

LOW LEVEL DETERMINATION OF PC-143 IN WATER

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## LOW LEVEL DETERMINATION FC-143 IN WATER

### Scope and Application

1.1 This method uses capillary gas chromatography with electron capture detection (GC/ECD) to analyze water samples for the presence of FC-143. FC-143 is a technical grade of ammonium perfluorooctanoate whose most abundant component has CAS Number 3825-26-1. The use of this method should be restricted to only those analysts who can demonstrate acceptable precision and accuracy with four spike replicates into laboratory water at the 1 ppb level. Sample results are reported without blank subtraction and without correction for surrogate recoveries.

1.2 The estimated limit of detection for FC-143 is lower than 0.1 ug/L.

### Summary of the Method

2.1 A one-liter aliquot of sample is fortified with surrogates, acidified, and extracted by partitioning with an organic solvent. The extract is concentrated to small volume before adding ammonium hydroxide and further concentrating to dryness. Alcoholic HCl is added to the dry residue and heated for one hour to form the ethyl ester. Several cleanups are described as part of the overall procedure to remove interferences from the extract before analysis using GC/ECD.

### Safety

3.1 The hazard of each reagent used in this method is not accurately known. Exposure to the chemicals should be held to the smallest practical level. Safety data sheets for all materials must be made available to the personnel involved in the chemical analysis.

3.2 Any unfamiliar water sample may offer dangerous native contents beyond the list of chemicals listed in this method.

### Apparatus and Materials

4.1 Samples should be collected in the field in accordance with the quality assurance project plan. Sample containers should be amber glass or similar inert material which will not offer interferences for the analysis.

#### 4.2 Glassware

4.2.1 Optional all glass continuous liquid/liquid extractor

designed to extract one liter of water sample using dichloromethane solvent.

4.2.2 Separatory funnel, 2-L size, PTFE stopcock.

4.2.3 Erlenmeyer flask, 1-L size with calibration mark etched at the 1.0 liter volume.

4.2.4 Concentrator tube, Kuderna-Danish, 10-mL size, graduated, ground glass joint.

4.2.5 Evaporative flask, Kuderna-Danish, 500-mL size, purchased with a 24/40 ground glass stopper for the upper joint.

4.2.6 Snyder column, Kuderna-Danish, three-ball macro, ground glass joint, floodless type.

4.2.7 Snyder column, Kuderna-Danish, two-ball micro, ground glass joint, floodless type.

4.2.8 Pipets, various sizes and types.

4.2.9 Autosampler vials, 2-mL size, PTFE-faced septum.

4.2.10 Screw-cap vials, 4-dram size, PTFE-lined cap.

4.2.11 Micro Reaction Vessels, graduated 5-mL size (Catalog #3-3299M, Supelco Inc., Bellefonte, PA).

4.2.12 Replacement PTFE-faced Rubber Septa for Micro Reaction Vessels, 20 mm size. (Catalog #2-3264M, Supelco Inc., Bellefonte, PA)

4.3 Analytical Balance.

4.4 Centrifuge, IEC HN-SII or equivalent.

4.5 Heated water bath, steam delivery and temperature should be controllable, concentric ring covers for openings.

4.6 Nitrogen blowdown apparatus.

4.7 Gas Chromatographic System, ECD, split/splitless capillary injector and pneumatics, complete with a data system for collecting and processing chromatographic data.

4.8 Rtx-200 Primary column or equivalent, 100% trifluoropropyl methyl polysiloxane stationary phase, 30 meters in length, 0.32 mm inside diameter, 0.5 um film thickness. (Catalog #15039, Restek Corporation, Bellefonte, PA).

4.9 HP-5 Confirmation column or equivalent, 5% phenyl methyl polysiloxane stationary phase, 25 meters in length, 0.32 mm inside diameter, 0.5 um film thickness. (Hewlett Packard part number 10091J-112, Telephone 1-800-227-9770).

#### Reagents

5.1 Blank water which does not produce an interference for parameters of interest.

5.2 Ether, pesticide quality or equivalent.

5.3 Hexane, pesticide quality or equivalent. Cleaned prior to use by briefly shaking 100 mL of hexane against 2 grams of Activity I Neutral Alumina.

5.4 Dichloromethane, pesticide quality or equivalent.

5.5 Acetone, pesticide quality or equivalent.

5.6 3% Ethanolic HCl. Prepared by adding 2.4 mL of Acetyl chloride (Instant Methanolic HCl Kit, Stock #18053A, Alltech Associates, Deerfield, IL) dropwise to 56 mL of absolute ethanol (Florida Distillers, Lake Alfred, FL). Sealed tightly in an amber glass bottle, this reagent may be stored at room temperature for at least two weeks.

5.7 Sulfuric acid, ACS grade. Carefully dilute with an equal volume of laboratory blank water to prepare 1+1 sulfuric acid for use.

5.8 Sodium hydroxide, ACS grade. Dissolve in laboratory blank water to prepare both 10 N NaOH and 1 N NaOH for use.

5.9 Ammonium hydroxide, ACS grade. Use as received.

5.10 Florisil, Selective Adsorbent for Gas Chromatography, 100/200 mesh. (Baker Analyzed Catalog #M369-07). Activate at 130 °C in a shallow tray overnight prior to use.

5.11 Alumina N (Neutral) Super I Activity. (Catalog #04580, Universal Scientific Inc., Atlanta, GA). Use as received.

5.12 Boiling Chips, 10/40 mesh, heat at 400 °C for 30 minutes before use to insure freedom from contamination.

5.13 Wide and narrow range of pH paper.

#### Standards

6.1 Concentrated stock solutions may be prepared by weighing the primary neat materials and dissolving in ethanol. Primary

standards may be secured from the following vendors.

