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Determination of Residues of *cis*- and *trans*-3-Chloroacrylic Acid in Soil by Capillary Gas Chromatography with Mass Selective Detection

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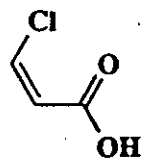
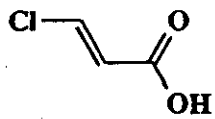
A. Scope

This method is applicable for the quantitation of residues of the 1,3-dichloropropene metabolites, *cis*- and *trans*-3-chloroacrylic acid (CAAC) in soil. The method was validated over the concentration range 0.20 ng/g to 2.0 µg/g with a limit of quantitation of 0.20 ng/g.



cis-3-Chloroacrylic Acid
CAS 1609-93-4



trans-3-Chloroacrylic Acid
CAS 2345-61-1

B. Principle

Residues of CAAC in soil are extracted with acidified acetone. The extract is partially evaporated to remove acetone, diluted with deionized (DI) water, and adjusted to a neutral pH. CAAC residues are concentrated using an ion-exchange solid-phase extraction column (SPE). The CAAC is eluted from the SPE in 0.1 N hydrochloric acid. The eluent is further acidified, saturated with sodium chloride and CAAC residues are partitioned into methyl-*t*-butyl ether (MTBE). The MTBE is passed through a silica gel SPE column to remove water and particulate. Isooctane is added and the MTBE is evaporated. CAAC residues in isooctane are derivatized with N-methyl-N-(*t*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) to their corresponding *t*-butyldi-methylsilyl esters (CAAC TBDMSE) and analyzed by capillary gas chromatography with mass selective detection (GC/MSD). Soils containing levels of CAAC above 20 ng/g are diluted 100-fold with isooctane, rederivatized and reanalyzed.

C. Safety Precautions

1. Each analyst must be acquainted with the potential hazards of the reagents, products, and solvents used in this method before commencing laboratory work. SOURCES OF INFORMATION INCLUDE: MATERIAL SAFETY DATA SHEETS, LITERATURE, AND OTHER RELATED DATA. Safety information on non-DowElanco products should be obtained from the container label or from the supplier. Disposal of reagents, reactants, and solvents must be in compliance with local, state, and federal laws and regulations.

2. Acetic acid, acetone, isooctane, methanol and MTBE are flammable and should be used in well-ventilated areas away from ignition sources.

3. Concentrated acetic acid is corrosive and can cause severe burns. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

4. *cis*- and *trans*-3-Chloroacrylic Acid are corrosive and lachrymators. It is imperative that proper eye and personal protection equipment be used when handling these reagents.

5. MTBSTFA is irritating to eyes, respiratory system and skin. It is imperative that proper eye and personal protection equipment be used when handling this reagent.

D. Equipment (Note N.1.)

1. Automatic sampler, Model 7673, Hewlett-Packard, Wilmington, DE 19808.

2. Balance, analytical, Model AE200, Mettler Instrument Corporation, Hightstown, NJ 08520.

3. Balance, pan, Model BB2440, Mettler Instrument Corporation.

4. Centrifuge, with rotor to accommodate 12-mL vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.

5. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549.

6. Gas chromatograph, Model 5890 Series II, Hewlett-Packard.

7. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.

8. Mass selective detector data system, Model G1034B, Hewlett-Packard.

9. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

10. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.

11. Vacuum manifold box, Model spe-21, J.T. Baker, Inc., Phillipsburg, NJ 08865.

12. Vial Crimper, catalog number 8710-0979, Hewlett-Packard, Wilmington, DE 19808.

13. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.

14. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

E. Glassware and Materials (Note N.1.)

1. Column, capillary gas chromatography, Durabond-5 liquid phase, 30 m x 0.25 mm i.d., 0.25 µm film thickness, catalog number 122-5032, J&W Scientific, Folsom, CA 95630.

2. Column, silica gel SPE, catalog number 7086-07, J.T. Baker, Inc.

3. Column, strong anion-exchange (quaternary amine) SPE, catalog number 71225-6013, Varian Sample Preparation Products, Harbor City, CA 90710.

4. Column adapter, PTFE, catalog number 120-1100, Jones Chromatography, Inc., Lakewood, CO 80228.

5. Column inlet liner, deactivated, catalog number 5181-3315, Hewlett-Packard.

6. Column reservoir, 75 mL, catalog number 7120-03, J.T. Baker, Inc.

7. Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869. (Note N.2.)

8. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note N.2.)

9. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note N.2.)

10. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.

11. Gas, nitrogen, 99.99% purity, Airco.

12. Indicator strips, pH range 0.0 to 6.0, 0.5 pH gradation, product number 4391-01, J.T. Baker, Inc.

13. Indicator strips, pH range 4.5 to 10.0, 0.5 pH gradation, product number 4395-01, J.T. Baker, Inc.

14. Microdispenser, 25 µL, Drummond Dialamatic Microdispenser, catalog number 3000225, Drummond Scientific Company, Broomall, PA 19008.

15. Microdispenser replacement bore, 25 µL, catalog number 3000225G, Drummond Scientific Company.

16. Syringes, 100, 250, and 500 µL capacity, catalog numbers 80600, 80700, and 80800, Hamilton Co., Reno, NV 89520.

17. Tube, Pyrex brand culture tube with threaded end, catalog number 14-957-86D, Fisher Scientific, Pittsburgh, PA 15219.

18. Vial, autosampler, 2 mL, catalog number C4011-1, National Scientific Co., Lawrenceville, GA 30243.

19. Vial, 11-mL with PTFE-lined screw cap, catalog number 2504T, Qorpak, Pittsburgh, PA 15205.

20. Vial, 45-mL with PTFE-lined screw cap, catalog number 60958A-11, Kimble Glass, Vineland, NJ 08360.

21. Vial seal, catalog number C4011-1A, National Scientific Company.

F. Reagents and Chemicals (Note N.1.)

1. Reagents

a. Acetic acid, HPLC grade, catalog number A35-500, Fisher Scientific, Pittsburgh, PA 15219.

b. Acetone, Optima grade, catalog number A929-4, Fisher Scientific.

c. Hydrochloric acid, 0.1 N, ACS reagent grade, certified concentration, catalog number SA54-4, Fisher Scientific.

d. Hydrochloric acid, 2.0 N, ACS reagent grade, certified concentration, catalog number SA431-500, Fisher Scientific.

e. Isooctane, Optima grade, catalog number O301-4, Fisher Scientific.

f. Methanol, Optima grade, catalog number A454-4, Fisher Scientific.

g. MTBE, methyl-*t*-butyl ether, HPLC grade, catalog number E127-4, Fisher Scientific.

h. MTBSTFA, N-methyl-N-(*t*-butyldimethylsilyl)trifluoroacetamide, catalog number 48920, Pierce, Rockford, IL 61105.

i. Sodium chloride, ACS reagent grade, catalog number S271-1, Fisher Scientific.

j. Sodium hydroxide, 0.1 N, ACS reagent grade, certified concentration, catalog number SS276-4, Fisher Scientific.

k. Sodium sulfate (anhydrous), certified ACS grade, catalog number S421-500, Fisher Scientific.

l. Standards

(1) *cis*-3-Chloroacrylic acid

The *cis*-CAAC standard, TSN100370, used for this study was originally obtained from Aldrich Chemical Co., Milwaukee, WI 53233, catalog number 17,740-7, lot number 04926EW, with a purity of >98% (1).

(2) *trans*-3-Chloroacrylic acid

The *trans*-CAAC standard, TSN100371, used for this study was originally obtained from Aldrich Chemical Co., catalog number C2,235-0, lot number 09114TW, with a purity of >99% (2).

Obtain from Test Substance Coordinator, DowElanco, Indianapolis, IN 46268-1053.

2. Prepared Solutions

a. 90% acetone/10% 0.1 N hydrochloric acid solution

Pipet 200 mL of 0.1 N hydrochloric acid into a 2000-mL volumetric flask containing approximately 1000-mL of acetone. Swirl the flask and allow to equilibrate to room temperature. Dilute to volume with acetone.

b. 0.025% Acetic acid in MTBE

Deliver 250 µL of acetic acid into a 1000-mL volumetric flask containing approximately 500-mL of MTBE. Swirl the flask and dilute to volume with MTBE.

G. Preparation of Standards

All solutions prepared in Section G should be stored in amber bottles and sealed with PTFE-lined caps.

1. Preparation of *cis*- and *trans*-CAAC Stock Solutions

a. Weigh 0.1000 g of *cis*-CAAC analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

b. Weigh 0.1000 g of *trans*-CAAC analytical standard and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with acetone to obtain a 1000 µg/mL stock solution.

