

Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pesticide Name: Dichloropropene Degradate

MRID #: 445365-04

Matrix: Water

Analysis: GC/MS

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Appendix A

DowElanco Method GRM 94.14

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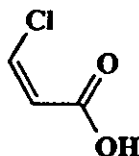
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SUPERSEDES: New

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

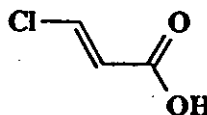
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Indianapolis, Indiana 46268-1053

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 water. The method was validated over the concentration range 0.05 to 5.0 ng/mL with a limit of quantitation of 0.05 ng/mL.



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



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

B. Principle

CAAC residues in water 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. 2,2,4-Trimethylpentane (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*-butyldimethylsilyl esters (CAAC TBDMSE) and analyzed by capillary gas chromatography with mass selective detection (GC/MSD).

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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 this reagent.
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 L.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, 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.

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

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

1. Column, capillary gas chromatography, Durabond-5 liquid phase, 30 m x 0.25 mm i.d., 0.25 μ m 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 7091-03, J.T. Baker, Inc.
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 L.2.)
8. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note L.2.)
9. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note L.2.)
10. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.
11. Gas, nitrogen, 99.99% purity, Airco.
12. Microdispenser, 25 μ L, Drummond Dialomatic Microdispenser, catalog number 3000225, Drummond Scientific Company, Broomall, PA 19008.
13. Microdispenser replacement bore, 25 μ L, catalog number 3000225G, Drummond Scientific Company.
14. Syringe, 100, 250, and 500 μ L capacity, catalog numbers 80600, 80700, and 80800, Hamilton Co., Reno, NV 89520.
15. Tube, Pyrex brand culture tube with threaded end, catalog number 14-957-86D, Fisher Scientific, Pittsburgh, PA 15219.
16. Vial, autosampler, 2 mL, catalog number C4011-1, National Scientific Co., Lawrenceville, GA 30243.
17. Vial, 11-mL with PTFE-lined screw cap, catalog number 2504T, Qorpak, Pittsburgh, PA 15205.
18. Vial, 45-mL with PTFE-lined screw cap, catalog number 60958A-11, Kimble Glass, Vineland, NJ 08360.

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

F. Reagents and Chemicals (Note L.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 sulfate (anhydrous), certified ACS grade, catalog number S421-500, Fisher Scientific.

k. 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. 0.025% Acetic acid in MTBE.

Deliver 250 μ L of acetic acid into a 1000-mL volumetric flask containing

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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, 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 G.1.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 preparation of spiking and calibration solutions.
- b. Solutions for spiking water samples are prepared by adding 10.0 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 mL	Final Soln. Volume mL	Spiking Soln. Final Conc. ng/mL	Equivalent Sample Conc. ^a ng/mL
0.100	100	10.0	0.250
0.200	100	20.0	0.500
0.500	100	50.0	1.25
1.00	100	100.	2.50
2.00	100	200.	5.00

^a The equivalent sample concentration is based on fortifying a 40-mL water sample with 1.0 mL of spiking solution.

- c. A 2.00 ng/mL spiking solution is prepared by adding 10.0 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 1.00-mL aliquot of the 200 ng/mL spiking solution from Section G.2.b. above is added and diluted to volume with acetone. A 1.00-mL aliquot of this solution will fortify a 40-mL water sample at the limit of quantitation, 0.05 ng/mL.

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

- a. Solutions for calibration are prepared by adding 10 mL of isooctane and 100 µL of acetic acid to a 100-mL volumetric flask. The flask is agitated to allow acetic acid

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to contact the glass surface. The appropriate aliquot of the 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	Aliquot of Acetone	Final Soln. Volume	Calibrn. Soln. Final Conc.	Equivalent Sample Conc. ^a
mL	mL	mL	ng/mL	ng/mL
0.200	4.8	100	20.0	0.250
0.500	4.5	100	50.0	0.625
2.00	3.0	100	200.	2.50
5.00	0.0	100	500.	6.25

^a The equivalent sample concentration of the standard is based on taking the 40-mL water sample extract to a final volume of 0.5 mL.

- b. A 2.00 and 4.00 ng/mL calibration solution are prepared by adding 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/mL
1.00	5.0	100	2.0	0.025
2.00	5.0	100	4.0	0.050

^a The equivalent sample concentration of the standard is based on taking the 40-mL water sample 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 G1034B 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

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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 L.3.)
Electron Multiplier	1647 volts (tune voltage plus 200)
Ions Monitored:	
<i>cis</i> -CAAC TBDMSE	<i>m/z</i> 163 (quantitation), <i>m/z</i> 165 (confirmation)
<i>trans</i> -CAAC TBDMSE	<i>m/z</i> 163 (quantitation), <i>m/z</i> 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 ³⁵Cl and ³⁷Cl, respectively.

3. Calibration Curves

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

4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 0.050 ng/mL recovery sample for *cis*- and *trans*-CAAC in water are shown in Figures 5-10.

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

1. Preparation of Recovery Samples

- Pipet 40.0 mL of control water samples into a series of 45-mL vials.
- For preparing fortified samples, use some of the samples as controls and fortify the remaining samples by adding 1.0-mL aliquots of the appropriate spiking solutions (Section G.2.) in acetone to obtain concentrations ranging from 0.050 to 5.0 ng/mL. A reagent blank, containing no water sample, should be carried through the method with the samples.

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- c. 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 (Section D.11.).
 - (2) Attach a 75-mL reservoir to the top of the column using an SPE column adapter.
 - (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.b. 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 eluant in an 11-mL vial. Discard the SPE column. (Note L.4.)
- d. Add 100 μ L of 2.0 N HCl and approximately 2 to 3 grams of NaCl to the 11-mL vial.
- e. 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.
- f. Centrifuge the vial for 5 minutes at 2500 rpm.
- g. The samples are then dried and purified further using the following silica gel SPE procedure:
- (1) Weigh a 12-mL tube to 4 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.j. 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 iso-octane. (Do not allow the column bed to dry.)
 - (5) Transfer the MTBE layer (top layer) from the vial in Step I.1.f. to the SPE column and allow the MTBE to pass by gravity flow through the column.
 - (6) Repeat Steps I.1.e. and f. 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.

