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Determination of Endothall In Water

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Reference: Elf Atochem North America, Inc., "Endothall in Sediment and Soil" November 18, 1992

1.0 Synopsis

Endothall is extracted from water samples by taking 100 g (100 mL) of sample and evaporating to dryness. The residue is dissolved in phosphoric acid and is transferred to a centrifuge tube containing heptafluoro-p-tolylhydrazine (HFTH) and heated to form the endothall-HFTH derivative. The derivatized endothall is partitioned into methyl t-butyl ether and eluted through solid phase extraction (SPE) columns. The samples are analyzed using a gas chromatograph equipped with an electron capture detector.

2.0 Reagents

1. Phosphoric Acid 85% (H_3PO_4), Fisher A260-500
   6N H_3PO_4: 135 mL H_3PO_4 in 865 mL D.I. water
   3N H_3PO_4: 67.5 mL H_3PO_4 in 932.5 mL D.I. water

2. Potassium Phosphate Monobasic, Mallinckrodt 7100 (Extraction Buffer: 13.61 g KH_2PO_4 in one liter D.I. water. Adjust pH to 2.5 ± 0.1 with concentrated H_3PO_4).

3. Potassium Phosphate Dibasic, Mallinckrodt 7088 or (Saturated K_2HPO_4: Add K_2HPO_4 to 1000 mL D.I. water until saturated).

4. Water, de-ionized (D.I.)

5. Hexane, Burdick & Jackson 216-4

6. Petroleum Ether, EM Science PX0424-1

7. Acetonitrile, EM Science AX0142-1 (to make endothall monohydrate standard solution)

8. *Acetonitrile, EM Science AX0155-1*

9. HPLC Water, Fisher W5-4 (to make fortification solutions)

10. Methanol, EM Science MX0488-1 (to elute columns)

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11. Methanol, Burdick & Jackson 230-4 (to make endothall-HFTH standard solution)

12. Methyl t-Butyl Ether (MTBE), Burdick & Jackson 242-4

13. Anhydrous Sodium Sulfate (Na_2SO_4), EM Science SX0760-3

14. Whatman GF/C 9.0 cm Glass Microfibre filters, 1822090

15. Amine solid phase extraction (SPE) column, 5 gram, Varian 1225-6028

16. Florisil solid phase extraction (SPE) column, 5 gram, Varian 1225-6030

17. Dry ice

18. *Heptafluoro-p-tolylhydrazine (HFTH), Sigma Chemical Co., 5 gram bottle, H-2642.* To recrystallize, dissolve 5 grams in approximately 60 mL of hexane, add heat. The solution is allowed to cool, about one hour, and filtered through a glass filter. Repeat this recrystallization two additional times. The HFTH is recrystallized to remove impurities.

19. *Celite 545, Fisher C212-500*

20. Endothall Monohydrate Pesticide Residue Analytical Standard

21. Endothall-HFTH Pesticide Residue Analytical Standard

Equivalent Reagents may be substituted.

3.0 Apparatus

1. *Wrist Action Shaker (Burrell)*

2. Sonicator (American Scientific Products, C64550-11)

3. Heat Block (VWR Scientific, 13259-007)

4. J & W Scientific, DB-5, 30 m x 0.32 mm I.D. x 0.25 µm, GC column or equivalent.

5. *HP 5890 Series II Gas Chromatograph equipped with ^{63}Ni; Electron Capture Detector.*

6. N-Evap analytical evaporator (Organomation, Model No. 112)

7. Büchi Rotovaps

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8. 100 mL graduated cylinder

9. Büchner Funnels

10. 500 mL graduated mixing cylinders, glass stoppered

11. Volumetric flasks, glass stoppered

12. Centrifuge tubes, screw capped with Teflon cap liner 15 mL and 50 mL

13. Pasteur Disposable Pipets

14. 500 mL boiling flask

15. 10 mL Manostat syringe

16. Centrifuge (Damon/IEC Division)

Equivalent Apparatus may be substituted

4.0 Procedure

4.1 Preparation of the Standard Solution and Standard Curve.

1. Standard Endothall Monohydrate Solution - To prepare a 100 µg/mL solution of endothall monohydrate as endothall acid equivalents the molecular weights need to be taken into consideration. The following formula is used:

$$0.010\ g \times \frac{204.18 \text{ molecular weight endothall monohydrate}}{186.16 \text{ molecular weight endothall acid}} = \frac{0.01097\ g}{\%\text{ purity of endothall monohydrate}} \times 100$$

Weigh the appropriate amount of standard (from the above equation) into a 100 mL volumetric flask add 2 mL acetonitrile and 98 mL HPLC grade water, mix well. Dilutions of this standard should be made in Fisher water to provide fortification solutions where a maximum of 5 mL can be added to the recovery samples.