6.1.1 PCR Incorporated, Gainesville, FL 1-800-331-6313

6.1.2 MTM Research Chemicals, Lancaster Synthesis, Windham, NH, 1-800-238-2324

6.1.3 ChemService, West Chester, PA 215-692-3026

6.2 Surrogate spike solution. Prepare a three-component surrogate spiking solution by diluting the concentrated stock of perfluorononanoic acid, perfluorodecanoic acid, and 11H-Eicosafluoroundecanoic acid. The final concentration of each surrogate should be 10 ug/mL in ethanol.

Note: Early work did not include the perfluorodecanoic acid surrogate.

6.3 Matrix spiking solution. Prepare an FC-143 spiking solution by diluting the concentrated stock to 10 ug/mL using ethanol.

6.4 Internal standard stock solution. Prepare a three-component internal standard solution by diluting concentrated stocks of 1,2-Dibromoethane, 1,2-Dichlorobenzene, and 1,2,4-Trichlorobenzene. The final concentration of each internal standard should be 2 ug/mL, 40 ug/mL, and 4 ug/mL respectively in hexane.

EXCEPTION - Recent work has discontinued 1,2-Dibromoethane as an internal standard and substituted Ethyl perfluoroheptanoate at 20 ug/mL in this solution. A concentrated stock solution of the ester may be prepared by weighing approximately 10 mg of the acid directly into a reactor vial and following the derivatizing procedure beginning at procedure step 7.22.

6.5 Instrument calibration working standards may be prepared from either the acids or the salt primary materials by processing known masses through the derivatization steps of the sample preparation.

#### Sample Extraction - Separatory Funnel Technique

7.1 Set up a different 2-L separatory funnel for each sample and the blank. Using a graduated 1-L Erlenmeyer flask place 1 liter of sample into the separatory funnel.

7.2 Fortify each sample and the blank with 0.1 mL of the surrogate spiking solution (see 6.2). Fortify any matrix or blank spike aliquots with both surrogate and 0.1 mL of matrix spiking solution (see 6.3).

7.3 Adjust the sample to pH >12 with 10 N NaOH. Test the pH with pH paper.

7.4 Add 100 mL of dichloromethane to the separatory funnel and shake for two minutes to reach equilibrium. Vent the funnel frequently especially for the first few seconds of shaking to release the vapor pressure which will be produced. Allow the liquid phases to separate and discard the lower organic phase. If a significant emulsion develops, use a centrifuge to separate the phases.

7.5 Adjust the sample to pH <1.5 with 1+1 sulfuric acid.

7.6 Rinse the 1-L Erlenmeyer flask used to aliquot the sample with 200 mL of ether before adding this ether to the separatory funnel.

7.7 Assemble a Kuderna-Danish (KD) apparatus by connecting a 10-mL concentrator tube to a 500-mL evaporative flask. Place two or three boiling chips into the unit.

7.8 Shake the separatory funnel for two minutes and allow the phases to separate before draining the sample (lower phase) back into its original Erlenmeyer. Drain the ether extract directly into the assembled KD apparatus.

7.9 Return the aqueous sample to its separatory funnel. Rinse the Erlenmeyer flask with 100 mL of fresh ether before adding the ether to the separatory funnel for the last extraction. Repeat step 7.8.

7.10 Add 4.0 mL of 1 N NaOH and approximately 20 mL of fresh ether to the "empty" separatory funnel and shake (roll around) for several seconds. Allow at least two minutes for the liquid to drain down to the stopcock before adding the base and the ether to the KD apparatus.

7.11 Attach a three-ball macro Snyder column to the KD flask.

7.12 Concentrate the combined extract on the steam bath until boiling slows considerably and the KD begins to get warm. Only the aqueous liquid should be present in the receiver with ether refluxing in the Snyder column.

7.13 With the KD still on the steam bath slowly add at least 5 mL of dichloromethane into the macro Snyder column. The dichloromethane should be added slowly to avoid superheating with any contact against the warm receiver region. Continue heating for approximately one minute to exchange most of the residual ether.

7.14 Remove the KD from the heat and cool for two minutes or more before pouring 5 mL of dichloromethane down the macro Snyder column. Remove the Snyder column and add two or three new boiling chips. Replace the Snyder column and return to the steam bath. Heat the KD until only the aqueous phase appears to be present in

the receiver and the KD begins to warm. This procedure should remove the dissolved ether without floating the aqueous keeper out of the receiver into the KD regions.

7.15 Remove the KD from the heat for the last time and allow it to cool. Replace the Snyder column with a 24/40 stopper. Disassemble the receiver and transfer all liquid to a 4-dram vial. Add three mL of 1 N NaOH and three mL of fresh dichloromethane to the receiver before reconnecting it to the KD flask. Gently rotate the apparatus to rinse the interior walls. Vapor pressure will be produced which should be released frequently at the stoppered joint. After several seconds of rotation, return the apparatus to the upright position and allow a few minutes for the liquids to drain into the receiver. Remove the receiver from the KD and transfer the rinse liquids to the 4-dram vial. Additional fresh dichloromethane may be required to establish equal volumes of the two liquid phases in the vial.

Note: The stopper, the KD, the Snyder, and the empty receiver should be rinsed with water without delay to avoid future problems with this reusable glassware. Do not allow the basic water to dry on the glass surfaces.

7.16 Secure the cap on the 4-dram vial. Vigorously shake the vial for 30 seconds to reach equilibrium. Remember to vent any pressure which may be produced. Briefly centrifuge the vial to secure a good phase separation. Use a disposable 5-mL pipet to remove the dichloromethane phase (lower) and discard it to waste.

7.17 Adjust the aqueous sample extract to pH <1.5 using 1+1 sulfuric acid. Verify the pH by placing a small drop of the adjusted sample onto pH indicator paper.

7.18 Place 5 mL of fresh ether into the vial containing the acidified aqueous extract. Recap the vial and shake for 30 seconds remembering to vent any pressure which may develop. After equilibration and phase separation, transfer the upper ether phase to a clean 10-mL receiver. **CAUTION:** Do not approach the liquid/liquid interface too closely. Severe problems may result from the transfer of any aqueous phase.