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- (7) Elute the CAAC by passing 10.0 mL of the 0.025% acetic acid in MTBE solution (Section F.2.) through the column, collecting the eluant in the 12-mL tube previously weighed. (Note L.4.)
- h. Add 0.5 mL of isooctane, seal the tube with a PTFE-lined cap and vortex the sample for 5 seconds.
 - i. 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 to dryness.)
 - j. Add 25 μ L of acetone and bring the volume of the sample to 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.
 - k. Add approximately 0.1 g of anhydrous sodium sulfate and vortex the sample for 15 seconds.
 - l. Add 25 μ L of MTBSTFA and vortex the sample for 15 seconds.
 - m. Centrifuge the sample for 5 minutes at 2500 rpm.
 - n. Transfer the sample to a 2-mL autosampler vial and seal the vial with a cap and crimper.
 - o. Transfer 0.5 mL of each of the calibration standards in Section G.3.a and b. to autosampler vials. Derivatize by adding 25 μ L of MTBSTFA to each vial and seal with a cap and crimper.
 - p. Analyze the samples and calibration standards by capillary gas chromatography/mass spectrometry 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 from the weight of the tube and sample.
- 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:

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

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

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

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

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

d. $\frac{\text{final sample weight (g)}}{\text{final sample weight (g)}} = 11.82 - 11.48$

$$\text{final sample weight (g)} = 0.340$$

e. $\frac{\text{final sample volume (mL)}}{\text{final sample volume (mL)}} = \left[\frac{0.340 \text{ g} - (0.025 \text{ mL} \times 0.7899 \text{ g/mL})}{0.687 \text{ g/mL}} \right] + 0.025 \text{ mL}$
 $\text{final sample volume (mL)} = 0.4912$

f. $\frac{\text{normalized } m/z \text{ 163 peak area}}{\text{normalized } m/z \text{ 163 peak area}} = \frac{0.4912 \text{ mL}}{0.5 \text{ mL}} \times 633$

$$\text{normalized } m/z \text{ 163 peak area} = 622$$

$$\frac{\text{normalized } m/z \text{ 165 peak area}}{\text{normalized } m/z \text{ 165 peak area}} = \frac{0.4912 \text{ mL}}{0.5 \text{ mL}} \times 231$$

$$\text{normalized } m/z \text{ 165 peak area} = 227$$

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.o.
- 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 water samples.

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

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

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

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$$\text{Confirmation Ratio} = \frac{244}{673}$$

$$\text{Confirmation Ratio} = 0.3626$$

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

- c. Prepare *cis*- and *trans*-CAAC standard curves by plotting the equivalent sample concentration (ng/mL) 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:

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

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

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

$$\text{trans-CAAC Conc. (ng/mL)} = \left(\frac{\text{trans-CAAC TBDMSE peak area}}{15347} \right)^{1/1.0089}$$

- d. Determine the net concentration in each recovery sample 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 above 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:

$$\text{trans-CAAC Conc. (ng/mL)} = \left(\frac{\text{net trans-CAAC TBDMSE peak area}}{15347} \right)^{1/1.0089}$$

$$\text{trans-CAAC Conc. (ng/mL)} = \left(\frac{703 - 52}{15347} \right)^{1/1.0089}$$

$$\text{trans-CAAC Conc.} = 0.0436 \text{ ng/mL}$$

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

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

$$\text{Recovery} = \frac{0.0436 \text{ ng/mL}}{0.050 \text{ ng/mL}} \times 100\%$$

$$\text{Recovery} = 87\%$$

The average of all the recovery samples can be used to correct for method efficiency.

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

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.
2. Prepare a standard calibration curve for *cis*- and *trans*-CAAC and determine the percentage recovery as described in Section I.3.
3. Determine the concentration of *cis*- and *trans*-CAAC in each treated sample by substituting the normalized (Section I.2.) *cis*- and *trans*-CAAC TBDMSE *m/z* 163 peak area obtained into the equation for the corresponding standard calibration curve, and calculate the uncorrected residue results.

For example, using the normalized *m/z* 163 peak area for *cis*-CAAC data from Figure 7, the uncorrected concentration is calculated as follows:

$$\text{cis-CAAC Conc. (ng/mL)} = \left(\frac{\text{cis-CAAC TBDMSE peak area}}{14562} \right)^{1/1.0148}$$

$$\text{cis-CAAC Conc. (ng/mL)} = \left(\frac{622}{14562} \right)^{1/1.0148}$$

$$\text{cis-CAAC Conc.} = 0.0447 \text{ ng/mL}$$

4. To correct for method recovery, the following procedure is used:
 - a. Determine the *cis*-CAAC concentrations in the water samples as described in Section J.3.
 - b. Determine the corrected analyte concentration in the water samples, using the average % recovery for the set, as follows:

$$\text{cis-CAAC Conc. (corrected ng/mL)} = \text{cis-CAAC Conc. (ng/mL)} \times \frac{100}{\% \text{ Recovery}}$$

$$\text{cis-CAAC Conc. (corrected ng/mL)} = 0.0447 \text{ ng/mL} \times \frac{100}{91}$$

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cis-CAAC Conc. = 0.0491 ng/mL
(corrected)

K. Results and Discussion

1. Method Validation

a. Recovery Levels and Precision

A method validation study was conducted to determine the recovery levels and the precision of the method for *cis*- and *trans*-CAAC in surface water. The results are summarized in Tables I and II, respectively.

Recovery values of *cis*-CAAC from surface water fortified over the concentration range 0.05 to 5.0 ng/mL averaged 91% with one standard deviation equal to 7% (Table I).

Recovery values of *trans*-CAAC from surface water fortified over the concentration range 0.05 to 5.0 ng/mL averaged 92% with one standard deviation equal to 7% (Table II).

b. Standard Curve Fit

The average correlation coefficient (r^2) for the power regression equations describing the detector response as a function of the standard calibration curve concentration was greater than 0.999 for both *cis*- and *trans*-CAAC.

c. Calculated Limits of Quantitation and Detection

Following established guidelines (4), the limits of quantitation (LOQ) and detection (LOD) were calculated using the standard deviation from the 0.05 ng/mL recovery results. The LOQ was calculated as ten times the standard deviation ($10s$), and the LOD was calculated as three times the standard deviation ($3s$) of the results of the analysis of eight samples. The results are summarized in Tables III and IV.