2. Standard Endothall-HFTH Solution - To prepare a 100 µg/mL solution of endothall-HFTH as endothall acid equivalents the molecular weights need to be taken into consideration. The following formulation is used:

$$0.010\ g \times \frac{398.24\ g \text{ molecular weight endothall-HFTH}}{186.16\ g \text{ molecular weight endothall acid}} = \frac{0.02139\ g}{\%\text{ purity of endothall HFTH}} \times 100$$

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Weigh the appropriate amount of standard (from the above equation) into a 100 mL volumetric flask and dilute to the mark with MeOH. Mix the solution well. Dilutions of this standard should be made in MTBE to provide "shooting standards" to generate the standard curve.

4.2 Sample Preparation

1. No preparation needed for water samples.

4.3 Sample Extraction

1. Measure a representative 100 mL (100 g) sample into a 100 mL graduated cylinder.

2. Vacuum filter into a 500 mL boiling flask using a Buchner funnel containing a Whatman GF/C filter paper. Fortify control samples at this point.

   NOTE: A 1/4" bed of celite can be placed in the funnel to aid in filtering

3. Add 75 mL of acetonitrile and 25 mL of extraction buffer to each sample aliquot and evaporate to dryness on the buchi rotovap. The water bath temperature should be 35-45°C. (Note: If the water bath exceeds 45°C, endothall anhydride formation may form. This anhydride is stable and difficult to re-hydrate. Acetonitrile is added to form an azeotrope and facilitate the removal of water).

4.4 Derivatization

1. Transfer the residue to a 15 mL centrifuge tube containing 50 mg HFTH. Transfer using 3 mL of 3N H_3PO_4, then 2 mL of 3N H_3PO_4. Use a pasteur pipet to transfer from the boiling flask to the centrifuge tube.

2. Cap the tube and sonicate briefly to dissolve the HFTH.

3. Place the tube in a heating block, preheated between 100°C and 105°C, for one hour, occasionally swirl the tube.

4. Remove the sample from the heating block and allow the sample to cool.

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4.5 Partition

1. Partion the aqueous extract by adding 5 mL of MTBE to each sample. Shake 1 minute. Allow the phases to separate (centrifuge if phases do not separate) and transfer the top MTBE layer, to a clean 15 mL centrifuge tube using a pasteur pipet.

2. Repeat the partion with an additional 5 mL of MTBE. Again transfer the MTBE layer into the tube containing the first 5 mL portion of MTBE.

3. Add approximately 1 mL of MTBE to the remaining layer of MTBE in the aqueous extract and swirl the tube. Transfer the MTBE and combine it with the two 5 mL portions. Discard the aqueous extract. (Note: a small amount of the aqueous layer may transfer into the MTBE, this will be removed in step 4.5.6).

4. Add 5 mL of 6N H_3PO_4 to the MTBE extract and shake 30 seconds. Allow the phases to separate and discard the bottom acid layer using a disposable pasteur pipet.

5. Add 5 mL of saturated K_2HPO_4 to the MTBE and shake 30 seconds. Allow the phases to separate and discard the bottom buffer layer using a disposable pasteur pipet.

6. Add Na_2SO_4 to a depth of 2-5 mm in each tube. Invert several times and quantitatively transfer to a clean 15 mL centrifuge tube. Do not transfer the Na_2SO_4.

7. Evaporate the extracts to dryness on the N-evap.

8. Dissolve the residue with 2 mL of 50:50 (v:v) petroleum ether:MTBE.

4.6 Purification

1. Rinse a 5 g amine SPE column with 20 mL of 50:50 (v:v) petroleum ether:MTBE.

2. As the last of the solvent enters the top of the column, add the 2 mL sample extract to the column. Allow it to elute into the column.

