under each sample. Prepare a 1% ethyl acetate in hexane solution. Measure out separate 10 ml amounts and add to the separatory funnels above that were used to prepare and hold the solutions for solid phase extraction. Shake the separatory funnels to wash the inside surface of the funnels. Add this 1% EA/hexane solution to the C18 mini-columns and use this solution for elution volume. Use no vacuum for the first 10 minutes. Let the solution penetrate the C18 bed slowly to ensure no channeling occurs. Sometimes a positive pressure of clean air or nitrogen is needed to start the elution. After the 10 minutes of equilibration, slowly increase the vacuum for a flow of about 1 to 2 mls per minute to complete the elution. Add about 0.5 g of anhydrous sodium sulfate to the solution in the collecting tubes and shake. Quantitatively transfer the solution to a clean graduated 15 ml tube and concentrate using compressed air

blow down and water bath at 60°C to 1.0 ml. The water samples can be further concentrated to 0.5 ml for analysis at 500 g/ml at this point in the method if the water is fairly clean and without interferences. For the sediment samples proceed to the Liquid/Solid Chromatography Cleanup in the next section. Also, if the water samples show interferences, continue with the L/S Cleanup.

LIQUID/LIQUID PARTITION CLEANUP

Transfer 2 g aliquot of the hexane fish extract (24 mls = 2 g) to a 250 ml separatory funnel and add hexane to make a total volume of 50 mls. Add 50 mls of hexane saturated acetonitrile and shake vigorously for about 1 minute. Drain the lower acetonitrile phase into a rotary evaporator flask. Add a second 50 ml aliquot of hexane saturated acetonitrile to hexane extract and shake vigorously again for 1 minute. Combine the acetonitrile in the evaporation flask and concentrate using the rotary evaporator to about 3 ml. Add 100 mls of hexane and concentrate again to 3 ml. Add 100 mls of hexane a second time and again concentrate using the rotary evaporator to about 3 mls. Quantitatively transfer the hexane to a 15 ml centrifuge tube and concentrate using compressed air blow down and a water bath at 60°C to 2 ml. Proceed to the Liquid/Solid Chromatography section.

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LIQUID/SOLID CHROMATOGRAPHY CLEANUP

Silica Column Cleanup

Condition enough Bond Elut® Si columns for the samples from the Solid Phase Extraction and/or the Liquid/Liquid Partition Cleanup steps above. Using a vacuum box apparatus and reservoirs that mount above the Si columns, condition with 10-15 mls of hexane. Place the sample solutions from above through the Si columns using an quantitative rinse of hexane and adjusting the vacuum for flow of about 1-2 ml per minute and elute to waste. Add 10 ml of hexane and elute to waste. Make up a solution of 2% ethyl acetate in hexane. Place clean labelled 15 ml collecting tubes in the vacuum box under each sample. Elute with 15 mls of the 2% EA/hexane at 1-2 mls per minute. Concentrate (air blow down and water bath) this solution to 0.5 ml (500 g/ml) for the water samples, to 0.5 ml (10 g/ml) for sediment samples (if 5 g used), and to 2.5 ml (2 g/ml) for the fish samples (if 5 g was used). Proceed to the Sample Analysis section. If preliminary analysis indicates that more cleanup is needed, go to the Florisil column or the combined column cleanup given below.

Florisil Column Cleanup

Condition enough Baker Florisil columns for the samples from liquid/solid cleanup step above. Using a vacuum box apparatus and reservoirs that mount above the Florisil columns, condition with 10-15 mls of hexane. Place the sample solutions from above through the Florisil columns using a quantitative rinse with hexane and

adjusting the vacuum for a flow of about 1-2 mls per minute. Elute this hexane plus an additional 10 ml of hexane to waste. Make up a solution of 2% ethyl acetate in hexane solution. Place clean 15 ml labelled collecting tubes in the vacuum box under each sample. Elute with 12 mls of the 2% ethyl acetate in hexane solution at 1-2 mls per minute. Concentrate (air blow down and water bath) this solution to the same sample volumes as given above for the Si Bond Elut cleanup step. Proceed to the Sample Analysis section.

Combined Column Cleanup

A combined or piggyback cleanup has been used for both the sediment and fish samples. Condition both the Si and Florisil columns separately as above using 10-15 mls of hexane. Using the mini-column adapters, attach the Si columns in series above the Florisil columns. Place the sample solutions from the solid phase extraction cleanup section through the tandem columns using a quantitative rinse with hexane and adjusting the vacuum for a flow of about 1-2 mls per minute. Elute this hexane plus an additional 10 ml of hexane to waste. Make up a solution of 2% ethyl acetate in hexane solution. Add 10 mls of the 2% EA/hexane to the tandem column and elute to waste. Place clean labelled 15 ml collecting tubes in the vacuum box under each sample. Elute with 12 mls of the 2% ethyl acetate in hexane solution at 1-2 mls per minute. Concentrate this solution to the same sample volumes as given above for the Si and/or Florisil cleanup step. Proceed to the Sample Analysis section.