7.19 Repeat step 7.18 for the last extraction. To the combined ether extract add 0.1 mL of concentrated ammonium hydroxide. Add two or three boiling chips to the receiver and attach a micro two-ball Snyder column. Take the extract to the steam bath and concentrate it to approximately 0.5 mL volume. Remove the sample from the heat and allow it to cool.

7.20 Transfer the extract to a clean 5-mL reactor vial. Complete the transfer quantitatively using 0.5 mL of acetone.

7.21 Further concentrate the sample to dryness using a gentle

stream of clean nitrogen at room temperature. This process should take approximately one hour due to the small amount of water in the sample. Many samples will offer a visible residue at dryness. Each sample will require careful inspection to determine that all water has been removed. Some samples may have tiny droplets of water covered by a thin membrane of solid phase which can be broken by adjusting the stream of nitrogen. Experience will dictate the best way of judging the dryness of the sample. Nitrogen blowdown may be continued as long as one hour past dryness without loss of target compounds.

7.22 Add 1.0 mL of ethanolic HCl reagent to the dry sample residue. Secure the screw cap to the vial and shake it for several seconds. Place the sample into an oven at 65 °C for one hour to derivatize the compounds of interest to their respective ethyl esters.

7.23 Remove the samples from the oven and allow the reactor vials to cool. Remove the cap and add 1.0 mL of clean hexane and 1.0 mL of laboratory blank water. Recap and shake vigorously for several seconds. Allow the phases to separate and discard most of the lower liquid phase keeping the upper "hexane" phase.

7.24 Add Florisil to a clean empty 2-mL autosampler vial. The amount of Florisil should provide approximately 1 mm of depth in the vial. Transfer the "hexane" phase from the reactor vial in procedure step 7.23 to the vial containing Florisil. Be careful not to transfer any residual aqueous phase which may still be present. Shake the Florisil vigorously for a several seconds and then allow it to settle.

**IMPORTANT NOTE:** The extract is stable against the Florisil for less than one hour. Leaving the extract against Florisil for more than several minutes will result in gradual loss of the compounds of interest. The "hexane" phase from procedure step 7.23 contains a significant amount of ethanol and should not be diluted with pure hexane before performing the Florisil adsorption step. If any dilutions are needed, then they should be performed after removing the extract from the Florisil vial.

7.25 Transfer a measured portion of the final extract away from the Florisil and place it into an autosampler vial already containing internal standards. This final extract should be stable for at least forty days. The vial should be clearly labeled and is ready for analysis.

#### Sample Extraction - Continuous Extractor Technique

8.0 An alternative to separatory funnel extraction is continuous liquid/liquid extraction. FC-143 may be successfully extracted from an acidified water sample using a continuous extractor and dichloromethane solvent.

8.1 Set up a different 1-Liter continuous extractor unit for each sample and a blank. Place a few boiling chips in the round bottom flask supported snugly by the heating mantle. Attach the extraction reservoir to the flask with sufficient support for safe operation. Add approximately 300 mL of dichloromethane to the reservoir.

8.2 Use a graduated Erlenmeyer flask to measure 1 liter of the sample and transfer it into the continuous extractor reservoir. Fortify the sample with 0.1 mL of surrogate solution (see 6.2). Fortify any matrix spike or blank spike sample with both surrogate and 0.1 mL of matrix spiking solution (see 6.3).

8.3 Adjust the sample to pH <1.5 using 1+1 sulfuric acid.

8.4 Add extra dichloromethane to the reservoir if necessary to insure proper solvent circulation during the extraction. Attach the condenser to the reservoir and start cool water flow through the condenser jacket. Energize the heating mantle to maintain gentle solvent boiling for sixteen hours of continuous extraction.

8.5 When extraction time has expired, discontinuing heating and allow the unit to cool. Turn off the water to the condenser jacket. Disconnect the collection flask and transfer the extract directly to a KD apparatus using a small amount of acetone to complete the transfer quantitatively.

8.6 Concentrate the extract using the steam bath. When the apparent volume of the extract reaches 2 mL, remove the KD from the heat and allow it to cool.

8.7 Replace the 3-ball Snyder column with a 24/40 stopper. Disassemble the receiver and transfer the extract to a 4-dram vial. Add seven mL of 1 N NaOH and three mL of fresh dichloromethane to the receiver before reconnecting it to the KD flask. Gently rotate the apparatus to rinse the interior walls. Vapor pressure will be produced which should be released frequently at the stoppered joint. After several seconds of rotation, return the apparatus to the upright position and allow a few minutes for the liquids to drain into the receiver. Remove the receiver from the KD and transfer the rinse liquids to the 4-dram vial. Additional fresh dichloromethane may be required to establish equal volumes of the two liquid phases in the vial.

Note: The stopper, the KD, the Snyder, and the empty receiver should be rinsed with water without delay to avoid future problems with this reusable glassware. Do not allow the basic water to dry on the glass surfaces.

8.8 Finish the sample preparation in the same manner as that used for separatory funnel extractions. Continue at procedure step 7.16.

## Instrument Calibration - Primary Analysis

9.1 Figure 1 through Figure 4 are example chromatograms with recommended instrument conditions included in the legends. Figure 1 is a typical instrument calibration standard. Figure 3 illustrates the multiple peak pattern of FC-143 using the primary analytical column.

9.2 Instrument calibration and sample analysis must be performed using multiple internal standards. The three internal standard compounds listed in section 6.4 are recommended to establish both relative retention times (RRT) and relative response factors (RRF). Internal standards appearing in a chromatogram will establish primary search windows for those target compounds nearby in the chromatogram. Relative retention times are calculated using equation 2.

$$RRT = RT^{\text{target}} / RT^{\text{IS}} \quad \text{Eq. 2}$$

The relative response factor is calculated as follows.

$$\text{Absolute Response Factor} = RF = \text{Amount} / \text{Area} \quad \text{Eq. 3}$$

$$\text{Relative Response Factor} = RRF = RF^{\text{target}} / RF^{\text{IS}} \quad \text{Eq. 4}$$

Note: Amount in equation 3 refers to the mass (e.g. ug) of compound mixed into the solution injected.