3. Rinse the tube with 5 mL of 50:50 (v:v) petroleum ether:MTBE and add it to the column.

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4. As the last of the 5 mL rinse enters the top of the column, add an additional 10 mL 50:50 (v:v) petroleum ether:MTBE to the column.

5. Discard all the solvent to this point.

6. Elute the endothall-HFTH from the column with 20 mL of 10:90 (v:v) methanol: MTBE. Collect the eluate in a 50 mL centrifuge tube.

7. Evaporate the extract on the N-evap to dryness.

8. Dissolve the residue with 2 mL of 50:50 (v:v) petroleum ether:MTBE.

9. Rinse a 5 gram florisil SPE column with 20 mL of 50:50 (v:v) petroleum ether:MTBE.

10. As the last of the solvent enters the top of the column, add the 2 mL sample extract to the column. Allow it to elute into the column.

11. Rinse the tube with 5 mL of 50:50 (v:v) petroleum ether:MTBE and add it to the column.

12. As the last of the 5 mL rinse enters the top of the column, add an additional 10 mL 50:50 (v:v) petroleum ether:MTBE to the column.

13. As the last of 10 mL rinse enters the top of the column add an additional 5 mL of 50:50 (v:v) petroleum ether:MTBE to the column.

14. Discard all the solvent to this point.

15. Elute the endothall-HFTH from the column with 15 mL of MTBE and collect in a 50 mL centrifuge tube.

16. As the last of the 15 mL MTBE enters the top of the column add 15 mL of 20:80 (v:v) methanol: MTBE, this is collected in the same 50 mL tube as the 15 mL MTBE.

17. Evaporate extract to approximately 5 mL and transfer to 15 mL centrifuge tube premarked at 10 mL.

18. Adjust the volume to the 10 mL mark using MTBE. A final volume of 50 mL is required for a detection limit of 0.01 ppm.

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19. Make the necessary dilutions to adjust the final volume to 50 mL for GC analysis.

4.7 Recovery and Control Sample Requirements

1. Method spikes are assayed with each set of authentic experimental samples. The method spikes are prepared by adding an appropriate amount of endothall monohydrate standard, where the volume added is ≤ 5 mL, to 50 g of control matrix. See step 4.3.1.

2. Recovery and control samples are assayed exactly as an authentic experimental sample.

4.8 Gas Chromatography

Analyze a 1-2 µL portion by gas chromatography using a electron capture detector. (These conditions are nominal).

1.	Instrument::	Hewlett Packard HP 5890 Series II equipped with an electron capture detector
2.	Column:	J & W Scientific, DB5, 30 m x 0.32 mm I.D. x 0.25 µm
3.	Conditions:	Column Temperature
	Initial temperature:	130°C
	Initial time:	6.0 min
	Rate:	11°C/min
	Final temperature:	200°C
	Initial time A:	4.0 min
	Rate A:	11°C/min
	Final temperature:	240°C
	Initial time B:	6.0 min
	Rate B:	50°C/min
	Final temperature B:	265°C
	Final time:	2.5 min
	Injector temperature:	250°C
	Detector temperature:	310°C
4.	Gas flows:	Carrier - He at 1 mL/min (130°C) Detector Make-up Gas Ar/CH_4 at 40 mL/min
5.	Attn:	2^3

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6. Approx. retention time: 23.5 min

7. Chart speed: *0.5 cm/min*

4.9 Calculations

1. ppm Found

$$\text{ppm found} = \frac{\text{ng found}}{\text{mg inj}}$$

ng found from standard curves regression line

$$\text{mg inj} = \frac{\mu\text{L x gm (final weight)}}{\text{mL (final volume)}}$$

3. Percent Recovery

$$\% \text{ recovery} = \frac{\text{ppm found}}{\text{ppm fortified}} \times 100$$

VII. Method Summary

The following methods were used to analyze samples for endothall residue:

McKenzie Laboratories, Inc. Method Number PRM-042 entitled "Determination of Endothall in Water" written by Jean Butterfield, McKenzie Laboratories, 03 February 1993.

McKenzie Laboratories, Inc. Method Number PRM-040 entitled "Determination of Endothall in Sediment and Soil" written by Jean Butterfield, McKenzie Laboratories, 28 December 1992.