For *cis*- and *trans*-CAAC, calculated statistics support an LOQ of 0.030 and 0.029 ng/mL, respectively. Results should not be quantified, however, at levels below which no recovery samples have been analyzed.

For both *cis*- and *trans*-CAAC, calculated statistics support an LOD of 0.009 ng/mL.

2. Confirmation of Residue Identity

Confirmation of the presence of residues is described in Section I.3.b. For *cis*- and *trans*-CAAC, confirmation is by comparison of the chromatographic retention time as well as the peak area ratios resulting from mass selective ion monitoring. Positive confirmation of *cis*- and *trans*-CAAC is indicated when the confirmation ratio for the sample is in the range of $\pm 20\%$ of the average found for the standards.

3. Assay Time

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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.e. An acceptable stopping point with the analytes in the MTBE extract.
- b. Step I.1.g.(7). An acceptable stopping point with the analytes in the 0.025% acetic acid in MTBE eluent solution.
- c. Step I.1.h. An acceptable stopping point with the analytes in the 0.025% acetic acid in MTBE eluent solution.

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 μ L of the 10 μ g/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 K.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 μ L 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 μ L of

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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.o. and p.
- (13) Calculate the percentage recoveries for each analyte as described in Section I.2. 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 μ L of the 10 μ g/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.d. through f.
- (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 K.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.e. and f. 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 K.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 mL with isooctane by comparison to two 8-mL vials each containing a measured volume of 1 mL isooctane. Add 25 μ L 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.o. and p.
- (14) Calculate the percentage recoveries for each analyte as described in Section I.2. Use actual standard concentrations in determination of the standard curve equation and fraction recoveries.

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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 preceding suggestions fail to give acceptable results.

L. 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.

M. References

1. Certificate Of Analysis, Aldrich Chemical Co., January 20, 1994.
2. Certificate Of Analysis, Aldrich Chemical Co., January 20, 1994.
3. *HP-41C/41CV Standard Applications Handbook*, Hewlett-Packard Publication No. 00041-90402, 1982, pp 42-48.
4. Keith, L. H.; Crummett, W. B.; Deegan, J.; Libby, R. A.; Taylor, J. T.; Wentler, G., "Principles of Environmental Analysis", *Anal. Chem.*, 1983, 55, 2210-2218.

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Table I. Recovery of *cis*-CAAC from Water

Sample Number	Date of Analysis	<i>cis</i> -CAAC, ng/mL		Percent Recovery
		Added	Found ^a	
14734601	07-June-1994	0.000	0.0041	--
14734601	07-June-1994	0.000	0.0040	--
14734601	21-Oct-1994	0.000	0.0049	--
14734601	21-Oct-1994	0.009	0.0084	NA ^b
14734601	21-Oct-1994	0.009	0.0079	NA
14734601	07-June-1994	0.050	0.0409	82
14734601	07-June-1994	0.050	0.0469	94
14734601	07-June-1994	0.050	0.0439	88
14734601	07-June-1994	0.050	0.0459	92
14734601	07-June-1994	0.050	0.0417	83
14734601	07-June-1994	0.050	0.0501	100
14734601	07-June-1994	0.050	0.0449	90
14734601	07-June-1994	0.050	0.0432	86
14734601	07-June-1994	0.25	0.223	89
14734601	07-June-1994	0.25	0.232	93
14734601	07-June-1994	0.50	0.374	75
14734601	07-June-1994	0.50	0.467	93
14734601	07-June-1994	1.25	1.29	103
14734601	07-June-1994	1.25	1.14	91
14734601	07-June-1994	2.50	2.22	89
14734601	07-June-1994	2.50	2.39	96
14734601	07-June-1994	5.00	5.09	102
14734601	07-June-1994	5.00	4.19	84
			\bar{x} =	91
			<i>s</i> =	7
			<i>n</i> =	18

^a Fortified samples corrected for average of controls in set.

^b NA = not applicable. The residue was below the 0.05 ng/mL limit of quantitation.

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Table II. Recovery of *trans*-CAAC from Water

Sample Number	Date of Analysis	<i>trans</i> -CAAC, ng/mL		Percent Recovery
		Added	Found ^a	
14734601	07-June-1994	0.000	0.0021	--
14734601	07-June-1994	0.000	0.0050	--
14734601	21-Oct-1994	0.000	0.0034	--
14734601	21-Oct-1994	0.009	0.0073	NA ^b
14734601	21-Oct-1994	0.009	0.0080	NA
14734601	07-June-1994	0.050	0.0453	91
14734601	07-June-1994	0.050	0.0499	100
14734601	07-June-1994	0.050	0.0426	85
14734601	07-June-1994	0.050	0.0436	87
14734601	07-June-1994	0.050	0.0422	84
14734601	07-June-1994	0.050	0.0488	98
14734601	07-June-1994	0.050	0.0430	86
14734601	07-June-1994	0.050	0.0455	91
14734601	07-June-1994	0.25	0.228	91
14734601	07-June-1994	0.25	0.236	94
14734601	07-June-1994	0.50	0.378	76
14734601	07-June-1994	0.50	0.479	96
14734601	07-June-1994	1.25	1.31	105
14734601	07-June-1994	1.25	1.18	94
14734601	07-June-1994	2.50	2.28	91
14734601	07-June-1994	2.50	2.44	98
14734601	07-June-1994	5.00	5.22	104
14734601	07-June-1994	5.00	4.26	85
			\bar{x} =	92
			<i>s</i> =	7
			<i>n</i> =	18

^a Fortified samples corrected for average of controls in set.

^b NA = not applicable. The residue was below the 0.05 ng/mL limit of quantitation.

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Table III. Calculated Limits of Detection and Quantitation for the Determination of *cis*-CAAC in Water

Sample Number	Date of Analysis	<i>cis</i> -CAAC, ng/mL	
		Added	Found
14734601	07-June-1994	0.050	0.0409
14734601	07-June-1994	0.050	0.0469
14734601	07-June-1994	0.050	0.0439
14734601	07-June-1994	0.050	0.0459
14734601	07-June-1994	0.050	0.0417
14734601	07-June-1994	0.050	0.0501
14734601	07-June-1994	0.050	0.0449
14734601	07-June-1994	0.050	0.0432
		\bar{x} =	0.0447
		s =	0.0030
		LOD ^a (3s) =	0.009
		LOQ ^b (10s) =	0.030

- ^a LOD = Limit of Detection.
^b LOQ = Limit of Quantitation.