9.3 Prepare initial calibration standards at a minimum of three concentration levels for both FC-143 and the surrogates. Analyze each level of calibration standard. For each compound, tabulate the RRF at each level (see equation 4). If the RRF over the working range is constant enough (less than 20% relative standard deviation), then the midpoint value may be used for calculation. Alternatively, the calibration data may be used to construct a calibration curve of relative response against relative amounts.

9.4 Continuing calibration standards must be injected after every ten sample extracts and at the end of an analytical sequence. Relative response factors must be compared to the initial calibration values. Treat the continuing calibration standard as an unknown sample and compute the concentration injected. If the calculated concentration differs more than 20% from the true value, then initial calibration must be repeated and all affected samples must be reanalyzed.

## Instrument Calibration - Confirmation Analysis

10.0 FC-143 tentatively identified by the primary analysis should be confirmed by at least one qualitative technique. Confirmation data may be supplied by analysis using a dissimilar analytical

column, or by gas chromatography with mass spectrometry detection (if the target concentration is adequate), or by a second derivatization forming a different ester. Confirmation analysis must be qualitative and may be quantitative.

#### 10.1 GC Dissimilar Column Confirmation

10.1.1 Column: HP-5 (or equivalent) 5% phenyl methyl polysiloxane stationary phase, 25 meters in length, 0.32 mm inside diameter, 0.5 um film thickness.

##### 10.1.2 Chromatographic conditions:

Oven Program: 50 °C for 2 minutes  
to 90 °C at 5 °C/minute  
to 280 °C at 10 °C/minute  
(hold 2 minutes)

Injector Zone: 200 °C

Detector Zone: 320 °C

Helium carrier inlet pressure: 5.5 psig

1.0 uL splitless injection, split on at 0.5 minutes

10.1.3 The primary initial and continuing calibration standards and criteria are used for GC dissimilar column confirmation (sections 9.2 - 9.4)

10.1.4 The sample extracts requiring confirmation and the associated method blanks should be analyzed at the same dilutions as for the primary analysis.

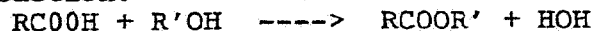
#### 10.2 GC/MS Confirmation

10.2.1 GC/MS analysis normally requires a minimum concentration of 10 ug/mL in the field sample extract. The sample extracts requiring confirmation and the associated method blanks must be analyzed. A reference standard for the target compound must also be analyzed by GC/MS. The concentration of the reference standard must be at a level that would demonstrate the ability to confirm the target.

10.2.2 To confirm the identification of FC-143, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions.

#### 10.3 Confirmation by Analysis of an Alternate Ester

10.3.1 Both standards and sample extracts may be derivatized to an alternate ester before analysis according to the following reaction.



Choice of the alcohol to which acetyl chloride is added (section 5.6) will determine which ester is prepared at procedure step 7.22. The methyl, ethyl, n-propyl, and n-butyl esters of FC-143 have been prepared by using the corresponding alcoholic HCl. Only the ethyl ester has been processed through full method validation.

10.3.2 Before samples are confirmed by this technique, the laboratory must demonstrate the ability to successfully process and analyze representative standards.

#### 10.4 Simultaneous Primary and Confirmation Analysis

10.4.1 A gas chromatograph using only one injection port, one column oven, and one detector can produce a chromatogram from two dissimilar columns installed in parallel. The resulting chromatogram is a superimposed (overlaid) composite of the separations achieved with each independent column. Each compound in the sample will produce two chromatographic peaks thus providing enhanced qualitative information for every injection.

10.4.2 The recommended primary and confirmation columns may be connected in parallel using two "Y" connectors (part number 20405, Restek Corporation, Bellefonte, PA) and short sections of uncoated deactivated fused silica tubing. Alternatively, a 2-hole ferrule may be used at the injection port and at the detector.

10.4.3 An example chromatogram is presented in Figure 4. The "#" symbol precedes the name of a chromatographic peak produced by the confirmation column. Named peaks produced by the primary column will not include the "#" symbol. Therefore, the FC-143 peak was produced by the Rtx-200 column, and the # FC-143 peak was produced by the HP-5 column.

10.4.4 When a 2-hole graphite ferrule is used at the detector, peak identification may be assisted by temporarily removing one column from the detector for selected injections.

#### Quality Control

11.1 Every laboratory must operate a formal quality control program which meets or exceeds the requirements of the data quality objectives.

11.2 Before using this method, laboratory capability must be demonstrated by analyzing spiked samples. Seven replicates spiked at sufficiently low FC-143 concentration must be analyzed to estimate the method detection limit (Table 1 and Table 2). Figure 2 is an example chromatogram of blank water spiked to estimate the method detection limit using the separatory funnel extraction

technique. Also four additional replicates must be spiked at the matrix spike level and analyzed to create additional precision and accuracy data available for audits (Table 3).

11.3 Ongoing performance evaluation will be accomplished by analyzing a matrix spike and a matrix spike duplicate for every twenty field samples. A control sample will be extracted and analyzed with every set of matrix spikes. Surrogates will be spiked into every field sample, matrix spike, matrix spike duplicate, control sample, and laboratory blank. Surrogate recoveries will be control charted to determine control limits for corrective action.

### Gas Chromatography

12.1 The gas chromatographic conditions listed in Figure 1 are recommended for primary analysis. Peaks tentatively identified in a sample require confirmation analysis (section 10.0).