2. Preparation of *cis*- and *trans*-CAAC Spiking Solutions

a. Transfer 1.0 mL of each of the stock solutions in Sections G.1.a. and b. to a 100-mL volumetric flask and bring to volume with acetone to obtain an initial solution of 10.0 µg/mL for each *cis*- and *trans*-CAAC. This solution is used for spiking and preparation of diluted spiking and calibration solutions.

b. Solutions for spiking soil samples are prepared by adding approximately 10 mL of acetone and 10 µL of acetic acid to a 100-mL volumetric flask. The flask is agitated to allow acetic acid to contact the glass surface. The appropriate aliquot of the initial solution from Section G.2.a. is then added and diluted to volume with acetone as follows:

Aliquot of 10.0 µg/mL Soln.	Final Soln. Volume	Spiking Soln. Final Conc.
mL	mL	ng/mL
0.100	100	10.0
0.200	100	20.0
0.500	100	50.0
1.00	100	100.
2.00	100	200.
10.0	100	1000.

c. A 2.00-ng/mL spiking solution is prepared by adding approximately 10 mL of acetone and 10 µL of acetic acid to a 100-mL volumetric flask. The flask is agitated to allow acetic acid to contact the glass surface. A 1.0 mL aliquot of the 200 ng/mL spiking solution from Section G.2.b. above is added and diluted to volume with acetone. A 1.0 mL aliquot of this solution will fortify a 10-g soil sample at the limit of quantitation, 0.20 ng/g.

d. A summarization of the spiking solutions prepared, aliquots to be delivered to a 10-g soil sample and the resulting sample concentration is shown below.

Spiking Soln. Concentration	Control Soil Mass	Aliquot to Deliver	Equivalent Sample Conc.[a]
	g	mL	ng/g
2.0 ng/mL	10.0	1.0	0.20
10.0 ng/mL	10.0	1.0	1.0
20.0 ng/mL	10.0	1.0	2.0
50.0 ng/mL	10.0	1.0	5.0
100.0 ng/mL	10.0	1.0	10.0
200.0 ng/mL	10.0	1.0	20.0
1000.0 ng/mL	10.0	1.0	100.
10.0 µg/mL	10.0	0.5	500.
10.0 µg/mL	10.0	2.0	2000.

[a] The equivalent sample concentration is based on fortifying a 10-g soil sample with the specified aliquot of spiking solution.

3. Preparation of *cis*- and *trans*-CAAC Calibration Solutions

a. Solutions for calibration are prepared by adding approximately 10 mL of isooctane and 100 µL of acetic acid to a 100-mL volumetric flask. The flask is agitated to allow acetic acid to contact the glass surface. The appropriate aliquot of the initial solution from Section G.2.a. and acetone is then added and diluted to volume with isooctane as follows:

Aliquot of 10.0 µg/mL Soln.	Aliquot of Acetone	Final Soln. Volume	Calibrn. Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	mL	ng/mL	ng/g
0.200	4.8	100	20.0	1.00
0.500	4.5	100	50.0	2.50
2.00	3.0	100	200.	10.0
5.00	0.0	100	500.	25.0

[a] The equivalent sample concentration of the standard is based on taking the 10-g soil extract to a final volume of 0.5 mL.

b. A 2.00 and 4.00 ng/mL calibration solution are prepared by adding approximately 10 mL of isooctane and 100 µL of acetic acid to a 100-mL volumetric flask. The flask is agitated to allow acetic acid to contact the glass surface. The appropriate aliquot of the 200 ng/mL solution from Section G.3.a. above and acetone is then added and diluted to volume with isooctane as follows:

Aliquot of 200 ng/mL	Aliquot of Acetone	Final Soln. Volume	Calibrn. Soln. Final Conc.	Equivalent Sample Conc.[a]
mL	mL	mL	ng/mL	ng/g
1.00	5.0	100	2.0	0.100
2.00	5.0	100	4.0	0.200

[a] The equivalent sample concentration of the standard is based on taking the 10-g soil extract to a final volume of 0.5 mL.

H. Gas Chromatography/Mass Spectrometry

1. Column

Install the splitless column inlet liner (Section E.5.) and the capillary column (Section E.1.) in the split/splitless injection port of the GC/MSD following the manufacturer's recommended procedure.