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Table IV. Calculated Limits of Detection and Quantitation for the Determination of *trans*-CAAC in Water

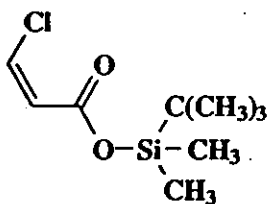
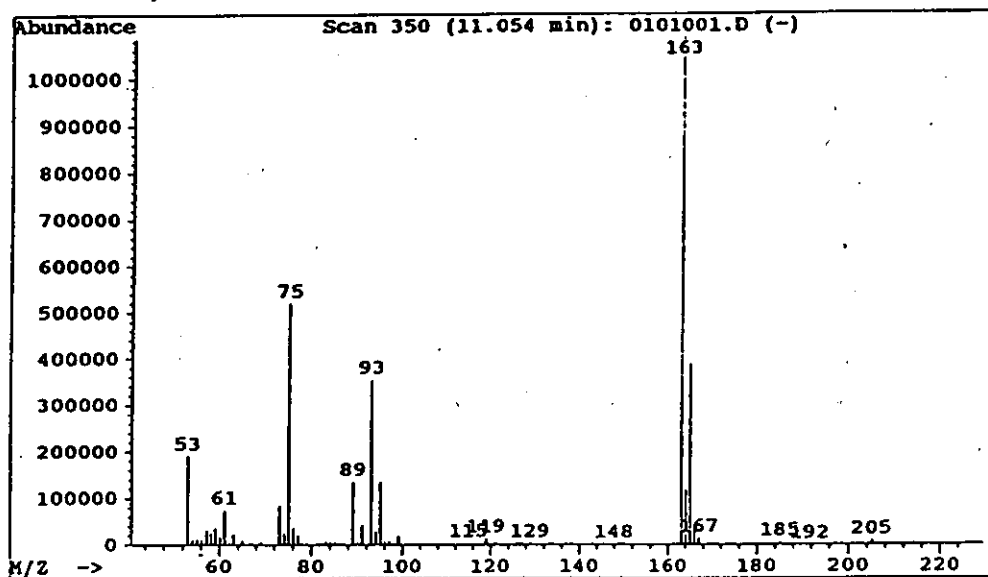
Sample Number	Date of Analysis	<i>trans</i> -CAAC, ng/mL	
		Added	Found
14734601	07-June-1994	0.050	0.0453
14734601	07-June-1994	0.050	0.0499
14734601	07-June-1994	0.050	0.0426
14734601	07-June-1994	0.050	0.0436
14734601	07-June-1994	0.050	0.0422
14734601	07-June-1994	0.050	0.0488
14734601	07-June-1994	0.050	0.0430
14734601	07-June-1994	0.050	0.0455
		\bar{x} =	0.0451
		s =	0.0029
		LOD ^a (3s) =	0.009
		LOQ ^b (10s) =	0.029

^a LOD = Limit of Detection.

^b LOQ = Limit of Quantitation.

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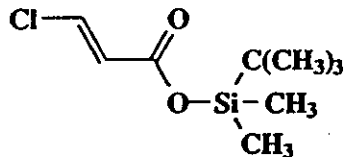
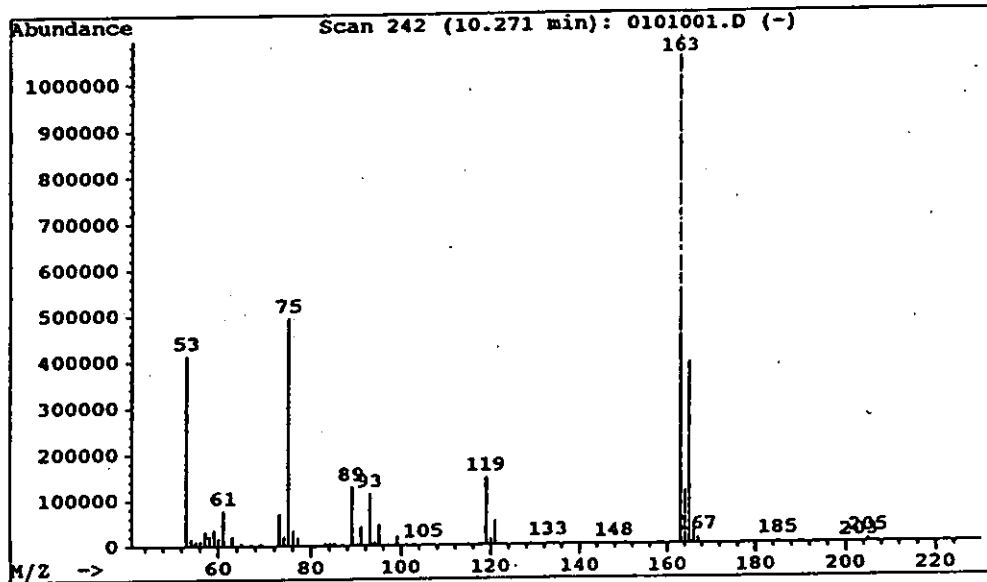