### 12.2 Qualitative Analysis.

12.2.1 Peak identification is based upon relative retention time comparison to calibration data. Library retention times are established on a daily basis. A nearby internal standard serves as a time reference for all chromatographic acquisitions. A target compound should be identified in a sample only if the chromatographic peak matches the predicted retention time within 0.05 minutes.

$$\text{Predicted RT} = (\text{RRT}^{\text{library}})(\text{RT}_{\text{IS}}^{\text{sample}}) \quad \text{Eq.5}$$

12.2.2 Equation 5 should serve only as a recommended treatment for peak identification. The experienced analyst must consider other chromatographic features such as peak shape, resolution, distance from the time reference, and multiple peak pattern recognition.

### 12.3 Quantitative Analysis.

12.3.1 Sample extracts should be prepared for analysis by mixing 50 uL of internal standard solution (see 6.4) into 0.5 mL of the sample extract.

12.3.2 The concentration of FC-143 identified in a sample may be calculated using equation 6. It is recommended that the nearest internal standard not suffering from co-elution be used for calculation, but the discretion of the analyst should prevail.

$$\text{PPB}_x^{\text{sample}} = \frac{(\text{ug}_{\text{IS}}^{\text{sample}})(\text{Area}_x^{\text{sample}})(\text{RRF}_x)}{(\text{Area}_{\text{IS}}^{\text{sample}})(\text{L extracted})(\text{D.F.})} \quad \text{Eq.6}$$

$PPB_x^{sample}$  = concentration of target (in the sample)  
 $ug_{IS}^{sample}$  = mass of material (I.S.) mixed with the solution injected.  
 $Area_x^{sample}$  = peak area of target (in the sample)  
 $RRF_x$  = relative response factor (equation 4)  
 $Area_{IS}^{sample}$  = peak area of internal standard (in the sample)  
 $L_{extracted}$  = Total raw sample producing total extract  
 $D.F.$  = dilution factor of the extract

12.3.3 The concentration of FC-143 identified in a sample may alternatively be calculated using the initial calibration curve.

TABLE 1  
LOW LEVEL SPIKING STUDY - SEPARATORY FUNNEL

| <u>FC-143<br/>Spiked</u> | <u>FC-143 Conc.<br/>Found (ug/L)</u> | <u>FC-143 %<br/>Recovery</u> | <u>ET-C9<br/>SURR.</u> | <u>ET-C11<br/>SURR.</u> |
|--------------------------|--------------------------------------|------------------------------|------------------------|-------------------------|
| 0.0 ppb                  | ND                                   | N/A                          | 52%                    | 47%                     |
| 0.1 ppb                  | 0.062                                | 62%                          | 51%                    | 48%                     |
| 0.1 ppb                  | 0.052                                | 58%                          | 44%                    | 41%                     |
| 0.1 ppb                  | 0.061                                | 61%                          | 49%                    | 46%                     |
| 0.1 ppb                  | 0.059                                | 59%                          | 53%                    | 47%                     |
| 0.1 ppb                  | 0.058                                | 58%                          | 50%                    | 48%                     |
| 0.1 ppb                  | 0.062                                | 62%                          | 51%                    | 47%                     |
| 0.1 ppb                  | 0.062                                | 62%                          | 54%                    | 50%                     |
| 0.2 ppb                  | 0.11                                 | 55%                          | 48%                    | 48%                     |
| 0.4 ppb                  | 0.23                                 | 58%                          | 53%                    | 46%                     |

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ND - Not detected

Mean Concentration FC-143 (seven 0.1 ppb replicates) = 0.059 ppb  
 Standard Deviation FC-143 (seven 0.1 ppb replicates) = 0.0036 ppb  
 Three std. dev. FC-143 (seven 0.1 ppb replicates) = 0.011 ppb

One liter of blank water was spiked and extracted

TABLE 2  
LOW LEVEL SPIKING STUDY - CONTINUOUS EXTRACTOR

| <u>FC-143<br/>Spiked</u> | <u>FC-143 Conc.<br/>Found (ug/L)</u> | <u>FC-143 %<br/>Recovery</u> | <u>ET-C9<br/>SURR.</u> | <u>ET-C11<br/>SURR.</u> |
|--------------------------|--------------------------------------|------------------------------|------------------------|-------------------------|
| 0.0 ppb                  | ND                                   | N/A                          | 50%                    | 49%                     |
| 0.0 ppb                  | ND                                   | N/A                          | 61%                    | 64%                     |
| 0.1 ppb                  | 0.041                                | 41%                          | 64%                    | 65%                     |
| 0.1 ppb                  | 0.039                                | 39%                          | 64%                    | 58%                     |
| 0.1 ppb                  | 0.045                                | 45%                          | 69%                    | 67%                     |
| 0.1 ppb                  | 0.042                                | 42%                          | 57%                    | 50%                     |
| 0.1 ppb                  | 0.045                                | 45%                          | 67%                    | 60%                     |
| 0.1 ppb                  | 0.043                                | 43%                          | 64%                    | 64%                     |
| 0.1 ppb                  | 0.041                                | 41%                          | 67%                    | 70%                     |
| 0.2 ppb                  | 0.076                                | 38%                          | 64%                    | 68%                     |
| 0.4 ppb                  | 0.155                                | 39%                          | 60%                    | 65%                     |
| 1 ppb                    | 0.670                                | 67%                          | 61%                    | 68%                     |
| 10 ppb                   | 8.94                                 | 89%                          | 81%                    | 81%                     |
| 100 ppb                  | 96.3                                 | 96%                          | 90%                    | 83%                     |

ND - Not detected

Mean Concentration FC-143 (seven 0.1 ppb replicates) = 0.042 ppb  
 Standard Deviation FC-143 (seven 0.1 ppb replicates) = 0.0022 ppb  
 Three std. dev. FC-143 (seven 0.1 ppb replicates) = 0.0066 ppb