2. Typical Operating Conditions

Instrumentation:	Hewlett-Packard Model 5890 (II) Gas Chromatograph Hewlett-Packard Model 5971A Mass Selective Detector Hewlett-Packard Model G1034C Data System Software
Column:	J&W Scientific fused silica capillary Durabond-5 liquid phase 30 m x 0.25 mm i.d. 0.25 µm film thickness
Temperatures:	
Column	45 °C for 1.0 min 45 °C to 220 °C at 10 °C/min
Injector	230 °C
Interface	300 °C

Carrier Gas:	helium
Head Pressure	50 kPa
Linear Velocity	approximately 40 cm/sec at an oven temperature of 140 °C
Injection Mode:	splitless
Purge Delay	0.5 min
Splitter Flow	50 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	1 µL
Detector:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune (Note N.3.)
Electron Multiplier	1647 volts (tune voltage plus 200)
Ions Monitored:	
cis-CAAC TBDMSE	*m/z* 163 (quantitation), *m/z* 165 (confirmation)
trans-CAAC TBDMSE	*m/z* 163 (quantitation), *m/z* 165 (confirmation)
Dwell Time	100 msec

Typical mass spectra of *cis*- and *trans*-CAAC TBDMSE are shown in Figures 1 and 2, respectively. Nominal *m/z* 163 and 165 ions monitored result from loss of the *t*-butyl radical (mass 57) and reflect the isotopic contributions of ^{35}Cl and ^{37}Cl, respectively.

3. Calibration Curves

Typical calibration curves for the determination of *cis*- and *trans*-CAAC in soil are shown in Figures 3 and 4, respectively.

4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 0.20 ng/g recovery sample for *cis*- and *trans*-CAAC in soil are shown in Figures 5-10.

I. Determination of Recovery of *cis*- and *trans*-CAAC from Soil

To minimize the potential for cross contamination, equipment used to process samples and reusable glassware should be thoroughly rinsed with the 90% acetone/10% 0.1 N hydrochloric acid solution followed by acetone prior to use.

1. Preparation of Recovery Samples

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

b. For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding the specified aliquots of the appropriate spiking solutions (Section G.2.d.) in acetone to obtain concentrations ranging from 0.20 to

2000 ng/g. A reagent blank, containing no soil sample, should be carried through the method with the samples.

c. Add 15.0 mL of the 90% acetone/10% 0.1 N hydrochloric acid solution (F.2.a.) to each sample vial and seal with a PTFE-lined cap.

d. Vortex the samples briefly and sonicate 10-15 seconds.

e. Shake the samples for a minimum of 30 minutes on a reciprocating shaker at approximately 180 excursions/minute.

f. Centrifuge each sample for 10 minutes at 2500 rpm.

g. Carefully decant each extract to a clean 45-mL vial.

h. Extract each sample a second time by repeating Steps I.1.c., d. and f. Combine the extracts by decanting to the vial in Step I.1.g.

i. Evaporate the acetone in the extract by placing the vial in an N-Evap evaporator set at 40 °C. Evaporate the sample under nitrogen to approximately 4.0 mL.

j. Add approximately 20 mL of deionized water to the vial and seal with a PTFE-lined cap.

k. Vortex and sonicate the samples for 10-15 seconds.

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

(1) Determine the pH using an indicator strip (Section E.12.).

(2) Add a volume (mL) of 0.1 N sodium hydroxide equal to 240×10^{-pH}. For example, if the pH was determined to be three, the amount of 0.1 N sodium hydroxide to add would be 240×10^{-3} or 0.24 mL.

(3) Vortex and sonicate the sample for 5 seconds.

(4) Determine the pH using an indicator strip (Section E.13.).

(5) If the pH falls within the acceptable range proceed with Step I.1.m. If the pH is below the acceptable range add 0.1 N sodium hydroxide dropwise with thorough mixing until an acceptable pH is reached. If the pH is above the acceptable range add 0.1 N hydrochloric acid dropwise with mixing until an acceptable pH is reached. Mixing is critical to ensure that the pH determined represents the total solution.

m. The samples are then concentrated and purified using the following ion-exchange SPE procedure:

(1) Place an ion-exchange (quaternary amine) SPE column (Section E.3.) on the vacuum manifold box.

(2) Attach a 75-mL (Section E.6.) reservoir to the top of the column using an SPE column adapter (Section E.4.).

(3) Rinse the SPE column and reservoir with approximately 5 mL of methanol. (Do not allow the column bed to dry.)

(4) Condition the SPE column with approximately 5 mL of deionized water. (Do not allow the column bed to dry.)

(5) Transfer the sample solution from Step I.1.l(5). to the reservoir and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min.