cis-CAAC *t*-Butyldimethylsilyl Ester
Formula: C₉H₁₇ClO₂Si
Molecular Weight: 220

Figure 1. Mass Spectrum of *cis*-CAAC *t*-Butyldimethylsilyl Ester

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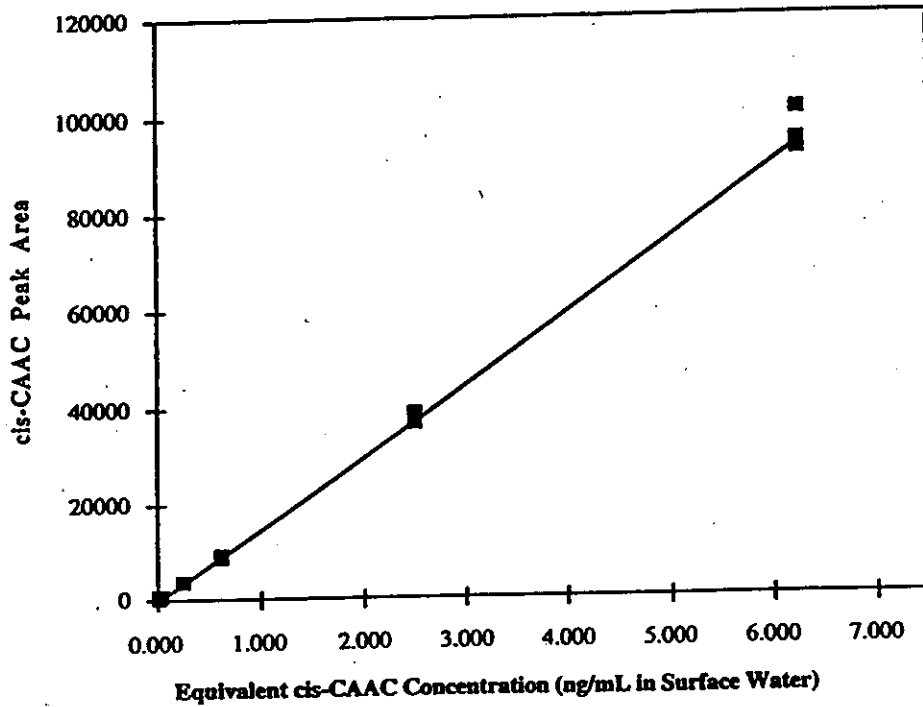
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trans-CAAC *t*-Butyldimethylsilyl Ester
Formula: C₉H₁₇ClO₂Si
Molecular Weight: 220

Figure 2. Mass Spectrum of *trans*-CAAC *t*-Butyldimethylsilyl Ester

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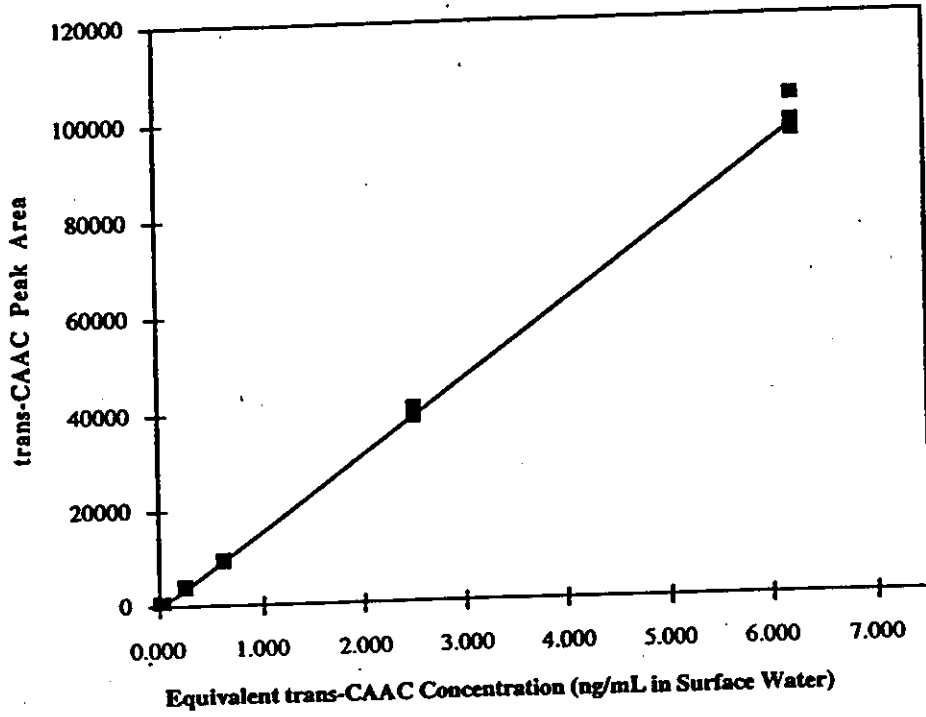
Equivalent <i>cis</i> -CAAC Conc. ng/mL	<i>m/z</i> 163 Peak Area Position in Sequence		
	Start	Middle	End
0.025	340	349	380
0.050	673	658	727
0.250	3447	3450	3684
0.625	8624	8613	9110
2.50	36680	36509	38212
6.25	91681	100291	94107

Power Regression Equation: $X = \left[\frac{Y}{14562} \right]^{1/1.0148}$

Coefficient of Determination (r^2): 0.9996

Figure 3. Typical Calibration Curve for the Determination of *cis*-CAAC in Water Samples

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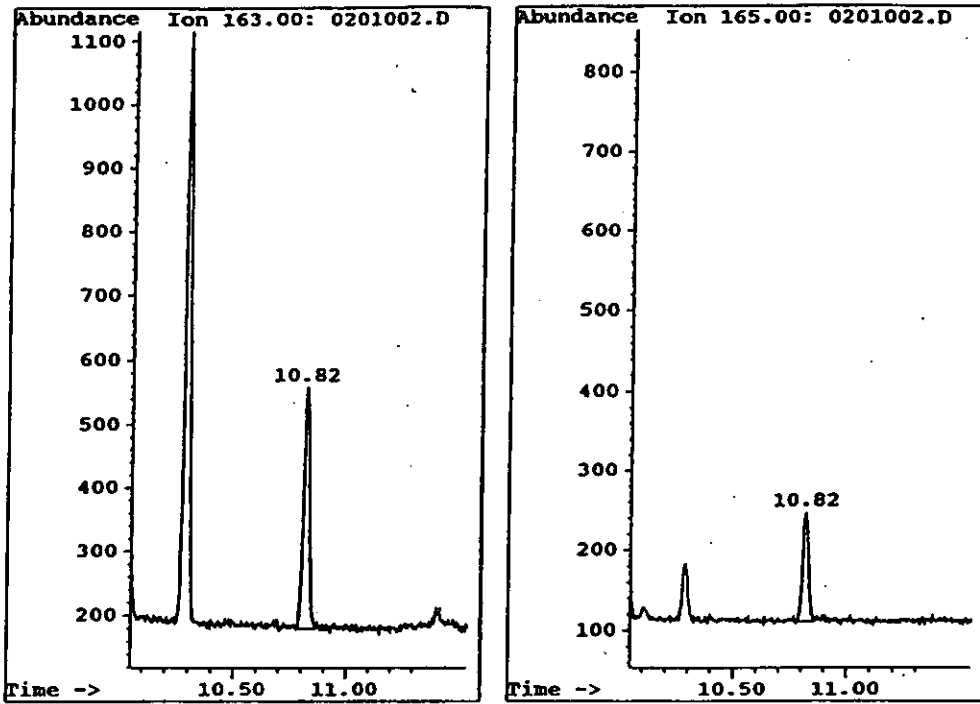
Equivalent <i>trans</i> -CAAC Conc. ng/mL	<i>m/z</i> 163 Peak Area Position in Sequence		
	Start	Middle	End
0.025	378	374	403
0.050	693	716	785
0.250	3631	3676	3927
0.625	9156	9168	9680
2.50	38430	38262	40105
6.25	95935	103548	98073