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PREPARATION OF STANDARDS AND STANDARD CURVE

Weigh 0.1 gram (nearest mg) of pure (>99%) analytical standard of DPX-32293 into a 100 ml volumetric flask and bring up to the mark with acetone. This gives a stock solution of 1000 µg/ml.

Fortification Solution - Make dilutions with acetone to give spiking solutions containing 100 ng/ml (0.1 µg/ml) and 10 ng/ml.

Analytical Standard For Determination Step - Make dilutions using hexane to give GC calibration standards containing 1.0 ng/ml, 2.0 ng/ml, 5.0 ng/ml, 10 ng/ml, and 20 ng/ml.

The standard (calibration) curve is prepared by injecting 2-4 microliters of each standard with each set of samples in the sequence of standard/sample, standard/sample, etc. The average value for each standard injected during the analysis is plotted using an linear regression plot to calculate (average response factor value) each sample response.

FORTIFICATION OF RECOVERIES

A control (untreated if possible) sample fortified with DPX-32293 should be run with each set of samples. These samples are prepared by pipetting a known amount of the acetone spiking solution (prepared above) over the surface of the control samples just before extraction. These samples are then analyzed along with the actual analytical samples to monitor the analytical method. Typical

recoveries for this method are given in the Validation of the Method Section below.

GAS CHROMATOGRAPHY CONDITIONS

Two instruments have been used for analysis of DPX-32293 in the water, sediment, and fish samples. A Hewlett Packard Model 5880 and a Varian Model 3700 both equipped with Electron Capture detectors. GLC operating conditions were as follows:

Column:	Fused Silica Capillary, 15 meters X 0.32 mm coated with methyl silicone
Temp.:	240°C, Isothermal
Inlet Temp:	260°C
Detector Temp.:	325°C
Carrier Gas:	Helium at about 3 ml/min column flow Split mode: About 1/5
Detector Purge:	Hewlett Packard - Argon/10% methane Varian - Nitrogen Flow: About 30 ml/min

DPX-32293 gives a two peak response with the first peak about 20% of the second peak. DPX-32293 is made up of four enantiomers which elute as two discrete peaks under the GLC conditions referenced in this method. The sum of both peaks are used for analysis. A typical response for DPX-32293 under the above conditions should be at least 40% full scale for the 0.01 µg/ml standard to get the 1-2 ppt (3% Full Scale) sensitivity when injecting a volume of the 500 g/ml solution for water samples.

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SAMPLE ANALYSIS

Inject a 2 - 4 microliter aliqudt of the sample solutions and the standard solutions alternately into the GLC. Plot a calibration curve using all the standard injections. Carefully measure the chromatogram for the samples at the elution times for DPX-32293 and if the retention time correlations are good, measure the combined response and compare with the calibration curve to find the weight of DPX-32293 in each sample.

Calculate the DPX-32293 concentration in the water sample by means of the following equation:

$$C = \frac{W}{S}$$

Where C = Concentration of DPX-32293 found, ppb

W = Weight of DPX-32293 in ng/ml in each sample

S = Amount of sample in g/ml in each sample

TABLE II

GC INSTRUMENT CONDITIONS

Instrument:	Hewlett-Packard 5890 Gas Chromatograph with an electron-capture detector (EC). Data collection and processed with a Hewlett-Packard 3396A integrator.
Column:	30 meter x 0.32 mm DB-1 0.25-µm micron film thickness (J & W Scientific, Inc.)
Gases:	Carrier: Helium 4-5 mL/min. at 55 psi Detector Argon/Methane 70 mL/min. at 50 psi
Injection:	5 µl, 1:8.5 split injection technique
Temperatures:	Injector: 280°C Column 260°C Isothermal
Integrator:	Hewlett-Packard 3396A
Integrator Parameters:	ZERO = 18 ATT 2^ = -5 through -1 CHT SP = 2.0 AR REJ = 0 THRSH = -6 PK WD = 0.10
Approximate Ratio*:	30:70 ratio for ASANA 1 (first peak): ASANA 2 (second peak) isomer pair.
Retention Times:	Approximately 6.1 minutes for ASANA 1 and 6.5 minutes for ASANA 2.

* The "total toxic residues" of Asana® Insecticide (esfenvalerate) is expressed as the sum of all the fenvalerate isomers and are calculated based on the sum of the two diasteomeric peaks [(SR,RS) and (SS,RR)] eluted from the GC. For esfenvalerate, the first peak is much smaller than the second peak since esfenvalerate contains a much reduced concentration of the (RS) and (SR) isomers. Pydrin® Insecticide, racemic fenvalerate, containing approximately an equal mixture of the two diasteomeric peaks and would give approximately a 55/45 peak ratio on GC analysis.