(6) Rinse the sample vial with approximately 2 mL of deionized water and transfer the rinse to the reservoir. With the aid of vacuum pull the sample through the column at a flow rate of approximately 2 mL/min.

(7) Elute the CAAC by passing 5.0 mL of 0.1 N hydrochloric acid solution through the column, collecting the eluent in a 11-mL vial. Discard the SPE column. (Note N.4.)

n. Add 100 µL of 2.0 N HCl and 2-3 g of sodium chloride to the 11-mL vial.

o. Add 2.5 mL of MTBE to the vial and seal with a PTFE-lined cap. Vortex the vial for 15 seconds and shake the sample for 10 minutes on a reciprocating shaker at approximately 180 excursions/minute.

p. Centrifuge the vial for 5 minutes at 2500 rpm.

q. The samples are then dried and purified further using the following silica gel SPE procedure:

(1) Weigh a 12-mL tube to four significant figures. This tube will be used to collect the eluent at the end of this procedure and the difference between the empty tube weight and the weight at Step I.1.t. will be used to calculate the final sample volume.

(2) Place a silica gel SPE column (Section E.2.) on the vacuum manifold box.

(3) Rinse the SPE column with approximately 5 mL of MTBE.

(4) Condition the SPE column with approximately 5 mL of isooctane. (Do not allow the column bed to dry.)

(5) Transfer the MTBE layer (top layer) from the vial in Step I.1.p. to the SPE column and allow the MTBE to pass by gravity flow through the column.

(6) Repeat Steps I.1.o. and p. without the shaking procedure and add the MTBE layer to the SPE column. With the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/min..

(7) Elute the CAAC by passing 10.0 mL of the 0.025% acetic acid in MTBE solution (Section F.2.b.) through the column, collecting the eluent in the 12-mL tube previously weighed. (Note N.4.)

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

s. Evaporate the solution at ambient temperature to a volume of approximately 0.25 mL under a gentle flow of nitrogen. (Do not allow sample to evaporate significantly below 0.25 mL.)

t. Add 25 µL of acetone and bring the volume of the sample to approximately 0.5 mL with isooctane by comparison to a set of 12-mL tubes containing a measured volume of 0.5 mL of isooctane. Weigh the tube with sample for use in calculation of final volume. Seal the tube with a PTFE-lined cap after weighing.

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

v. Add 25 µL of MTBSTFA and vortex the sample for 15 seconds.

w. Centrifuge the sample for 5 minutes at 2500 rpm.

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

y. Transfer 0.5 mL of each of the calibration standards in Section G.3.a. and b. to autosampler vials. Derivatize by adding 25 µL of MTBSTFA to each vial and seal with a cap and crimper.

z. Analyze the samples and calibration standards by capillary gas chromatography/ mass spectrometry as described in Section H. Samples that demonstrate CAAC levels above 20 ng/g are diluted 100-fold and reanalyzed as follows:

(1) Transfer 0.100 mL of the sample to a 10-mL volumetric flask, add 10 µL of acetic acid, approximately 0.5 mL acetone and dilute to volume with isooctane.

(2) Transfer 0.5 mL of the diluted sample to a 2-mL autosampler vial.

(3) Add 25 µL of MTBSTFA and seal the vial with a cap and crimper.

(4) Reanalyze as described in Section H.

2. Calculation of Normalized *m/z* 163 and 165 Sample Peak Area Response

a. Calculate the final sample weight by subtracting the weight of the empty 12-mL tube (Section I.1.q.) from the weight of the tube and sample (Section I.1.t.).

b. The final sample volume contains a known volume of 0.025 mL of acetone and an approximate volume of isooctane. The weight contribution of 0.025 mL acetone is subtracted from the sample final weight (Step I.2.a.) giving the weight of isooctane present. The volume of isooctane can then be determined using the density of isooctane. The final sample volume is the addition of the calculated isooctane volume and the 0.025 mL of acetone. Calculate the final sample volume using the following equations:

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

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

c. Normalize the sample *m/z* 163 and 165 peak areas to 0.5 mL using the following equation:

$$\text{normalized peak area} = \frac{\text{final sample volume (mL)}}{0.5\ \text{mL}} \times \text{sample peak area}$$