Power Regression Equation: $X = \left[\frac{Y}{15347} \right]^{1/1.0089}$

Coefficient of Determination (r^2): 0.9996

Figure 4. Typical Calibration Curve for the Determination of *trans*-CAAC in Water Samples

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Data File : 0201002.D
ALS Bottle : 2
Date : 7 Jun 94 2:42 pm
Data Path : C:\CHEMPC\DATA\F060194A\
Instrument : GC/MSD - GC serial#3126A36485

Sample Name: 4 ng/ml cis/trans 3-Chloroacrylic acid
Sample Info:
Operator : SEF

cis-3-Chloroacrylic acid Retention Time: 10.80
PEAK AREA (M/Z 163) : 673
PEAK AREA (M/Z 165) : 244

cis-3-Chloroacrylic Acid:
RATIO OF M/Z 165/163: 0.3626

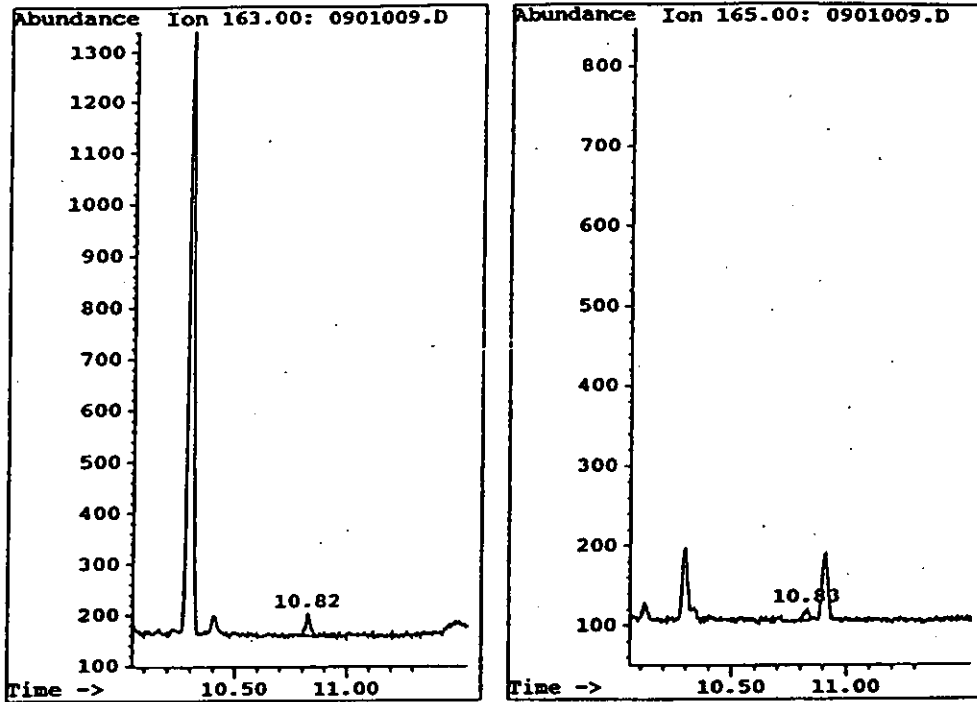
Equivalent cis-CAAC Concentration: 0.050 ng/mL

Average Standard Confirmation Ratio: 0.3623

Figure 5. Typical Chromatogram of a 4.0 ng/mL Standard, Equivalent to 0.050 ng/mL cis-CAAC in Surface Water

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Data File : 0901009.D
ALS Bottle : 9
Date : 7 Jun 94 5:30 pm
Data Path : C:\CHEMPC\DATA\F060194A\
Instrument : GC/MSD - GC serial#3126A36485

Sample Name: control
Sample Info:
Operator : SEF

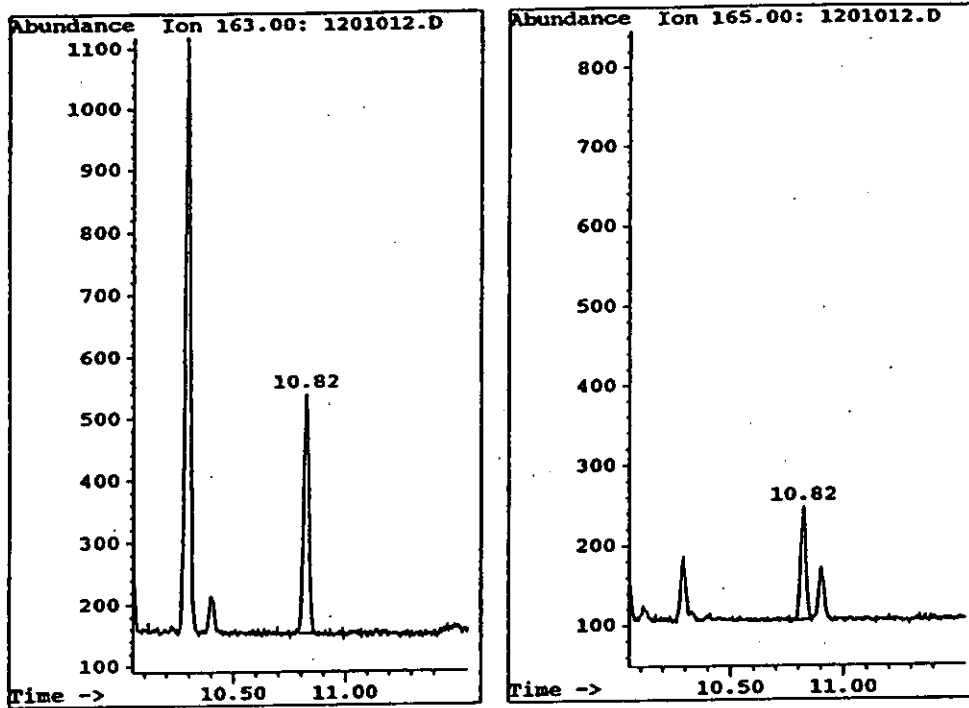
cis-3-Chloroacrylic acid Retention Time: 10.80
PEAK AREA (M/Z 163) : 59
PEAK AREA (M/Z 165) : 22

