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McKenzie Laboratories, Inc., Phoenix
Modification of AMR-720-87 (MMS-R-494-Z)

Residue Determination of Vendex Miticide and Its Organotin Metabolites, SD 31723 and SD 33608 in Soils and Agriculture Commodities

Modified by Kathryn Koktavy and Diana Price on 4/1/88.

Reference: "Residue Determination of Vendex Miticide and Its Organotin Metabolites, SD 31723 and SD 33608 in Agricultural Commodities." AMR-720-87 (MM-R-494-S)

Apparatus and Reagents:

- Boiling flasks, 500 mL and 250 mL
- 250 mL Nalgene Bottles (HDPE)
- Separatory Funnels, 125 mL and 500 mL capacity equipped with Teflon stopcocks
- 50 mL Centrifuge Tubes with Teflon lined lids
- 5.0 mL Gas Tight Syringe (or 2.5 mL)
- Wrist Action Shaker
- N-Evap Analytical Evaporator
- Buchi Rotary Evaporator
- Centrifuge
- Gas Chromatograph
- Chromatographic Column 15 m x 0.53 mm x 0.1 μ dimethyl polysiloxane
- Reference Standards:
  - Vendex Miticide: DPX-CG296 (SD14114), Generic-Fenbutatin oxide
  - IN-CG200 (SD31723) metabolite of Vendex Miticide
  - IN-DP387 (SD33608) metabolite of Vendex Miticide
- Ethyl Acetate, N-Hexane, Chloroform, Acetonitrile, Isopropyl Alcohol, "Distilled in glass" Solvents, Omni-Solv.
- Hydrochloric Acid, EM Science
- Diethyl Ether, Burdick and Jackson High Purity Solvent
- Dry Chemicals, Florisil, Sodium Sulfate
- Methyllithium in Diethyl Ether, 1.4 (CH_3Li) (from Aldrich Chemical Company)
- Tropolone, 98% (from Aldrich Chemical Company)

PROCEDURE:

Isolation

The day before:

1. Weigh 10 g of sample into a 250 mL Nalgene bottle
2. Add 50 mL of concentrated HCl to each sample.
3. Add 150 mL of chloroform to each sample

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4. Fortify controls where necessary with a mixed spiker (containing SD 33608, SD 31723, SD 14114) at appropriate level.

5. Tightly cap bottles and place them on their sides on a wrist action shaker and shake vigorously for two hours. If the shaker is not capable of vigorous shaking, double the shaking time.

6. Allow samples to sit for at least 20 hours.

The next day:

1. Shake samples vigorously, by hand, for about 30 seconds.

2. Centrifuge for 3 minutes at setting of 1300 RPM.

3. Pour entire contents into 500 mL or 1 L separatory funnel.

Solvent Partition Cleanup

4. Measure an aliquot of the lower chloroform layer into a 250 mL boiling flask. (60 mL aliquot for crop and 90 mL aliquot for soils). Add 3 drops of keeper (1% mineral oil in toluene) and a boiling chip. Evaporate off the chloroform on a Buchi equipped with a 40°C bath.

5. Remove the residual chloroform traces using a stream of N_2.

6. Add 20 mL of hexane and swirl the sample.

7. Prepare hexane saturated acetonitrile containing 0.5 g/L tropolone. (It is important to add the tropolone just before it is going to be used)

8. Add 20 mL of the hexane saturated acetonitrile solution containing tropolone to the sample and swirl

9 Transfer the sample to a 125 mL separatory funnel and shake for 1 minute.

10. Drain the lower acetonitrile phase into a clean 500 mL boiling flask.

11 Partition 3 additional times using 20 mL of the saturated acetonitrile solution containing tropolone for each partition. Shake for two minutes between each partition and combine all four partitions.

12. Add approximately 3 drops of keeper and concentrate on a Buchi at 40°C to approximately 50 mL.

13. Add 150 mL ethyl acetate and concentrate to approximately 20 mL.

14. Quantitatively transfer samples with ethyl acetate into a 50 mL centrifuge tube with Teflon lined lids that do not leak.

15. At this point make the methylated standard. The best way is to combine SD 14114 and SD 33608 for one standard and prepare a separate standard containing only SD 31723.

16. Add approximately 2-3 drops of keeper and evaporate all samples (including methylated standards) to near dryness (there should be a small drop left in the tube) on N-evap with a water bath set below 40°C.

Methylation

1. Immediately add 4 mL of diethyl ether, tightly cap centrifuge tubes with Teflon lined lids and swirl.

2. Add methylithium in diethyl ether DROPWISE (a vigorous reaction may occur!). Use 5 mL of methylithium for crops and 2.5 mL for soils. Swirl gently to ensure thorough mixing and let stand for 20 minutes.

3. Add 3 mL isopropyl alcohol DROPWISE to destroy any excess reagent. Note that if no reaction occurs the methylation is not complete!

