METHOD OUTLINE

RESIDUE DETERMINATION METHOD: GRM 94.14

Independent Laboratory Validation of Method GRM 94.14 -
Determination of Residues of *cis*- and *trans*-3-Chloroacylic Acid in Water by
Capillary Gas Chromatography with Mass Selective Detection

Pipet 40.0 mL of control water samples into a series of labeled 45-mL vials.

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Use some of the samples as controls and fortify the remaining samples by adding 1.0-mL aliquots of the appropriate spiking solutions 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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Place an anion-exchange SPE column on the vacuum manifold box.

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Attach a 75-mL reservoir to the top of the column using an SPE column adapter.

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Rinse the SPE column and reservoir with approximately 5 mL of methanol.

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Condition the SPE column with approximately 5 mL of deionized water.

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Transfer the sample solution to the reservoir and, with the aid of vacuum, pull the sample through the column at a flow rate of approximately 2 mL/min.

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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.

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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.

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Add 100 µL of 2.0 N HCl and approximately 2 to 3 grams of NaCl to the 11-mL vial.

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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.

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METHOD OUTLINE (continued)

Centrifuge the vial for 5 minutes at 2500 rpm.
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Weigh a 12-mL tube to collect the eluant.
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Place a silica gel SPE column on the vacuum manifold box.
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Rinse the SPE column with approximately 5 mL of MTBE.
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Condition the SPE column with approximately 5 mL of isooctane.
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Transfer the MTBE layer from the vial to the SPE column and allow the MTBE to pass by gravity flow through the column.
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Again, add 2.5 mL of MTBE to the vial and seal with a PTFE-lined cap. Vortex the vial for 15 seconds. Centrifuge the vial for 5 minutes at 2500 rpm. 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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Elute the CAAC by passing 10.0 mL of the 0.025% acetic acid in MTBE solution through the column, collecting the eluant in the 12-mL tube.
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Add 0.5 mL of isooctane, seal the tube with a PTFE-lined cap and vortex the sample for 5 seconds.
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Evaporate the solution at ambient temperature to a volume of approximately 0.25 mL under a gentle flow of nitrogen.
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Add 25 µL 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, then seal with a PTFE-lined cap.
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Add approximately 0.1 g of anhydrous sodium sulfate and vortex the sample for 15 seconds.
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Add 25 µL of MTBSTFA and vortex the sample for 15 seconds.
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Centrifuge the sample for 5 minutes at 2500 rpm.
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Transfer the sample to a 2-mL autosampler vial and seal the vial with a cap and crimper.
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METHOD OUTLINE (continued)

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

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Analyze the samples and calibration standards by capillary gas chromatography with mass spectrometry.

ANALYTICAL RESULTS

Calculations

Calibration standards were analyzed with each sample set. Power regression equations were generated for each analyte using the log of the concentrations of the calibration standards versus the log of the respective peak area responses. The least squares coefficient of determination (r^2 value) of each power regression equation was 0.99 or greater. Concentrations of the analytes in the final solutions were determined by substituting the peak area responses into the applicable power regression equation as shown below:

$$\text{ng/mL} = \left[\frac{(\text{m/z 163 CAAC TBDMSE peak area})}{\text{constant}}\right] \wedge (1/\text{exponent})$$

where the exponent is the coefficient and the constant is the inverse log of the Y-intercept generated from the power regression equation.

For example, using the *trans*-CAAC data from Figure 2, 8 and 10.

1) Final sample weight (g) = weight of vial and sample (g) - weight of vial (g)

   Final sample weight (g) = 13.1406 - 12.7785

   Final sample weight (g) = 0.3621

2) Final sample volume (mL) =

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

$$\text{Final sample volume (mL)} = \left[\frac{(0.3621\ \text{g} - (0.025\ \text{mL} * 0.7899\ \text{g/mL}))}{0.6870\ \text{g/mL}}\right] + 0.025\ \text{(mL)}$$

Final sample volume (mL) = 0.5233

3) Normalized peak area = $\left[\frac{\text{final sample volume (mL)}}{0.5\text{ (mL)}}\right]$* sample peak area

Normalized m/z 163 peak area = $\left[\frac{0.5233\text{ (mL)}}{0.5\text{ (mL)}}\right]$* 4868

Normalized m/z 163 peak area = 5095

4) net m/z 163 *trans*-CAACTBDMSE peak area
= Normalized m/z 163 peak area (sample) - peak area (control)

net m/z 163 *trans*-CAACTBDMSE peak area = 5095 - 0

net m/z 163 *trans*-CAACTBDMSE peak area = 5095

5) *trans*-CAAC conc. ng/mL =

$$\left[\frac{(\text{net m/z 163 } \textit{trans}-\text{CAACTBDMSE peak area})}{\text{constant}}\right] \wedge (1/\text{exponent})$$

trans-CAAC conc. ng/mL = $\left[\frac{(5095)}{22044}\right] \wedge (1/1.0143)$

trans-CAAC conc. ng/mL = 0.2360 ng/mL

Percent recovery was calculated using the equation shown below:

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

$$\text{Recovery} = \frac{0.2360\text{ ng/mL}}{0.2500\text{ ng/mL}} \times 100$$

Recovery = 94%

Statistics

The mean recovery was calculated for each analyte by dividing the sum of the percent recoveries of each analyte by the number of samples in the set.

The standard deviation(s) was calculated for each analyte by summing the squares of the individual deviations from the mean, dividing by the number of degrees of freedom, and extracting the square root of the quotient.

Summary of Key Dates

Sample Identification	Bench Sheet	Extracted	Analyzed
Reagent Blank	CAAC-006	03-15-95	03-29-96
Ctrl -1	CAAC-006	03-15-95	03-29-96
Ctrl -2	CAAC-006	03-15-95	03-29-96
Ctrl -3	CAAC-006	03-15-95	03-30-96
Ctrl -4	CAAC-006	03-15-95	03-30-96
Ctrl -5	CAAC-006	03-15-95	03-30-96
Ctrl -6	CAAC-006	03-15-95	03-30-96