APPENDIX II

METHOD AMR-1086-88

TRACE METHOD FOR TOTAL ESFENVALERATE RESIDUES IN WATER SAMPLES

Pond Water Method

1.0 Extraction

1.1 Remove the sample from storage and equilibrate to room temperature. Weigh the sample, bottle and contents. After decanting the sample (250-mL) into a 500-mL separatory funnel, weigh the empty bottle and calculate the sample weight.

1.2 Rinse the empty bottle with 10 mL of acetone, shake vigorously for 30 seconds, and add to the sample in the separatory funnel.

1.3 Rinse the empty bottle with 25 mL of hexane, shake vigorously for 30 seconds, and add to the separatory funnel. Shake for one minute and let the phase separate.

1.4 Add an additional 25 mL of hexane to the empty bottle, shake for 30 seconds, and add to the separatory funnel. Shake for one minute and let the phase separate.

1.5 Decant the aqueous layer to waste. Quantitatively transfer the 50-mL hexane to an appropriate vessel and evaporate to incipient dryness. Reconstitute to an appropriate final volume with hexane.

If interferences are encountered proceed to Liquid/Solid Chromatography (Appendix III, Section 2.2)

APPENDIX III

METHOD AMR-1086-88

TRACE METHOD FOR TOTAL ESFENVALERATE RESIDUES IN SEDIMENT/SOIL SAMPLES

Pond Sediment Method

1.0 Extraction

1.1 Cut one inch from the top end of the frozen sediment core and weigh into a 250-mL Nalgene® bottle (25 grams ± 0.1 grams).

1.2 After the sample thaws, add 150 mL of acetonitrile to the bottle and extract by sonication for five minutes at 200 watts.

1.3 Centrifuge the sample at 2000 rpm for five minutes. Decant the extract into a 250-mL graduated cylinder.

1.4 Add another 100 mL of acetonitrile and extract by sonication for five minutes at 200 watts.

1.5 Centrifuge the sample at 2000 rpm for five minutes. Decant the extract into the 250-mL graduated cylinder with the first extract.

1.6 Bring the combined extract up to a volume of 250 mL with acetonitrile. Combine a five-gram aliquot (50-mL) with 170 mL of ultrapure water (Burdick and Jackson) and swirl.

2.0 Clean-up

2.1 Solid Phase Extraction

2.1.0 Take the sample through a 500 mg Analytichem C_{18} reversed-phase cartridge.

2.1.1 Using a vacuum box apparatus, condition the cartridge with 12-mL of acetonitrile followed by 12 mL of ultrapure water and discard eluant.

2.1.2 Place the sample solution through the C_{18} cartridge at a rate of 2-4 mL per minute and discard the eluant. After complete elution of the sample, increase the vacuum to maximum for five minutes. After drying, turn the vacuum off.

2.1.3 Rinse the 250-mL graduated cylinder which previously held the sample with 10 mL of a 99:1 hexane:ethyl acetate solution. Add this rinsate to the C_{18} cartridge.

2.1.4 Use no vacuum for the first 10 minutes, then slowly increase the vacuum to achieve a flowrate of 1-2 mL per minute. This fraction will contain any esfenvalerate residues that may be present.

2.1.5 Concentrate to 1.0 mL under a gentle stream of nitrogen at a bath temperature of 35°C. Add a few grains of anhydrous sodium sulfate to dry the solution, if needed.

2.2 Liquid/Solid Chromatography: Silica Column Clean-up

2.2.0 Take the sample through a Bond Elut® Silica cartridge.

2.2.1 Using a vacuum box apparatus, condition the cartridge with 10 mL of ethyl acetate followed by 20 mL of hexane and discard the eluant.

2.2.2 Place the sample solution through the silica cartridge using a 10-mL hexane quantitative rinse at a rate of 1-2 mL per minute. Elute to waste.

2.2.3 Elute the analyte with 12 mL of a 98:2 hexane:ethyl acetate solution at a rate of 1-2 mL per minute.

2.2.4 Concentrate to 1.0 mL under a gentle stream of nitrogen at a bath temperature of 35°C. Proceed to the Baker Florisil® cartridge, if needed. If not, reconstitute to an appropriate final volume with hexane.

2.3 Liquid/Solid Chromatography: Florisil® Column Clean-up

2.3.0 Take the sample through a Baker Florisil® cartridge.

2.3.1 Using a vacuum box apparatus, condition the cartridge with 10 mL of ethyl acetate followed by 20 mL of hexane and discard the eluant.