cis-3-Chloroacrylic Acid:
RATIO OF M/Z 165/163: 0.3729

Vial Weight Empty:	11.44 g	Normalized Peak Area (m/z 163):	55
Vial Weight with Sample:	11.76 g	Normalized Peak Area (m/z 165):	20
Final Sample Weight:	0.32 g	cis-CAAC Concentration:	0.0041 ng/mL
Final Sample Volume:	0.462 mL	Average Std. Confirmation Ratio:	0.3623
		Average Normalized Peak Area (m/z 163) of Controls:	54

Figure 6. Typical Chromatogram of a Control Surface Water Sample for the Determination of cis-CAAC

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Data File : 1201012.D
ALS Bottle : 12
Date : 7 Jun 94 6:42 pm
Data Path : C:\CHEMPC\DATA\F060194A\
Instrument : GC/MSD - GC serial#3126A36485

Sample Name: 0.05 ng/ml spiked control
Sample Info:
Operator : SEF

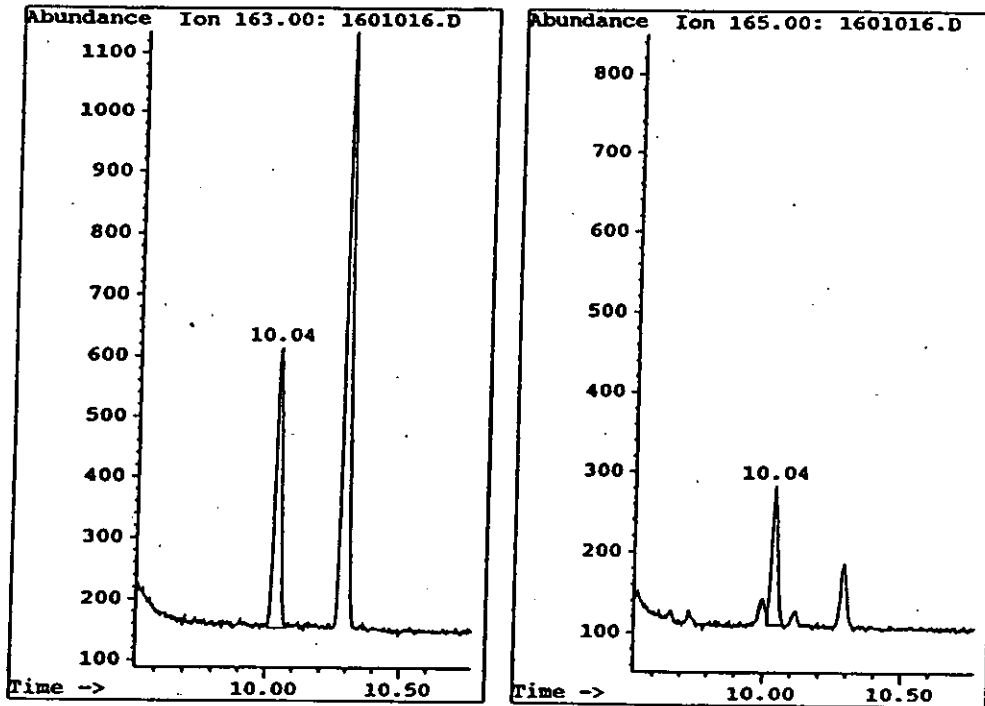
cis-3-Chloroacrylic acid Retention Time: 10.80
PEAK AREA (M/Z 163) : 633
PEAK AREA (M/Z 165) : 231

cis-3-Chloroacrylic Acid:
RATIO OF M/Z 165/163: 0.3649

Vial Weight Empty:	11.48 g	Normalized Peak Area (m/z 163):	622
Vial Weight with Sample:	11.82 g	Normalized Peak Area (m/z 165):	227
Final Sample Weight:	0.34 g	cis-CAAC Concentration:	0.0409 ng/mL
Final Sample Volume:	0.491 mL	Average Std. Confirmation Ratio:	0.3623
		Recovery:	82%

Figure 7. Typical Chromatogram of a Control Surface Water Sample Fortified with 0.050 ng/mL cis-CAAC

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Data File : 1601016.D
ALS Bottle : 16
Date : 7 Jun 94 8:18 pm
Data Path : C:\CHEMPC\DATA\F060194A\
Instrument : GC/MSD - GC serial#3126A36485

Sample Name: 4 ng/ml cis/trans 3-Chloroacrylic acid
Sample Info:
Operator : SEF

trans-3-Chloroacrylic acid Retention Time: 10.01
PEAK AREA (M/Z 163) : 716
PEAK AREA (M/Z 165) : 259

