

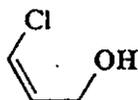
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SUPERSEDES: New

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

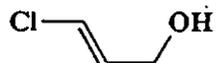
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A. Scope

This method is applicable for the quantitation of residues of the 1,3-dichloropropene metabolites, *cis*- and *trans*-3-chloroallyl alcohol (CAAL) in water. The method was validated over the concentration range 0.10 to 5.2 ng/mL with a limit of quantitation of 0.10 ng/mL.



cis-3-Chloroallyl Alcohol
CAS 4643-05-4



trans-3-Chloroallyl Alcohol
CAS 4643-06-5

B. Principle

CAAL residues in water are extracted with methyl-*t*-butyl ether (MTBE). The MTBE is dried and purified by passing over anhydrous magnesium sulfate and a silica gel solid phase extraction (SPE) column. Hexane is added and the sample is concentrated using a Snyder distillation column. The sample is further concentrated by evaporation and brought to a final volume of 1 mL with hexane. CAAL residues in hexane are derivatized with isobutyl chloroformate in the presence of pyridine to their corresponding *cis*- and *trans*-3-chloroallyl isobutyl carbonates (CAIBC) 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. Acetone, hexane, MTBE and 1-propanol are flammable and should be used in well-ventilated areas away from ignition sources.
3. *cis*- and *trans*-3-Chloroallyl alcohol are corrosive and lachrymators. It is imperative that proper eye and personal protection equipment be used when handling these compounds. Handling of neat material should be carried out in a fume hood.
4. Isobutyl chloroformate is highly toxic, irritating to eyes, respiratory system and skin. It is imperative that proper eye and personal protection equipment be used when handling this reagent. Handling of neat material should be carried out in a fume hood.

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. Centrifuge, with rotor to accommodate 8-mL vials, Model Centra-8, International Equipment Company, Needham Heights, MA 02194.
4. Centrifuge, with rotor to accommodate 2-oz bottles, Model CU-5000, International Equipment Company.
5. Evaporator, N-Evap, Model 111, Organomation Associates, Inc., South Berlin, MA 01549.
6. Gas chromatograph, Model 5890 Series II, Hewlett-Packard.
7. Heater, dry bath incubator, catalog number 11-718-2, Fisher Scientific, Pittsburgh, PA 15219.
8. Heater, dry bath incubator (aluminum) heating block, catalog number 11-718-16, Fisher Scientific.
9. Hot plate, Thermolyne extra-capacity hotplate, catalog number 11-496-5A, Fisher Scientific.
10. Mass selective detector, Model 5971A, Hewlett-Packard, Palo Alto, CA 94304.
11. Mass selective detector data system, Model G1034B, Hewlett-Packard.
12. Shaker, variable speed reciprocating with box carrier, Model 6000, Eberbach Corporation, Ann Arbor, MI 48106.

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13. Ultrasonic bath, Model 1200, Branson Cleaning Equipment Company, Shelton, CT 06484.
14. Vacuum manifold box, Model SPE-21, J.T. Baker, Inc., Phillipsburg, NJ 08865.
15. Vial crimper, catalog number 8710-0979, Hewlett-Packard, Wilmington, DE 19808.
16. Vortex mixer, Model G-560, Scientific Industries, Inc., Bohemia, NY 11716.
17. Water purification system, Model Milli-Q UV Plus, Millipore Corporation, Milford, MA 01757.

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

1. Bottle, 2 ounce, round, wide-mouth, clear, with PTFE-lined screw caps, catalog number 03-320-11C, Fisher Scientific, Pittsburgh, PA 15219.
2. Column, capillary gas chromatography, Durabond-17 liquid phase, 20 m x 0.18 mm i.d., 0.3 μ m film thickness, catalog number 121-1723, J&W Scientific, Folsom, CA 95630.
3. Column, silica gel SPE, catalog number 7086-07, 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, 25 mL, catalog number 71213-1011, Varian Sample Preparation Products, Harbor City, CA 90710.
7. Erlenmeyer flask, 50 mL, 19/22 joint, catalog number 296510-0050, Kontes, Vineland, NJ 08360.
8. Filter, charcoal, catalog number 7972, Chrompack, Inc., Raritan, NJ 08869. (Note L.2.)
9. Filter, moisture, catalog number 7971, Chrompack, Inc. (Note L.2.)
10. Filter, oxygen, catalog number 7970, Chrompack, Inc. (Note L.2.)
11. Gas, helium, 99.995% purity, Airco, Murray Hill, NJ 07974.
12. Gas, nitrogen, 99.99% purity, Airco.
13. Microdispenser, 10 μ L and 25 μ L, Drummond Dialomatic Microdispenser, catalog numbers 300210 and 300225, Drummond Scientific Company, Broomall, PA 19008.
14. Microdispenser replacement bore, 10 μ L and 25 μ L, catalog numbers 300210G and 300225G, Drummond Scientific Company.
15. Micro Snyder distilling column, 19/22 joint, catalog number 569001-0319, Kontes.