2.3.2 Place the sample solution through the Florisil® cartridge using a 10-mL hexane quantitative rinse at a rate of 1-2 mL per minute. Elute to waste.

2.3.3 Elute the analyte with 12 mL of a 98:2 hexane:ethyl acetate solution at a rate of 1-2 mL per minute.

2.3.4 Concentrate to 1.0 mL under a gentle stream of nitrogen at a bath temperature of 35°C. Reconstitute to an appropriate final volume with hexane.

APPENDIX IV

ALTERNATE CLEAN-UP PROCEDURE FOR SEDIMENT/SOIL SAMPLES

Alternate Clean-up Procedure for Sediment/Soil

1.0 Extraction (see Appendix III)

2.0 Clean-up

2.1 Solid Phase Extraction (see Appendix III)

2.2 Liquid/Solid Chromatography: Mixed Column Clean-up

2.2.0 Take the sample through a mixed Florisil®/silica column.

2.2.1 Add 25 mL of ethyl acetate to a 30 cm x 12 mm glass chromatography column with a glass wool plug.

2.2.2 Add two grams of Florisil®, followed by two grams of silica.

2.2.3 Elute the ethyl acetate to waste (the column cannot go dry beyond this point).

2.2.4 Add 25 mL of hexane and elute to waste.

2.2.5 Add the concentrated extract from the C_{18} cartridge (see Appendix III) and elute to waste.

2.2.6 Add ten mL of hexane and elute to waste.

2.2.7 Elute and save 50 mL of a 95:5 hexane:ethyl acetate solution (this portion contains any esfenvalerate residue present). Concentrate this eluant to 1.0 mL under a gentle stream of nitrogen at a bath temperature of 35°C and bring to an appropriate final volume with hexane.

APPENDIX V

METHOD AMR-1086-88

TRACE METHOD FOR TOTAL ESFENVALERATE RESIDUES IN FISH SAMPLES

Fish Method

1.0 Extraction

1.1 Remove samples from storage and let thaw to workable condition.

1.2 Macerate a representative sample in a blender to homogenize.

1.3 Weigh 5 grams of sample into a 250-mL Nalgene® jar.

1.4 Add 75 mL of hexane/isopropanol (75/25).

1.5 Extract using a Tekmar® Extractor for 1.5 minutes.

1.6 Centrifuge for about 5 minutes.

2.0 Clean-up

2.1 Liquid/Liquid Partition

2.1.0 A two-gram aliquot was placed in a 250-mL separatory funnel.

2.1.1 Partition with three 100-mL portions of ultrapure water.

2.1.2 Add hexane to make the total volume 50 mL.

2.1.3 Partition the hexane extract by vigorously shaking with three 50-mL portions of acetonitrile saturated with hexane for one minute each.

2.1.4 Concentrate the combined acetonitrile extracts using a Turbo-vap® evaporator to about 3 mL.

2.1.5 Add 100 mL of hexane and concentrate to about 3 mL.

2.1.6 Add 100 mL of hexane a second time and concentrate to about 3 mL.

2.1.7 Transfer to a 15-mL centrifuge tube and concentrate using compressed air blown down and a water bath at 60°C to 2 mL.

2.2 Liquid/Solid Chromatography: Silica Column Clean-up

2.2.0 Take the sample through a Bond Elut® Silica cartridge.

2.2.1 Using a vacuum box apparatus, condition the cartridge with 10-mL of ethyl acetate followed by 20 mL of hexane and discard the eluant.

2.2.2 Place the sample solution through the silica cartridge using a 10 mL hexane quantitative rinse at a rate of 1-2 mL per minute. Elute to waste.

2.2.3 Elute the analyte with 12 mL of a 98:2 hexane:ethyl acetate solution at a rate of 1-2 mL per minute.

2.2.4 Concentrate to 1.0 mL under a gentle stream of nitrogen at a bath temperature of 35°C. Proceed to the Baker Florisil® cartridge, if needed. If not, reconstitute to an appropriate final volume with hexane.

2.3 Liquid/Solid Chromatography: Florisil® Column Clean-up

2.3.0 Take the sample through a Baker Florisil® cartridge (20 mL).

2.3.1 Using a vacuum box apparatus, condition the cartridge with 30 mL of ethyl acetate followed by 60-70 mL of hexane and discard the eluant.

2.3.2 Place the sample solution through the Florisil® cartridge using a 10-mL hexane quantitative rinse at a rate of 1-2 mL per minute. Elute to waste.

2.3.3 Elute the analyte with 50 mL of a 98:2 hexane:ethyl acetate solution at a rate of 1-2 mL per minute.

2.3.4 Concentrate to 1.0 mL under a gentle stream of nitrogen at a bath temperature of 35°C. Reconstitute to an appropriate final volume with hexane.