METHOD OUTLINE

Residue Determination Method: GRM 94.17

Independent Laboratory Validation of Method GRM 94.17 - Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Soil by Capillary Gas Chromatography with Mass Selective Detection

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Rinse all reusable glassware with 90:10 acetone/0.1N HCl followed by acetone prior to use.

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Weigh 10.0 g of control soil into 45-mL labelled glass vials.

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Fortify soil as appropriate.

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Add 15.0 mL of 90:10 acetone/0.1N HCl to each vial and seal with a PTFE-lined cap. Vortex the samples briefly and place in an ultrasonic bath for 10-15 seconds. Shake the samples for at least 30 minutes at approximately 180 excursions/minute.

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Centrifuge samples for 10 minutes at 2500 rpm.

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Decant the extracts into clean 45-mL vials.

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Extract soil again with 15.0 mL of 90:10 acetone/0.1N HCl, vortexing, sonicating, and centrifuging for 10 minutes at 2500 rpm.

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Combine the extracts by decanting into the vials containing the first 15 mL of extract.

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Evaporate the extracts to approximately 4 mL (removing the acetone) under a gentle stream of nitrogen at 40°C.

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Add approximately 20 mL of deionized water to each vial and seal with a PTFE-lined cap. Vortex and sonicate for 10-15 seconds.

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Adjust the sample pH with 0.1 N NaOH to fall within a range of 6.5 to 8.0 pH units using the following procedure:

a)Determine the pH using the appropriate indicator strips.

b) Add a volume (mL) of 0.1 N NaOH equal to 240×10^{-pH}. For example, for a pH of 3, the amount 0.1 N NaOH would be 240×10^{-3} or 0.24 mL.

c)Vortex and sonicate for 5 seconds and check pH.

d)Adjust the pH with dropwise amounts of 0.1 N HCl if pH value is > 8 and with 0.1 N NaOH if the pH < 6.5. Mix thoroughly

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METHOD OUTLINE (continued)

Attach a 75-mL reservoir to the top of an ion-exchange (quaternary amine) SPE column and place on the vacuum manifold box.

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Rinse the column and reservoir with approximately 5 mL of methanol (do not allow column bed to dry). Condition with approximately 5 mL deionized water (do not allow the column bed to dry).

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Transfer the pH adjusted extract to the reservoir. With the aid of vacuum, pull the extract through the column at a flow rate of approximately 2 mL/minute.

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Rinse the extract vial with approximately 2 mL of deionized water and transfer the rinse to the reservoir. Pass through SPE column at approximately 2 mL/minute.

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Elute the analytes by passing 5.0 mL of 0.1 N HCl solution through the column, collecting the eluent in an 11-mL vial.

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Add 100 µL of 2.0 N HCl and 2-3 g of NaCl to the 11-mL vial. Add 2.5 mL of MTBE to the vial and seal with PTFE-lined cap. Vortex for 15 seconds and shake for 10 minutes at approximately 180 excursions/minute. Centrifuge for 5 minutes at 2500 RPM.

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Weigh a 12-mL tube to four significant figures and record the weight. This tube will be used to collect the eluent from the silica gel SPE clean-up.

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Place a silica gel SPE column on the vacuum manifold box. Rinse the SPE with approximately 5 mL of MTBE. Condition with 5 mL isooctane (do not allow the column bed to dry).

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Transfer the MTBE layer (top layer - from three steps back) to the silica gel SPE column. Allow the MTBE to pass by gravity flow through the column.

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Add another 2.5 mL of MTBE to the vial and vortex for approximately 15 seconds. Centrifuge the vial for 5 minutes at 2500 rpm. Transfer the MTBE layer to the silica gel SPE column. With the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/minute.

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Elute the analytes by passing 10.0 mL of 0.025% acetic acid in MTBE through the column, collecting the eluent in the previously weighed 12-mL tube.

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Add 0.5 mL of isooctane to the eluent, cap the tube with a PTFE-lined cap and vortex the sample for approximately 5 seconds.

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METHOD OUTLINE (continued)

Evaporate, at room temperature, to 0.25 mL under a **gentle** flow of N_2 (do not allow sample to evaporate significantly below 0.25 mL).

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Add 25 μl of acetone and bring the volume of the sample to approximately 0.5 mL with isooctane (compare with a new 12-mL tube containing 0.5 mL isooctane).