One liter of blank water was spiked and extracted

TABLE 3  
PRECISION & ACCURACY STUDY - SEPARATORY FUNNEL

| <u>FC-143<br/>Spiked</u> | <u>FC-143 Conc.<br/>Found (ug/L)</u> | <u>FC-143 %<br/>Recovery</u> | <u>ET-C9<br/>SURR.</u> | <u>ET-C11<br/>SURR.</u> |
|--------------------------|--------------------------------------|------------------------------|------------------------|-------------------------|
| 1 ppb                    | 1.10                                 | 110%                         | 100%                   | 88%                     |
| 1 ppb                    | 0.88                                 | 88%                          | 78%                    | 69%                     |
| 1 ppb                    | 1.01                                 | 101%                         | 94%                    | 87%                     |
| 1 ppb                    | 1.03                                 | 103%                         | 98%                    | 92%                     |
| 1 ppb                    | 0.84                                 | 84%                          | 78%                    | 65%                     |

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One liter of blank water was spiked and extracted

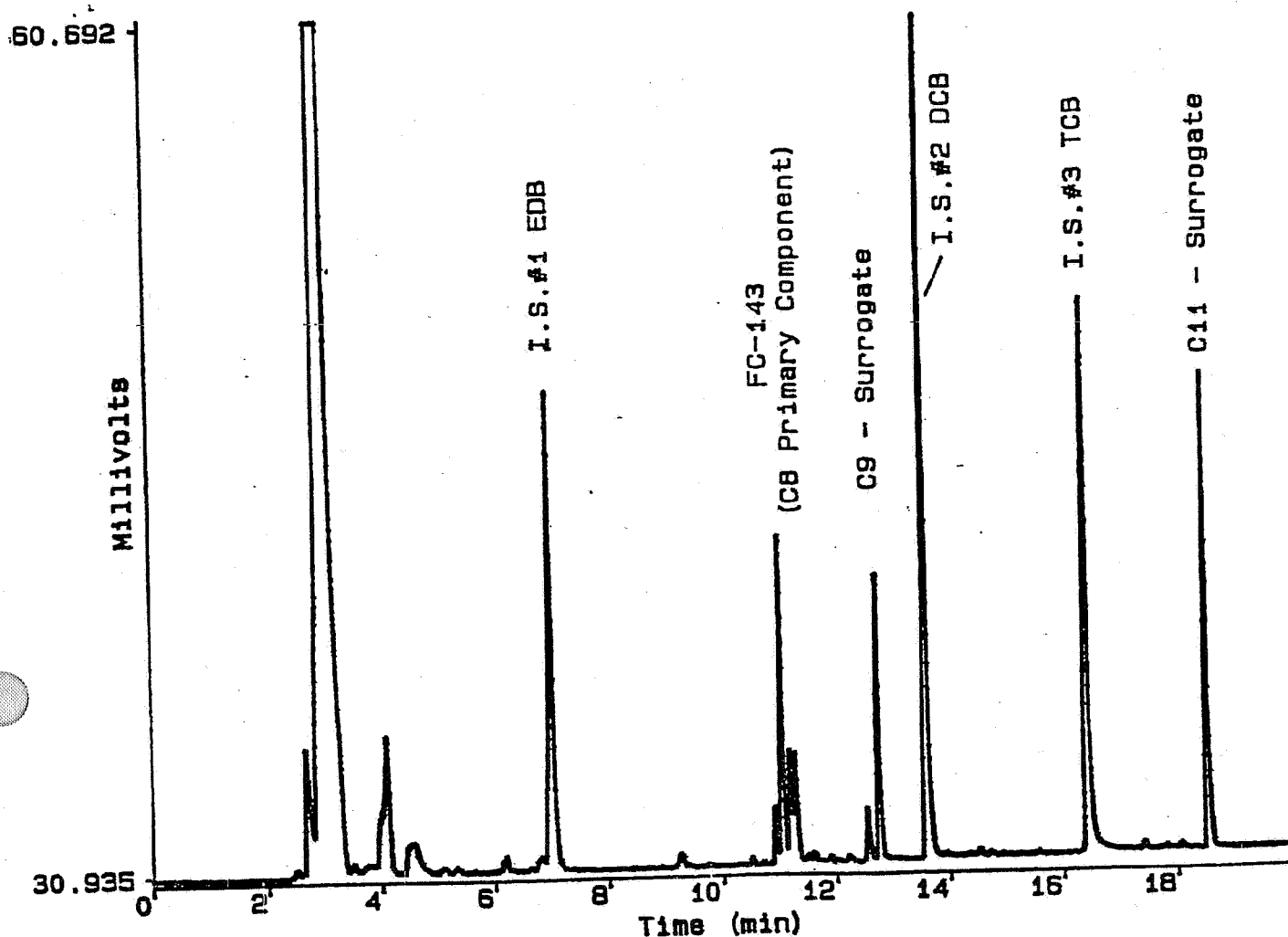


Figure 1. GC/ECD chromatogram of instrument standard at 1 ug/mL FC-143. Fused silica capillary column: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold 2 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes.