4. Add [illegible] mL of D.I. water — 10(?)

5. Add 25 mL hexane and gently invert several times to mix. Concentrate on N-evap until upper phase is approximately 5 mL.

6. Add more hexane to bring upper phase to 10 mL.

7. Tightly cap centrifuge tube and shake vigorously for 1 minute and remove hexane phase into a clean 50 mL tube. (Tubes without lids are used here).

8. Partition the aqueous solution two additional times with 10 mL of hexane and combine all three hexane extracts into a 50 mL centrifuge tube.

9. Discard aqueous solution and concentrate hexane extract to approximately 4-5 mL for methylated standards, crops, oil, and dislodgeables just prior to column cleanup.

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Column Cleanup

1 Weigh up 5 grams Florisil (heated at least 16 hours at 120°-160°F) and make a slurry with hexane.

2 Put a plug of glass wool into a 2.5 x 30 cm column and fill the column with hexane.

3 Add 2 cm of anhydrous sodium sulfate (approximately 1 tsp).

4 Add Florisil slurry and tap to remove air bubbles.

5 Add another teaspoon of sodium sulfate.

6 Drain hexane to the top of the sulfate.

7 Prepare a 5% diethyl ether in hexane solution.

8 Add the 4-5 mL sample to column and drain to the sulfate level.

9 Rinse the tube with 10 mL of the eluting solution (5% diethyl ether in hexane) and add to the column. Drain to the sulfate level into a 50 mL centrifuge tube.

10 Elute three additional times rinsing the tube each time. Columns should be run with the stopcock wide open when draining the column.

Dry down on N-evap to approximately 5 mL.

12 Transfer to 15 mL centrifuge tube previously marked at 2 mL and dry samples down to 2 mL. Transfer methylated standards to 100 mL volumetric flasks and bring to volume.

Helpful Hints for Running Vendex

Night before analysis:

Set up all glassware (separatory funnels, boiling flasks, and 100 mL graduated cylinders).

2 Saturate acetonitrile with hexane. Do not add tropolone until right before this solution is to be used.

3 Take the samples off the shaker and place by the centrifuge.

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G C Conditions

Instrument type:	Hewlett Packard 5890
Column.	15 m 0.53 mm x 0.1 μ
Column packing:	Dimethyl Polysiloxane
Detector type:	Flame Photometric Detector
	Sn Mode
He:	9.5 psi
Air:	50 psi
H_2:	40 psi
Attenuation:	2^2
Oven:	SD 14114 - 208°C
	SD 31723 - 174°C
	SD 33608 - 74°C
Inlet:	290°C

Make sure of the final volume and detection limit as they vary with matrix e.g. crops, soils and dislodgeables are different. The final sample weight and volume will determine your detection limit.

Appendix 4:

Sample Calculations

Calculations:

1. The parts per million Vendex® residues were calculated as follows:

$$ppm = \frac{ng\ found}{mg\ inj} \qquad mg\ inj = \frac{\mu L\ injected \times final\ weight\ (g)}{final\ volume\ (mL)}$$

The ng found was calculated from the standard curve's regression line.

2. The spike recoveries were calculated as follows:

$$percent\ recovery = \frac{ppm\ found}{ppm\ fortified} \times 100$$

Example:

1. Control S000 54112 + 0.40 ppm Vendex®, SD14114, analyzed on July 25, 1991.

The ppm calculated as follows:

$$ppm = \frac{ng\ found}{mg\ inj} \qquad ppm = \frac{1.00\ ng}{3.0\ mg\ inj} = 0.333$$

$$mg\ inj = \frac{2\ \mu L \times 6\ g}{4\ mL} = 3.0$$

The spike recovery was calculated as follows.

$$\frac{0.333\ ppm}{0.40\ ppm} \times 100 = 83\%$$

2. Control, S000 54114 + 0.40 ppm Vendex® SD31723 analyzed on July 26, 1991.

The ppm calculated as follows:

$$ppm = \frac{ng\ found}{mg\ inj} \qquad ppm = \frac{1.10\ ng}{3.0\ mg\ inj} = 0.367$$

$$mg\ inj = \frac{2\ \mu L \times 6\ g}{4\ mL} = 3.0$$

Appendix 4:

Sample Calculations, continued.

The spike recovery was calculated as follows:

$$\frac{0.367 \text{ ppm}}{0.40 \text{ ppm}} \times 100 = 92\%$$

3. Control, S000541-12 + 0.40 ppm Vendex®, SD33608 analyzed on July 25, 1991.

The ppm calculated as follows:

$$\text{ppm} = \frac{\text{ng found}}{\text{mg inj}} \qquad \text{ppm} = \frac{0.730 \text{ ng}}{2.0 \text{ mg inj}} = 0.365$$

$$\text{mg inj} = \frac{2 \text{ uL} \times 6 \text{ g}}{6 \text{ mL}} = 2.0$$

The spike recovery calculated as follows:

$$\frac{0.365 \text{ ppm}}{0.40 \text{ ppm}} \times 100 = 91\%$$