Brief descriptions of the methods are presented below; complete methods are contained in Appendix A of this report as Attachment A of Protocol Amendment 5.

Method validation was conducted under Elf Atochem Study Number BR-94-13.

For analysis of study samples, a typical sample set consisted of one reagent blank, one unfortified control, two fortified controls and study samples. The controls were typically fortified at levels of 0.10 ppm and 1.00 ppm for sediment analysis and 0.050 ppm and 3.00 ppm for water analysis. Procedural recoveries are discussed in Section XI.

A. Sample Extraction and Clean-Up

1. Water Analysis

A 100 mL (g) sample was measured/weighed into a 100 mL graduated cylinder. The sample was vacuum filtered and fortified, if necessary. Endothall present in the sample was extracted with acetonitrile and extraction buffer (potassium phosphate monobasic solution) and the extract was evaporated to dryness. The endothall was derivatized to Endothall-HFTH by transferring concentrated sample residue into a centrifuge tube containing HFTH, using 3N H_3PO_4, and heating. The sample was partitioned with MTBE and evaporated to dryness. The residue was dissolved in 2 mL of 50:50 (v:v) petroleum ether:MTBE. Sample clean-up was carried out with a florisil SPE column. (If sample needed additional clean-up,

an amine column was also used.) The florisil column eluent was adjusted to a final volume of 50 mL with MTBE for analysis by gas chromatograph.

2. **Sediment Analysis**

A 50 g sample was weighed into a nalgene bottle and fortified, if necessary. Endothall present in the sample was extracted with a potassium phosphate monobasic extraction buffer solution. The filtered extract was derivatized to Endothall-HFTH by transferring concentrated sample residue into a centrifuge tube containing HFTH, using 3N H_3PO_4, and heating. The sample was partitioned with MTBE and evaporated to dryness. The residue was dissolved in 2 mL of 50:50 (v:v) petroleum ether:MTBE. Sample clean-up was carried out with a florisil SPE column. (If sample needed additional clean-up, an amine column was also used.) The florisil column eluent was adjusted to a final volume of 50 mL with MTBE for analysis by gas chromatograph.

B. Gas Chromatography

During instrumental analysis and prior to sample injections, a standard curve was determined with standard injections of known analyte concentration. These standards ranged in concentration from 0.020 ng Endothall-HFTH to 0.30 ng Endothall-HFTH. A standard was injected at least every five sample injections to verify curve stability. Standard recoveries of 90% to 110% were deemed acceptable.

Nominal operating parameters of the GC are listed in Table III. For operating conditions of a particular analysis or matrix, the raw data should be referenced.

C. Equipment

For both matrices, chromatography was conducted on a Hewlett-Packard 5890 Series II Gas Chromatograph equipped with an electron capture detector. Integration of detector response (peak heights) was achieved with an HP 3396 Series II Integrator.

D. Method Modifications

Copies of the methods are contained in Appendix A as an attachment to Protocol Amendment 5. Method modifications are contained in Appendix B. Significant modifications include the elimination of the clean-up step from the method. In method PRM-040, the calculations using 'wet sample weight' were used in determining residue found.

Table III. Nominal Operating Parameters of the Gas Chromatograph and Additional Equipment.

NOTE: The raw data identifies all equipment used in the study and exact parameters used for each analysis. The equipment and parameters listed here are representative.

Parameter:	Condition:
Instrument Type:	HP 5890 Series II
Detector Type:	electron capture
Column Size:	(30m x 0.32 mm ID x 0.25 µm) dual columns
Column Packing:	5% diphenyl, 95% dimethyl polysiloxane
Temperatures:	
Inlet:	250°C
Detector:	300°C
Oven:	130°C for 6 min
Ramp:	11°C/min
Temperature:	200°C for 4 min
Ramp A:	11°C/min
Temperature:	240°C for 11 min
Ramp B:	50°C/min
Temperature:	285°C for 20 min
Flows:	
Carrier:	He = 1.0 mL/min
Make-up:	Ar/CH_4 = 37 psi
Integrator:	HP 3396 Series II
Chart Speed:	0.5 cm/min
Retention Time:	approximately 25.3 min
Attenuation:	2^4
Injection Volume:	2 µL
Balance:	Mettler H6T Analytical Balance