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Weigh the tube with the sample. Record the weight (value will be used to determine extract final volume). Seal the tube after weighing to prevent evaporation.

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Add approximately 0.1g of anhydrous sodium sulfate and vortex the sample for 15 seconds.

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Add 25 μL of MTBSTFA and vortex the sample for 15 seconds. Centrifuge the sample for 5 minutes at 2500 RPM.

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Transfer the sample to a 2-mL autosampler vial and seal with a cap and crimper.

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Transfer 0.5 mL of each of the calibration standards to autosampler vials. Derivatize by adding 25 μL of MTBSTFA to each vial and seal with a cap and crimper.

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Analyze by GC/MSD.

2. CALCULATIONS AND STATISTICAL METHODS

Calculations:

Final Sample Weight: The final sample weight was calculated by subtracting the weight of the empty 12-mL tube from the weight of the tube and sample.

Final Sample Volume: The final sample volume was calculated by first subtracting the weight contribution of acetone (0.025 mL) from the final sample weight giving the weight of isooctane present. The volume of isooctane present was calculated by dividing the weight of isooctane present by the density of isooctane. The final sample volume was calculated by the addition of the calculated isooctane volume and the 0.025 mL of acetone as represented in the equations below:

$$\text{Final Sample (mL)} = \left[\frac{\text{final sample weight - (0.025 mL x density of acetone)}}{\text{density of isooctane}}\right] + 0.025 \text{ mL}$$

$$\text{Final Sample (mL)} = \left[\frac{\text{final sample weight - (0.025 mL x 0.7899 g/mL)}}{0.687 \text{ g/mL}}\right] + 0.025 \text{ mL}$$

Normalized Peak Area: Peak area for *m/z* 163 was normalized to 0.5 mL by using the following equation:

$$\text{Normalized Peak Area} = \frac{\text{Final Sample Volume (mL)}}{0.5 \text{ mL}} \times m/z \text{ 163 peak area}$$

Confirmation Ratios: Confirmation ratios were calculated for each calibration standard and sample analyzed. Confirmation ratios were determined for both the *cis*- and the *trans*-3-chloroacrylic acid by dividing the peak area of the confirmation ion by the peak area of the quantitation ion.

For example, using the data from Figure 3:

$$\text{Confirmation Ratio} = \frac{\text{peak area of confirmation ion}}{\text{peak area of quantitation ion}}$$

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 165}}{\text{peak area at } m/z \text{ 163}}$$

$$\text{Confirmation Ratio} = \frac{327}{861}$$

$$\text{Confirmation Ratio} = 0.3798$$

Confirmation of the presence of *cis*- and *trans*-3-chloroacrylic acid is achieved when the confirmation ratio for the samples is in the range of ± 20% of the average found for the calibration standards.

Percent Recovery: The percent recovery was determined by dividing the net analyte concentration found in the sample by the theoretical concentration added.

$$\% \text{ Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100$$

For example, using the data from Figure 5:

$$\% \text{ Recovery} = \frac{0.1821 \text{ ng/g}}{0.2000 \text{ ng/g}} \times 100$$

$$\% \text{ Recovery} = 91\%$$

Statistical Methods:

Average recoveries for each analyte in the matrix was calculated by dividing the sum of the percent recoveries by the total number of fortified samples.

Standard deviations for each analyte in the matrix was also determined. The standard deviation was calculated by summing the squares of the individual deviations from the average recoveries, dividing by the number of degrees of freedom, and extracting the square root of the quotient.

3. FULL DESCRIPTION OF ANALYTICAL INSTRUMENTATION USED

Instrument:	Hewlett-Packard Model 5890 Series II Gas Chromatograph (GC) equipped with an Hewlett Packard 7673A Autosampler and a Model G1034B Chemstation
Detector:	Hewlett-Packard 5971A Mass Selective Detector (MSD) in the SIM mode
Analytical Column:	J & W Scientific DB-5 Capillary Column (30 m X 0.25 mm i.d., 0.25 μm film thickness)
Temperatures:	
Column:	45°C for 1.0 minutes 45° to 220°C at 10°C/minute
Injector:	230°C
Transfer Line:	300°C
Carrier Gas:	Helium
Head Pressure:	50 kPa
Injection Volume:	1 μL
Injection Mode	Splitless
Purge Time:	0.5 minute