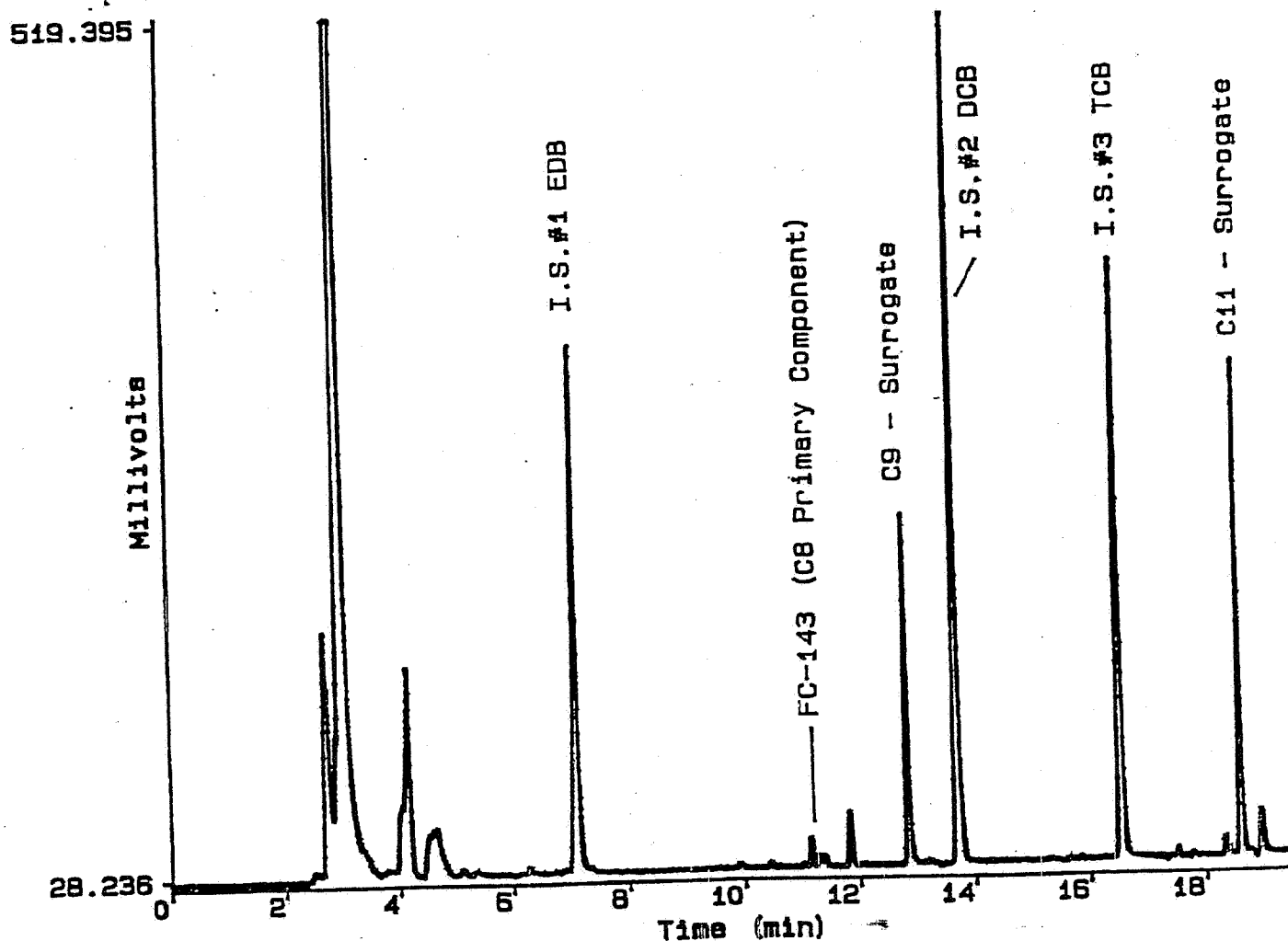


Figure 2. GC/ECD chromatogram of blank water (extracted by separatory funnel) spiked at 0.1 ug/L FC-143. Fused silica capillary column: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold 2 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes.