VIII. Calculation Method and Example Calculations

A. Method

A standard curve was derived each day of analysis from solutions of known concentration using the following equation:

Linear regression: $y = mx + b$

where, y = the detector response, peak height
m = the slope of the line
x = nanograms injected
b = y-intercept of the line

Peak heights were measured by the integrator. For samples, peak heights were used in the standard equation to calculate ng found. Matrix specific calculations are listed below.

final volume (all samples):

final volume = method final volume (50 mL) x dilution factor

µL injected (water samples):

$$\mu L \text{ injected} = \frac{\text{final sample vol (mL) x vol inj (µL)}}{\text{final vol (mL)}} \times \frac{1000 \text{ mg}}{\text{g}} \times \frac{\text{mL}}{1000 \text{ µL}}$$

ppm Found Endothall Free Acid (water samples):

$$\text{ppm Found Endothall Free Acid} = \frac{\text{ng found}}{\text{µL injected}}$$

mg injected (sediment samples):

$$\text{mg injected} = \frac{\text{final sample wt (g) x vol inj (µL)}}{\text{final vol (mL)}} \times \frac{1000 \text{ mg}}{\text{g}} \times \frac{\text{mL}}{1000 \text{ µL}}$$

ppm Found Endothall Free Acid (sediment samples):

$$\text{ppm Found Endothall Free Acid} = \frac{\text{ng found}}{\text{mg injected}}$$

ppm Found dipotassium salt of Endothall (all samples):

ppm Found dipotassium salt of Endothall = ppm Found Endothall Free Acid x 1.41

Calculations for % recoveries of fortified control samples were made with the following equation:

% recovery:

$$\% \text{ recovery} = \frac{\text{ppm Found Endothall Free Acid}}{\text{fortification level in ppm}} \times 100$$

B. Example Calculations

1. Water Treated Sample

Sample ID: IS-M2-W-6A
Analysis Date: 22 Dec 94

Standard Equation Derivation:

Standard Concentration	Peak Height
0.30 ng Endothall-HFTH	77149
0.20 ng Endothall-HFTH	47946
0.16 ng Endothall-HFTH	35973
0.040 ng Endothall-HFTH	6625
0.020 ng Endothall-HFTH	3454

Resulting Standard Equation:

$$y = 262{,}954.2285\,x + (-3{,}636.0089)$$
$$r = 0.9981$$

peak height:

peak height = 41457

ng found:

ng found (derived from above curve) = 0.171

mL final volume:

final volume = 50 mL x 50 dilution factor = 2500

µL injected:

$$\mu L \text{ injected} = \frac{100 \text{ mL sample vol} \times 2 \text{ µL vol inj}}{2500 \text{ mL final vol}} \times \frac{1000 \text{ mg}}{\text{g}} \times \frac{\text{mL}}{1000 \text{ µL}} = 0.08$$

ppm Found Endothall Free Acid (water samples):

$$\text{ppm Found Endothall Free Acid} = \frac{0.171 \text{ ng found}}{0.08 \text{ µL injected}} = 2.14$$

ppm Found dipotassium salt of Endothall (all samples):

ppm Found dipotassium salt of Endothall = 2.14 ppm x 1.41 = 3.02

3. Water Fortified Control

Sample ID: Control (R94-3649) + 3.0 ppm
Analysis Date: 20-21 Jan 95

Standard Equation Derivation:

Standard Concentration	Peak Height
0.30 ng Endothall-HFTH	168854
0.20 ng Endothall-HFTH	109171
0.16 ng Endothall-HFTH	82018
0.040 ng Endothall-HFTH	15473
0.020 ng Endothall-HFTH	6532