For example, using the data for *cis*-CAAC from Figure 7:

d. $\text{final sample weight (g)} = 12.22 - 11.74$

$\text{final sample weight (g)} = 0.480$

e. $$\text{final sample volume (mL)} = \left[\frac{0.480\ \text{g} - (0.025\ \text{mL} \times 0.7899\ \text{g/mL})}{0.687\ \text{g/mL}}\right] + 0.025\ \text{mL}$$

$$\text{final sample volume (mL)} = 0.6949$$

f. $$\text{normalized } m/z\ 163 \text{ peak area} = \frac{0.6949\ \text{mL}}{0.5\ \text{mL}} \times 415$$

$$\text{normalized } m/z\ 163 \text{ peak area} = 577$$

$$\text{normalized m/z 165 peak area} = \frac{0.6949\ \text{mL}}{0.5\ \text{mL}} \times 152$$

$$\text{normalized m/z 165 peak area} = 211$$

3. Calculation of Percent Recovery

a. Determine the *m/z* 163 and 165 response areas for both *cis*- and *trans*-CAAC TBDMSE in calibrations standards from Step I.1.y. and z.

b. For each standard, calculate the *cis*- and *trans*-CAAC confirmation ratios. The average confirmation ratio for each will be used to confirm the presence of the respective CAAC in the soil samples.

For example, using the data for *cis*-CAAC from Figure 5:

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

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

$$\text{Confirmation Ratio} = \frac{224}{621}$$

$$\text{Confirmation Ratio} = 0.3607$$

Positive confirmation of the presence of *cis*- and *trans*-CAAC is indicated when the confirmation ratio for the samples is in the range of ±20% of the average found for the respective standards.

c. Prepare *cis*- and *trans*-CAAC standard curves by plotting the sample equivalent concentration (ng/g) on the abscissa (x-axis) and the *cis*- and *trans*-CAAC TBDMSE *m/z* 163 peak area on the ordinate (y-axis) as shown in Figures 3 and 4, respectively. Using regression analysis, determine the equation for the curve with respect to the abscissa.

For example, using power regression (3) with the *trans*-CAAC data from Figure 4:

$$Y = \text{constant} \times X^{(\text{exponent})}$$

$$X = \left(\frac{Y}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\underset{\text{(ng/g)}}{\textit{trans}\text{-CAAC Conc.}} = \left(\frac{\textit{trans}\text{-CAAC TBDMSE peak area}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\underset{\text{(ng/g)}}{\textit{trans}\text{-CAAC Conc.}} = \left(\frac{\textit{trans}\text{-CAAC TBDMSE peak area}}{3424.0}\right)^{1/1.0147}$$

d. Determine the net concentration in each recovery sample that does not require dilution (less than or equal to 20 ng/g) by first subtracting the average *cis*- and *trans*-CAAC TBDMSE normalized *m/z* 163 peak area in the control sample from that of the recovery sample. Substitute the peak area obtained into the appropriate equation and solve for the concentration.

For example, using the normalized *m/z* 163 peak areas for *trans*-CAAC data from Figures 9 and 10 and the standard curve equation from Figure 4:

$$\underset{\text{(ng/g)}}{\textit{trans}\text{-CAAC Conc.}} = \left(\frac{\text{net } \textit{trans}\text{-CAAC TBDMSE peak area}}{3424.0}\right)^{1/1.0147}$$

$$\underset{\text{(ng/g)}}{\textit{trans}\text{-CAAC Conc.}} = \left(\frac{613 - 66}{3424.0}\right)^{1/1.0147}$$

$$\textit{trans}\text{-CAAC Conc.} = 0.1641 \text{ ng/g}$$

e. Determine the percent recovery by dividing the net concentration found for each recovery sample by the theoretical concentration added.

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

$$\text{Recovery} = \frac{0.1641 \text{ ng/g}}{0.200 \text{ ng/g}} \times 100\%$$

$$\text{Recovery} = 82\%$$

f. Determine the diluted concentration in each recovery sample fortified at levels above 20 ng/g by substituting the *cis*- and *trans*-CAAC TBDMSE normalized *m/z* 163 peak area into the appropriate standard curve and solve for the diluted concentration as described in Section I.3.d.

g. Determine the concentration in each diluted recovery sample by multiplying the diluted concentration by the dilution factor of 100.

$$\underset{(ng/g)}{\text{CAAC Conc.}} = \underset{(ng/g)}{\text{diluted CAAC Conc.}} \times 100$$

h. Determine the percent recovery for recovery samples fortified above 20 ng/g as described in Section I.3.e.

The average of all the recovery samples in a given analytical set can be used to correct sample results in the set for method efficiency.

J. Determination of *cis*- and *trans*-CAAC in Soil

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.