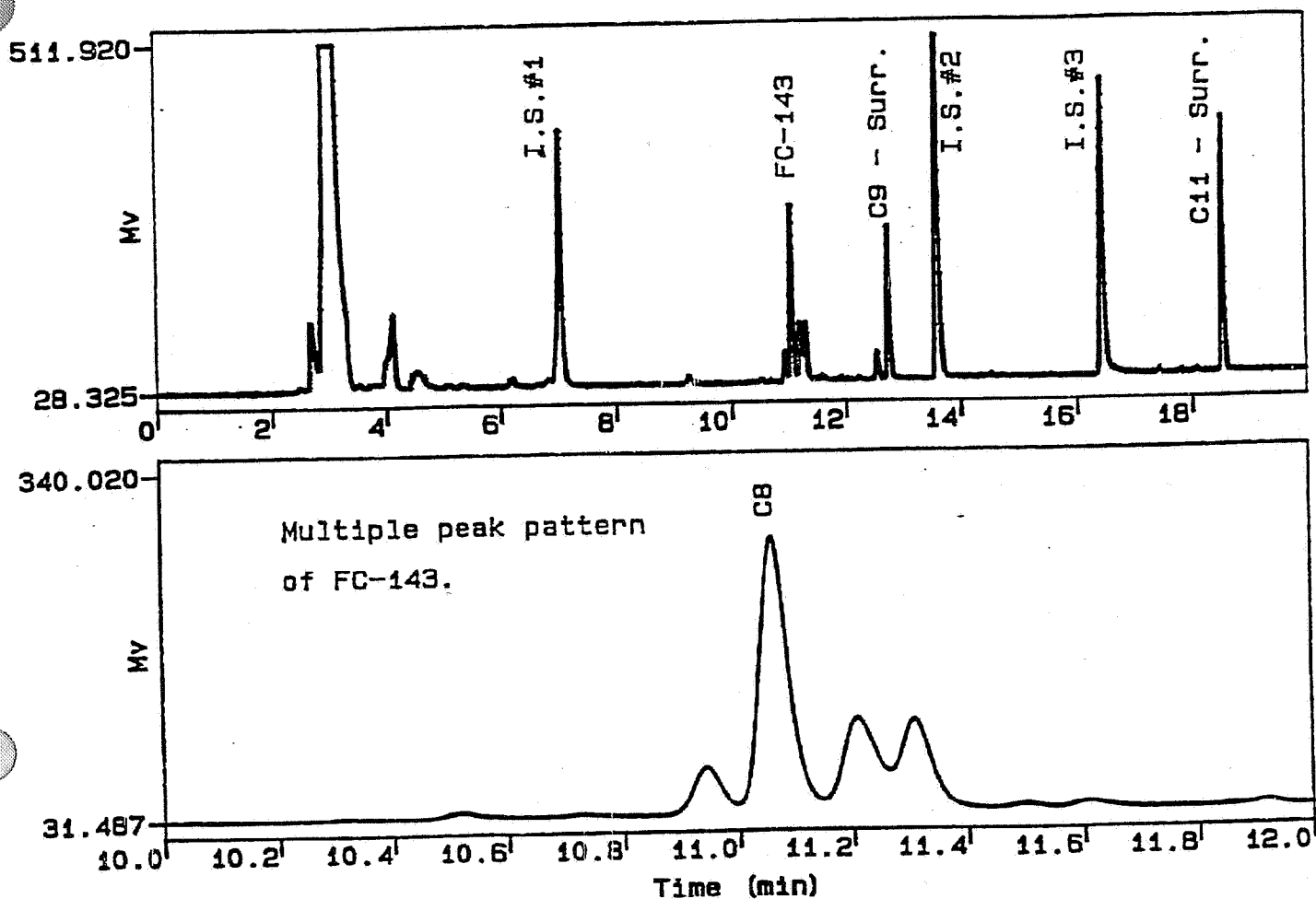


Figure 3. GC/ECD chromatogram of instrument standard at 1 ug/mL and multiple peak pattern of FC-143. Fused silica capillary column: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold 2 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes.

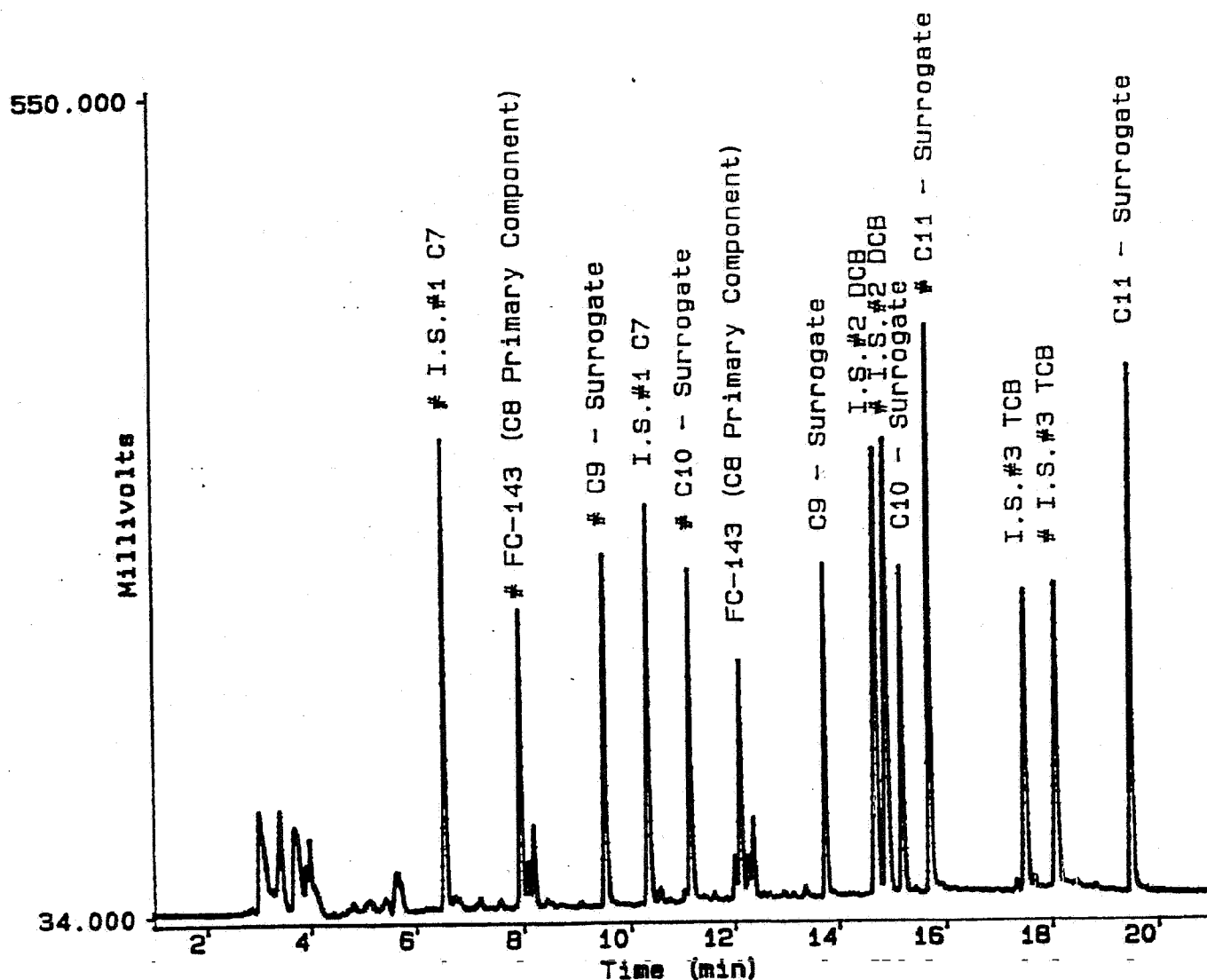


Figure 4. GC/ECD chromatogram of instrument standard at 1 ug/mL FC-143. Dual (parallel) capillary columns: Rtx-200 (Restek Corporation), 30m x 0.32mm I.D., 0.5um film thickness. HP-5 (Hewlett Packard), 25m x 0.32mm I.D., 0.5um film thickness. Column program: 50°C for 2 minutes to 90°C at 5°C/minute then to 280°C at 10°C/minute and hold for 4 minutes. Injector zone: 200°C. Detector zone: 320°C. Helium carrier inlet pressure = 5.5psig. 1uL splitless injection, split on at 0.5 minutes. Peak naming convention: The "#" symbol preceding the compound name indicates a peak produced by the HP-5 capillary column.