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16. Syringe, 100 and 500 μ L capacity, catalog numbers 80600 and 80800, Hamilton Co., Reno, NV 89520.
17. Vial, 8 mL, with PTFE-lined screw cap, catalog number B7800-3, National Scientific Company, Lawrenceville, GA 30243.
18. Vial, 45 mL, with PTFE-lined screw cap, catalog number 60958A-11, Kimble Glass, Vineland, NJ 08360..
19. Vial, autosampler, 2 mL, catalog number C4011-1, National Scientific Company.
20. Vial seal, catalog number C4011-1A, National Scientific Company.

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

1. Reagents

- a. Acetone, Optima grade, catalog number A929-4, Fisher Scientific.
- b. Hexane, Optima grade, catalog number H303-4, Fisher Scientific.
- c. Hydrochloric acid, 0.1 N, ACS reagent grade, certified concentration, catalog number SA54-4, Fisher Scientific.
- d. Isobutyl Chloroformate, 98%, catalog number 17798-9, Aldrich Chemical Company, Milwaukee, WI 53233.
- e. Magnesium sulfate (anhydrous), certified, catalog number M65-500, Fisher Scientific.
- f. Methyl-*t*-butyl ether, HPLC grade, catalog number E127-4, Fisher Scientific.
- g. 1-Propanol, 99.5+ %, HPLC grade, catalog number 29,328-8, Aldrich Chemical Company.
- h. Pyridine, HPLC grade, catalog number 27040-7, Aldrich Chemical Company.
- 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-Chloroallyl alcohol

The *cis*-CAAL standard, AGR164303, Lot Number GHC 0083-27, with a purity of 95.1% was used in this study (1).

(2) *trans*-3-Chloroallyl alcohol

The *trans*-CAAL standard, AGR159855, Lot Number GHC-2-12-119, with a purity of 94.8% was used in this study (2).

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

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*-CAAL Stock Solutions

cis- and *trans*-CAAL are volatile liquids, and pose some difficulty in weighing to a specific value. The following procedure is meant as a guideline to stress that the analyte be accurately weighed and recorded to four significant figures. Based upon an average density of 1.17 g/mL (3), a 100- μ L syringe was used to deliver 86 μ L of *cis*- or *trans*-CAAL in the preparation of stock solutions.

- a. Tare a 100-mL volumetric flask and sintered glass stopper. Deliver 86 μ L of *cis*-CAAL to the flask and stopper the flask. Weigh and record the amount of *cis*-CAAL in the flask. Dilute to volume with acetone to obtain a 1000 μ g/mL stock solution.
- b. Tare a 100-mL volumetric flask and sintered glass stopper. Deliver 86 μ L of *trans*-CAAL to the flask and stopper the flask. Weigh and record the amount of *trans*-CAAL in the flask. Dilute to volume with acetone to obtain a 1000 μ g/mL stock solution.

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

- a. Transfer 1.0 mL 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 μ g/mL for each *cis*- and *trans*-CAAL. This solution is used for preparation of spiking solutions.
- b. Solutions for spiking water samples are prepared by diluting the initial solution from Section G.2.a. with acetone as follows:

Aliquot of 10.0 μ g/mL Soln.	Final Soln. Volume	Spiking Soln. Final Conc.	Equivalent Sample Conc. ^a
mL	mL	ng/mL	ng/mL
0.100	250	4.00	0.100
0.100	100	10.0	0.250
0.500	250	20.0	0.500
1.00	250	40.0	1.00
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.

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

- a. Transfer 1.0 mL 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 hexane to obtain an initial

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solution of 10.0 µg/mL for each *cis*- and *trans*-CAAL. This solution is used for preparation of calibration solutions.

- b. Solutions for calibration are prepared by diluting the initial solution from Section G.3.a. with hexane as follows:

Aliquot of 10.0 µg/mL Soln. mL	Final Soln. Volume mL	Soln. Final Conc. ng/mL	Equivalent Sample Conc. ^a ng/mL
0.050	250	2.00	0.050
0.100	250	4.00	0.100
0.500	250	20.0	0.500
1.00	100	100.	2.50
2.00	100	200.	5.00
4.00	100	400.	10.0

^a The equivalent sample concentration is based on the concentration of a 40-mL water extract to a final volume of 1.0 mL.

- c. Calibration standards are prepared for capillary gas chromatography/mass spectrometry as described in Steps I.1.r. through I.1.x.