2. Prepare standard calibration curves for *cis*- and *trans*-CAAC and determine the percentage recovery for each as described in Section I.3.

3. Determine the concentration of *cis*- and *trans*-CAAC in each treated sample by substituting the *cis*- and *trans*-CAAC TBDMSE normalized *m/z* 163 peak areas obtained into the respective equations for the standard calibration curves, and calculate the uncorrected residue results.

   For example, using the *cis*-CAAC data from Figures 3 and 7, the uncorrected concentration is calculated as follows:

$$\underset{(ng/g)}{\textit{cis}\text{-CAAC Conc.}} = \left(\frac{\textit{cis}\text{-CAAC TBDMSE peak area}}{\text{constant}}\right)^{1/\text{exponent}}$$

$$\underset{(ng/g)}{\textit{cis}\text{-CAAC Conc.}} = \left(\frac{577}{3243.3}\right)^{1/1.0244}$$

$$\textit{cis}\text{-CAAC Conc.} = 0.1854 \text{ ng/g}$$

4. Samples that exceed a *cis*- or *trans*-CAAC concentration of 20 ng/g are diluted and reanalyzed as described in Section I.1.z.

5. Determine the concentration of *cis*- and *trans*-CAAC in each diluted, treated sample as described in Section I.3.f and g.

K. Determination of Soil Moisture

1. Weigh 10.00 g of soil into an aluminum or glass container.

2. Place the sample in an oven at approximately 130 °C and allow to dry for a minimum of 16 hours.

3. Remove the sample from the oven, place in a desiccator until the sample has cooled to ambient temperature, and then re-weigh.

4. Calculate the percent moisture on a dry weight basis as follows:

$$\text{Percent Moisture} = \frac{\text{soil moisture weight (g)}}{\text{dehydrated soil weight (g)}} \times 100$$

$$= \frac{\left(\begin{matrix}\text{soil weight} \\ \text{before drying}\end{matrix} - \begin{matrix}\text{soil weight} \\ \text{after drying}\end{matrix}\right)}{\begin{matrix}\text{soil weight} \\ \text{after drying}\end{matrix}} \times 100$$

L. Determination of Corrected *cis*- and *trans*-CAAC in Soil

1. Determine the *cis*- and *trans*-CAAC concentration in the soil samples as described in Section J.

2. Determine the soil moisture as described in Section K.

3. Determine the corrected *cis*- and *trans*-CAAC concentrations in soil samples as follows:

$$\begin{matrix}\text{CAAC} \\ \text{Corrected Conc. (ng/g)}\end{matrix} = \left(\begin{matrix}\text{CAAC} \\ \text{Conc. (ng/g)}\end{matrix}\right)\left(\frac{100}{\%\ \text{Recovery}}\right)\left(1 + \frac{\%\ \text{Moisture}}{100}\right)$$

3. Assay Time

A typical analytical run would consist of a minimum of four standards encompassing the expected range of sample concentrations, a reagent blank, a control (a non-fortified sample), a minimum of two fortified controls (one of which must be at the LOQ), and ten samples. This typical analytical run could be prepared in approximately 10 hours, and the chromatographic analysis take place the same evening.

There are several acceptable "stopping points" in the method where sample preparation (Section I.) may be suspended without deleterious effects on the sample analysis. These are indicated below:

a. Step I.1.o.

b. Step I.1.q.(7). If the samples are to be stored overnight, the vials should be sealed with PTFE-lined caps.

c. Step I.1.r.

4. Standardization of SPE Elution Profiles

Variation in the ion-exchange and silica gel SPE columns may influence the elution profile of *cis*- and *trans*-CAAC. If method performance degrades significantly with a change in the lot number of SPE columns used, an elution profile of the columns should be carried out. The following procedures can be used:

a. Ion-exchange SPE Profile

(1) In an 8-mL vial, add 25 µL of the 10 µg/mL *cis*- and *trans*-CAAC spiking solution (Section G.2.a.) to 5 mL of DI water.

(2) Place an ion-exchange SPE column on the vacuum manifold box.

(3) Rinse the SPE column with approximately 5 mL of methanol.

(4) Condition the SPE column with approximately 5 mL of DI water. (Do not allow the column bed to dry.)

(5) Transfer the sample solution from Step M.4.a.(1) to the SPE column and, with the aid of vacuum, slowly pull the sample through the column.

(6) Rinse the 8-mL vial with 2 mL of DI water, transfer the rinse to the SPE column and, with the aid of vacuum, slowly pull the rinse through the column.