trans-3-Chloroacrylic Acid:
RATIO OF M/Z 165/163: 0.3617

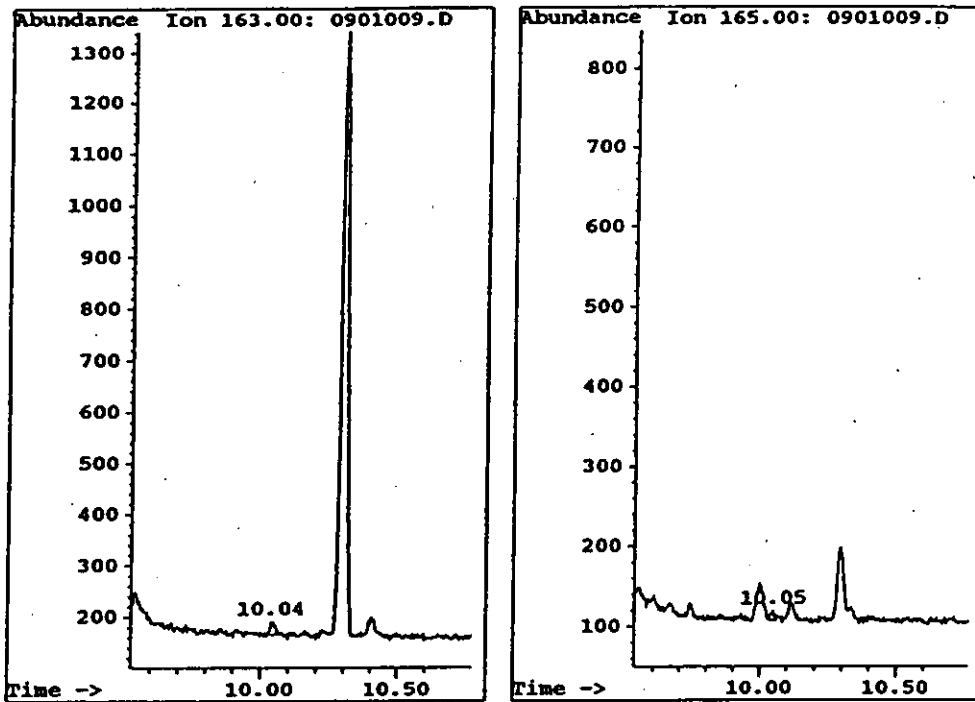
Equivalent *trans*-CAAC Concentration: 0.050 ng/mL

Average Standard Confirmation Ratio: 0.3640

Figure 8. Typical Chromatogram of a 4.0 ng/mL Standard, Equivalent to 0.050 ng/mL *trans*-CAAC in Surface Water

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Data File : 0901009.D
ALS Bottle : 9
Date : 7 Jun 94 5:30 pm
Data Path : C:\CHEMPC\DATA\F060194A\
Instrument : GC/MSD - GC serial#3126A36485

Sample Name: control
Sample Info:
Operator : SEF

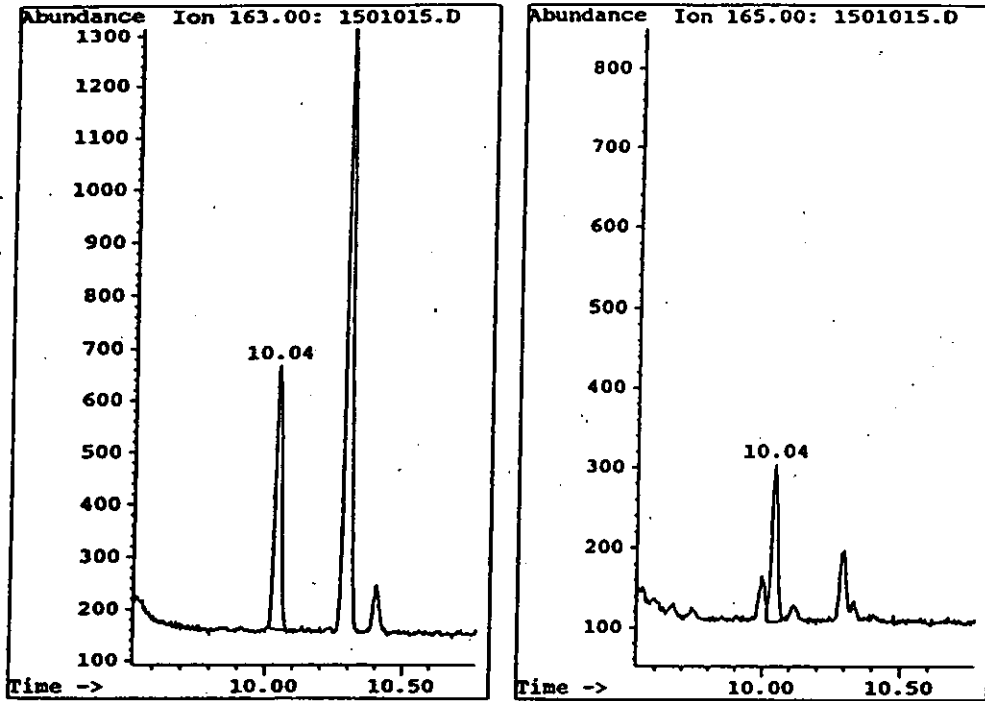
trans-3-Chloroacrylic acid Retention Time: 10.01
PEAK AREA (M/Z 163) : 32
PEAK AREA (M/Z 165) : 13

trans-3-Chloroacrylic Acid:
RATIO OF M/Z 165/163: 0.4063

Vial Weight Empty:	11.44 g	Normalized Peak Area (m/z 163):	30
Vial Weight with Sample:	11.76 g	Normalized Peak Area (m/z 165):	12
Final Sample Weight:	0.32 g	trans-CAAC Concentration:	0.0021 ng/mL
Final Sample Volume:	0.462 mL	Average Std. Confirmation Ratio:	0.3640
		Average Normalized Peak Area (m/z 163) of Controls:	52

Figure 9. Typical Chromatogram of a Control Surface Water Sample for the Determination of trans-CAAC

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Data File : 1501015.D
 ALS Bottle : 15
 Date : 7 Jun 94 7:54 pm
 Data Path : C:\CHEMPC\DATA\F060194A\
 Instrument : GC/MSD - GC serial#3126A36485

Sample Name: 0.05 ng/ml spiked control
 Sample Info:
 Operator : SEF

trans-3-Chloroacrylic acid Retention Time: 10.04
 PEAK AREA (M/Z 163) : 761
 PEAK AREA (M/Z 165) : 294

trans-3-Chloroacrylic Acid:
 RATIO OF M/Z 165/163: 0.3863

Vial Weight Empty:	11.30 g	Normalized Peak Area (m/z 163):	703
Vial Weight with Sample:	11.62 g	Normalized Peak Area (m/z 165):	272
Final Sample Weight:	0.32 g	trans-CAAC Concentration:	0.0436 ng/mL
Final Sample Volume:	0.462 mL	Average Confirmation Ratio:	0.3640
		Recovery:	87%

Figure 10. Typical Chromatogram of a Control Surface Water Sample Fortified with 0.050 ng/mL trans-CAAC