H. Gas Chromatography/Mass Spectrometry

1. Column

Install the splitless column inlet liner (Section E.5.) and the capillary column (Section E.2.) 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-17 liquid phase
 20 m x 0.18 mm i.d.
 0.3 µm film thickness

Temperatures:

Column 65 °C for 1.0 min
 65 °C to 150 °C at 5 °C/min, 0 min hold at 150 °C
 150 °C to 260 °C at 20 °C/min, 0 min hold at 260 °C

Injector 230 °C
 Interface 280 °C

Carrier Gas: helium

Head Pressure approximately 100 kPa
 Linear Velocity approximately 40 cm/sec at an oven temperature of 130 °C

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Injection Mode:	splitless
Purge Delay	0.7 min
Splitter Flow	50 mL/min
Septum Purge	1.0 mL/min
Injection Volume:	2 μ L
Detector:	electron impact ionization with selected ion monitoring
Calibration Program	maximum sensitivity autotune (Note L.3.)
Electron Multiplier	1412 volts (tune voltage plus 200)
Ions Monitored:	
<i>cis</i> -CAIBC	<i>m/z</i> 136 (quantitation) and <i>m/z</i> 75 (confirmation)
<i>trans</i> -CAIBC	<i>m/z</i> 136 (quantitation) and <i>m/z</i> 75 (confirmation)
Dwell Time	100 msec

Mass spectra of *cis*- and *trans*-CAIBC are shown in Figures 1 and 2, respectively. The nominal *m/z* 136 quantitation ion results from loss of 2-methyl-2-propene (mass 56). Both the quantitation and the nominal *m/z* 75 confirmation ions retain the chlorine functionality of CAAL.

3. Calibration Curves

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

4. Typical Chromatograms

Typical chromatograms of a standard, control sample, and a 0.10 ng/mL recovery sample for *cis*- and *trans*-CAAL in water are shown in Figures 5-10.

I. Determination of Recovery of *cis*- and *trans*-CAAL from Water

1. Preparation of Recovery Samples

- Pipet 40.0 mL of control water samples into a series of 2-oz bottles.
- 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.b.) in acetone to obtain concentrations ranging from 0.10 to 5.0 ng/mL. A reagent blank, containing no water sample, should be carried through the method with the samples.
- Add 10 μ L of 1-propanol, 15 g of sodium chloride, 15 mL of MTBE and seal the bottle with a PTFE-lined cap. (The addition of 1-propanol is critical to reduce evaporative losses of CAAL.)
- Shake the sample for 15 minutes on a reciprocating shaker at approximately 180 excursions/minute.

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- e. Centrifuge the bottle for 3 minutes at 1000 rpm.
- f. The samples are then dried and purified using the following silica gel SPE procedure (Note L.4.):
 - (1) Place a silica gel SPE column (Section E.3.) on the vacuum manifold box.
 - (2) Add approximately 2 g of magnesium sulfate (anhydrous) to the SPE column.
 - (3) Attach a 25-mL reservoir to the top of the column using an SPE column adapter.
 - (4) Wash the SPE column by adding approximately 10 mL of MTBE to the reservoir and, with the aid of vacuum, pull the MTBE through the column. Discard the column wash.
 - (5) Transfer the MTBE layer (top layer) of the sample solution from Step I.1.e. to the reservoir and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min. Collect the MTBE in a 45-mL vial.
 - (6) Add 15 mL of MTBE to the sample jar, shake for 5 minutes and repeat Steps I.1.e and I.1.f.(5).
 - (7) After the two MTBE extracts have passed through the column, add approximately 5 mL of MTBE to the reservoir and, with the aid of vacuum, pull the MTBE through the column at a flow rate of approximately 2 mL/min. Collect and combine with the MTBE in the vial from Step I.1.f.(5).
- g. Quantitatively transfer the MTBE in the 45-mL vial to a 50-mL Erlenmeyer flask (Section E.7.). Rinse the vial with approximately 2 mL of MTBE and add to the flask.
- h. Add approximately 3 mL of hexane and approximately 0.1 g of anhydrous sodium sulfate to the flask.
- i. Attach a Snyder column (Section E.15.) to the flask.
- j. In a fume hood, heat the flask on a hot plate to a steady boil.
- k. Allow the sample to concentrate to near dryness. Significant loss of CAAL will occur if the flask goes to dryness.
- l. Remove the flask from the hot plate, add 1 mL of hexane to the flask through the top of the Snyder column and allow the flask to equilibrate to ambient temperature.
- m. Remove the Snyder column from the flask and quantitatively transfer the sample to an 8-mL vial. Rinse the flask twice with approximately 1 mL MTBE, transferring each rinse to the 8-mL vial.
- n. Concentrate the sample at ambient temperature, on an N-Evap evaporator under a gentle flow of nitrogen to a volume of approximately 0.5 mL. Do not allow the volume to go significantly below 0.5 mL or loss of CAAL will occur.