(7) Elute the *cis*- and *trans*-CAAC with 10.0 mL of 0.1 N hydrochloric acid solution, collecting 1-mL aliquots in 8-mL vials.

(8) For each fraction collected, add 100 µL 2.0 N HCl, approximately 2 g of sodium chloride and extract two times with 2.5 mL of 0.025% acetic acid in MTBE.

(9) Combine both extracts in an 8-mL vial, add approximately 2 mL of isooctane and evaporate at ambient temperature under a gentle flow of nitrogen to approximately 0.5 mL.

(10) Adjust the volume to 1.0 mL with isooctane by comparison to two 8-mL vials each containing a measured volume of 1.0 mL isooctane. Add 25 µL of MTBSTFA and vortex the sample for 10 seconds.

(11) Transfer to an autosampler vial and seal the vial with a cap and crimper.

(12) Perform Steps I.1.y. and z.

(13) Calculate the percentage recoveries for each analyte as described in Section I.3. Use actual standard concentrations in determination of the standard curve equation and fraction recoveries.

Evaluation of results:

If less than 90% of the analytes are recovered in the first five 1-mL fractions, the method recoveries may fall below acceptable values. In such a case it would be imperative to check the validity of reagents, particularly the 0.1 N hydrochloric acid solution used to elute the column. If similar results are obtained after checking reagents, a second lot of SPE columns should be evaluated.

b. Silica gel SPE Profile

(1) In an 11-mL vial, add 25 µL of the 10 µg/mL *cis*- and *trans*-CAAC spiking solution (Section G.2.a.) to 5 mL of 0.1 N HCl.

(2) Proceed with Steps I.1.n. through p.

(3) Place a silica gel SPE column on the vacuum manifold box.

(4) Rinse the SPE column with 5 mL of MTBE.

(5) Condition the SPE column with 5 mL of isooctane. (Do not allow the column bed to dry.)

(6) Transfer the MTBE layer (top layer) of the sample solution from Step M.4.b.(2) to the SPE column and, with the aid of vacuum, slowly pull the sample through the column. (Do not allow the column bed to dry.)

(7) Repeat Steps I.1.o. and p. without the shaking procedure and transfer the

MTBE to the SPE column. With the aid of vacuum, slowly pull the sample through the column.

(8) Elute the analytes with 10 mL of the 0.025% acetic acid in MTBE solution, collecting 1-mL aliquots in 8-mL vials.

(9) Add 2 mL of isooctane to each of the elution fractions in Step M.4.b.(8).

(10) Concentrate the fractions at ambient temperature under a gentle flow of nitrogen to approximately 0.5 mL.

(11) Adjust the volume of the fractions to 1.0 mL with isooctane by comparison to two 8-mL vials each containing a measured volume of 1.0 mL isooctane. Add 25 µL of MTBSTFA and vortex the samples for 10 seconds.

(12) Transfer to an autosampler vial and seal the vial with a cap and crimper.

(13) Perform Steps I.1.y. and z.

(14) Calculate the percentage recoveries for each analyte as described in Section I.3. Use actual standard concentrations in determination of the standard curve equation and fraction recoveries.

Evaluation of results:

If less than 90% of the analytes are recovered in the ten 1-mL fractions, the method recoveries may fall below acceptable values. In such a case it would be imperative to check the validity of reagents, particularly the 0.025% acetic acid in MTBE solution used to elute the column. If similar results are obtained after checking reagents, a second lot of SPE columns should be evaluated. The presence of acetic acid in the MTBE is critical to eluting the analytes from the silica gel, increasing the eluting solution to 0.04% acetic acid in MTBE may be evaluated if the preceeding suggestions fail to give acceptable results.

N. Notes

1. Equipment, glassware, materials, reagents, and chemicals considered to be equivalent to those specified may be substituted with the understanding that their performance must be confirmed by appropriate tests. Common laboratory supplies are assumed to be readily available and are, therefore, not listed.

2. The filters are used in the carrier gas supply lines to purify the helium entering the gas chromatograph.

3. Several tuning, or calibration, options are available for the Model 597X series of MSDs. The "Maximum Sensitivity Autotune" feature was found to consistently yield approximately 5-10 times the sensitivity compared to that of the "Standard Autotune".

4. Depending on the number of samples being prepared, one may elute the CAAC from each SPE column individually, using either gravity-feed or pressurized elution, or as a group, using the vacuum manifold box.