Resulting Standard Equation:

$$y = 581{,}378.4866\,x + (-7{,}308.9021);\ r = 0.9994$$

peak height:

peak height = 68583

ng found:

ng found (derived from above curve) = 0.131

mL final volume:

final volume = 50 mL x 100 dilution factor = 5000

µL injected:

$$\mu L\ injected = \frac{100\ mL\ sample\ vol \times 2\ \mu L\ vol\ inj}{5000\ mL\ final\ vol} \times \frac{1000\ mg}{g} \times \frac{mL}{1000\ \mu L} = 0.040$$

ppm Found Endothall Free Acid:

$$ppm\ Found\ Endothall\ Free\ Acid = \frac{0.131\ ng\ found}{0.040\ \mu L\ injected} = 3.28$$

ppm Found dipotassium salt of Endothall:

ppm Found dipotassium salt of Endothall = 3.28 ppm x 1.41 = 4.62

% recovery:

$$\%\ recovery = \frac{3.28\ ppm\ Found\ Endothall\ Free\ Acid}{3.0\ ppm\ fortification\ level} \times 100 = 109$$

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Method Modification

Title: Determination of Endothall in Water, PRM-042

Protocol Title: Aquothal K®: An Aquatic Dissipation Study for Aquatic Non-Crop Uses

Elf Atochem Study No.: BR-94-17

Effective Date: 22 Nov 94

Requirement:

1) Section 4.4.1 states, "Transfer the residue to a 15 mL centrifuge tube containing 50 mg HFTH. Transfer using 3 mL of 3N H_3PO_4, then 2 mL of 3N H_3PO_4. Use a pasteur pipet to transfer from the boiling flask to the centrifuge tube."
2) Section 4.5.3 states, "Add approximately 1 mL of MTBE to the remaining layer of MTBE in the aqueous extract and swirl the tube. Transfer the MTBE and combine it with the two 5 mL portions. Discard the aqueous extract. (Note: a small amount of the aqueous layer may transfer into the MTBE, this will be removed in step 4.5.6).
3) Section 4.6.1-8 states,
    1) "Rinse a 5 g amine SPE column with 20 mL of 50:50 (v:v) petroleum ether:MTBE.
    2) As the last of the solvent enters the top of the column, add the 2 mL sample extract to the column. Allow it to elute into the column.
    3) Rinse the tube with 5 mL of 50:50 (v:v) petroleum ether:MTBE and add it to the column.
    4) As the last of the 5 mL rinse enters the top of the column, add an additional 10 mL 50:50 (v:v) petroleum ether:MTBE to the column.
    5) Discard all the solvent to this point.
    6) Elute the endothall-HFTH from the column with 20 mL of 10:90 (v:v) methanol:MTBE. Collect the eluate in a 50 mL centrifuge tube.
    7) Evaporate the extract on the N-evap to dryness.
    8) Dissolve the residue with 2 mL of 50:50 (v:v) petroleum ether:MTBE."
4) Section 4.6.16-18 states,
    16) "As the last of the 15 mL MTBE enters the top of the column add 15 mL of 20:80 (v:v) methanol:MTBE, this is collected in the same 50 mL tube as the 15 mL MTBE.

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17) Evaporate extract to approximately 5 mL and transfer to 15 mL centrifuge tube premarked at 10 mL.

18) Adjust the volume to the 10 mL mark using MTBE. A final volume of 50 mL is required for a detection limit of 0.01 ppm.

Description:

1) Quantitatively transfer the residue with 5 mL of 3N H_3PO_4, using a disposable pasteur pipet to a 15 mL centrifuge tube containing 100 mg HFTH.
2) **OMIT THIS STEP**
3) Amine SPE Column is only necessary if sample extracts need additional clean-up.
4) Elute the endothall-HFTH from the column with 15 mL of MTBE and collect in a 50 mL cylinder. As the last of the 15 mL MTBE enters the top of the column add 15 mL of 20:80 (v:v) methanol:MTBE. This is collected in the same 50 mL cylinder as the 15 mL MTBE. Bring volume to 50 mL, mix well and transfer to 15 mL centrifuge tube to store. Sample extracts should be stored in a refrigerator (0-10°C).

Reason:

1) Depending upon the lot number, additional HFTH is needed to derivatize the samples.
2) This step is not needed. Recoveries are acceptable without this step.
3) Recoveries are acceptable and chromatography is clean without Amine SPE Column.
4) More time efficient and less chance of dilution error to bring final volume to 50 mL.

Effect: These modifications should have no effect on the study.

Approved by:

Study Director — 12-7-94 Date

Serafima Shapiro — 07 Dec 94 Date

McKenzie Laboratories, Inc.