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- o. Adjust the volume to 1.0 mL with hexane by visual comparison to two 8-mL vials containing a measured volume of 1.0 mL hexane.
 - p. Add approximately 0.1 g of anhydrous sodium sulfate.
 - q. Add 25 μ L of pyridine and 25 μ L of isobutyl chloroformate, seal the vial with a PTFE-lined cap and vortex and sonicate the samples for 5 seconds.
 - r. Transfer 1.0 mL of each of the calibration standards in Section G.3.b. to 8-mL vials. Derivatize following Step I.1.q.
 - s. Heat the samples and calibration standards in an aluminum block heater (Section D.7. and D.8.) at 70 °C for 15 minutes.
 - t. Remove the vial from the aluminum block and allow the derivatized samples and standards to cool to ambient temperature.
 - u. Add approximately 1 mL of 0.1 N hydrochloric acid and vortex each vial for approximately 5 seconds.
 - v. Centrifuge the vial for 5 minutes at 2500 rpm.
 - w. Transfer the top hexane layer to a 2-mL autosampler vial and seal the vial with a cap and crimper.
 - x. Analyze the samples and calibration standards by capillary gas chromatography/mass spectrometry as described in Section H.
2. Calculation of Percent Recovery
- a. Determine the m/z 75 and 136 response areas for both *cis*- and *trans*-CAIBC in the calibration standards.
 - b. For each standard, calculate the *cis*- and *trans*-CAAL confirmation ratios. The average confirmation ratio for all calibration standards will be used to confirm the presence of the respective CAAL in the water samples.

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

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

$$\text{Confirmation Ratio} = \frac{\text{peak area at } m/z \text{ 136}}{\text{peak area at } m/z \text{ 75}}$$

$$\text{Confirmation Ratio} = \frac{981}{3133}$$

$$\text{Confirmation Ratio} = 0.3131$$

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

- c. Prepare *cis*- and *trans*-CAAL standard curves by plotting the equivalent sample concentration (ng/mL) on the abscissa (x-axis) and the *cis*- and *trans*-CAIBC *m/z* 136 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 (4) with the *trans*-CAAL data from Figure 4:

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

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

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

$$\text{trans-CAAL Conc. (ng/mL)} = \left(\frac{\text{trans-CAIBC peak area}}{11180} \right)^{1/1.0326}$$

- d. Determine the net concentration in each recovery sample by first subtracting the average *cis*- and *trans*-CAIBC *m/z* 136 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 *trans*-CAAL data from Figures 9 and 10:

$$\text{trans-CAAL Conc. (ng/mL)} = \left(\frac{\text{net trans-CAIBC peak area}}{11180} \right)^{1/1.0326}$$

$$\text{trans-CAAL Conc. (ng/mL)} = \left(\frac{911 - 0}{11180} \right)^{1/1.0326}$$

$$\text{trans-CAAL Conc.} = 0.0882 \text{ ng/mL}$$

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

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

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$$\text{Recovery} = \frac{0.0882 \text{ ng/mL}}{0.1039 \text{ ng/mL}} \times 100\%$$

$$\text{Recovery} = 85\%$$

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

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

1. Prepare reagent blank, control, recovery, and treated samples as described in Section I.1.
2. Prepare standard calibration curves for *cis*- and *trans*-CAAL and determine the percentage recoveries as described in Section I.2.
3. Determine the concentration of *cis*- and *trans*-CAAL in each treated sample by substituting the *cis*- and *trans*-CAIBC *m/z* 136 peak area obtained into the respective equations for the standard calibration curve, and calculate the uncorrected residue result.

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

$$\text{cis-CAAL Conc. (ng/mL)} = \left(\frac{\text{cis-CAIBC peak area}}{10884} \right)^{1/1.0453}$$

$$\text{cis-CAAL Conc. (ng/mL)} = \left(\frac{740}{10884} \right)^{1/1.0453}$$

$$\text{cis-CAAL Conc.} = 0.0764 \text{ ng/mL}$$

4. To correct for method recovery, the following procedure is used:
 - a. Determine the *cis*-CAAL concentrations in the water samples as described in Section J.3. Calculate the average percent recovery from recovery samples in the set (Table I).
 - b. Determine the corrected analyte concentration in the water samples as follows:

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

$$\text{cis-CAAL Conc. (corrected ng/mL)} = 0.0764 \text{ ng/mL} \times \frac{100}{83}$$

$$\text{cis-CAAL Conc. (corrected)} = 0.0920 \text{ ng/mL}$$

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

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 CAAL from the SPE column individually, using either gravity-feed or pressurized elution, or as a group, using the vacuum manifold box.