SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

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1.0 SCOPE AND APPLICATION

This analytical procedure applies to the determination of base, neutral, and acid (BNA) compounds in tissue matrices, using a gas chromatograph/mass spectrometer (GC/MS) method. The list of compounds of interest and their quantitation limits that are analyzed and reported by SERAS can be found in Appendix A.

2.0 METHOD SUMMARY

Ten-gram aliquots of a tissue homogenate sample are Soxhlet extracted with methylene chloride solvent. The extract is cleaned-up by Gel Permeation Chromatography (GPC), concentrated to 1 mL, an internal standard mixture added and analyzed by GC/MS. Compounds are identified by comparing their measured mass spectra and retention times to reference spectra and retention times obtained by the measurement of calibration standards under the same conditions used for samples. Quantitation of each identified analyte is calculated by the internal standard method. Appendix B lists the characteristic ions for each target compound and Appendix C lists the internal standards with corresponding target compounds assigned for quantitation.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING AND STORAGE

3.1 Sample Storage

Tissue samples must be protected from light and kept frozen in a freezer from the time of receipt, through homogenization until extraction and analysis.

Tissue samples and sample homogenates must be stored in an atmosphere demonstrated to be free of all potential contaminants.

Before and after analysis, extracts and unused samples must be protected from light. Extracts must be refrigerated at 4°C (± 2°C) and unused samples and homogenates frozen at -10°C, for the periods specified by the Task Leader and/or Work Assignment Manager.

Samples, sample extracts, and standards must be stored separately.

3.2 Holding Times

Extraction of tissue sample homogenates shall be completed within seven (7) days of sampling, and analysis completed within 40 days of sample extraction.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All of these

materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks on a routine basis. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source.

5.0 EQUIPMENT/APPARATUS

The following equipment/apparatus is required:

- Spatula, stainless steel or Teflon (or stainless steel spoons)
- Vials and caps, 2-mL for GC autosampler
- 500-mL Erlenmeyer flasks
- Balance capable of weighing 100 g to the nearest 0.01g
- Analytical balance capable of accurately weighing ± 0.001g
- Disposable Pasteur pipettes (1-mL) and Pyrex glass wool pre-rinsed with hexane
- Test tube rack
- Desiccator
- Beakers, 250-mL
- Zymark Workstation equipped for Gel Permeation Chromatography (GPC)
- 250-mL Zymark collection/concentration tubes
- 50-mL test tubes
- Filter paper, Whatman No. 541 or equivalent
- Soxhlet Extraction System
- Kuderna-Danish (K-D) apparatus consisting of 10-mL graduated concentrator tube, 500-mL evaporative flask, and three-ball macro Snyder column.
- Granular silicon carbide boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

- Water bath - heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

- Nitrogen evaporation device equipped with a water bath that can be maintained at 35-40°C. The N-Evap by Organomation Associations, Inc., South Berlin, MA (or equivalent) is suitable as well as the TurboVap II - Concentration Workstation by Zymark Corp., Hopkinton, MA.

- Hewlett-Packard (HP) 5995 GC/MS; equipped with a 7673 autosampler and controlled by an HP-1000 RTE-6/VM computer system.

- Restek Rtx-5 (cross bonded SE-54) column; 30 meter x 0.32 mm ID; 0.5 µm film thickness.

6.0 REAGENTS

1. Sodium Sulfate - anhydrous powdered reagent grade, heated at 400°C for four hours, cooled in a desiccator, and stored in a glass bottle.

2. Dichloromethane pesticide residue analysis grade or equivalent.

3. Base/Neutral and Acid Surrogate Spiking Solution:

   Surrogate standards are added to all samples and calibration solutions. The compounds specified are listed below. Store the spiking solutions at 4°C (± 2°C) in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after twelve months, or sooner if comparison with quality control check samples indicates a problem.

Bases/Neutrals		Acids	
Nitrobenzene-d_5	100 µg/mL	Phenol-d_6	200 µg/mL
2-Fluorobiphenyl	100 µg/mL	2-Fluorophenol	200 µg/mL
Terphenyl-d_{14}	100 µg/mL	2,4,6-Tribromophenol	200 µg/mL

4. Base/Neutral and Acid Matrix Spiking Solution:

   Prepare a spiking solution in methanol that contains the following compounds at a concentration of 100 µg/mL for base/neutrals and 200 µg/mL for acids. Store the spiking solutions at 4°C (± 2°C) in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after 6 months, or sooner if comparison with quality control check samples indicates a problem.

Base/Neutrals	Acids
1,2,4-Trichlorobenzene	Pentachlorophenol

Acenaphthene	Phenol
2,4-Dinitrotoluene	2-Chlorophenol
Pyrene	4-Chloro-3-methylphenol
N-Nitroso-di-n-propylamine	4-Nitrophenol
1,4-Dichlorobenzene	

5. Internal standards - 1,4-Dichlorobenzene-d_4, Naphthalene-d_8, Acenaphthene-d_{10}, Phenanthrene-d_{10}, Chrysene-d_{12}, Perylene-d_{12}.

   An internal standard solution mixture (2000 µg/mL), commercially available can be used. If necessary an internal standard solution can be prepared by dissolving 100 mg of each compound in 50 mL of methylene chloride. It may be necessary to use 5 to 10 percent benzene or toluene in this solution and a few minutes of ultrasonic mixing to dissolve all the constituents.

   The resulting solution will contain each standard at a concentration of 2000 ng/µL. Store at 4 °C or below when not being used. A 20-µL portion of this solution should be added to each 1 mL of sample extract. This will result in 40 ng of each internal standard in the 1-µL volume of extract injected into the GC/MS.

6. Calibration Standards:

   Prepare calibration standards at a minimum of six concentration levels (10, 20, 50, 80, 120 and 160 µg/mL). Each calibration standard should contain each compound of interest and each surrogate. Eight compounds, 2,4-Dinitrophenol, 2,4,5-Trichlorophenol, 2-Nitroaniline, 3-Nitroaniline, 4-Nitroaniline, 4-Nitrophenol, 4,6-Dinitro-2-methylphenol, and Pentachlorophenol will require only a five-point initial calibration at 20, 50, 80, 120, and 160 total ng, since detection at less than 20 ng per injection is difficult. Great care must be taken to maintain the integrity of all standard solutions. Store all standard solutions at 0 °C to -10 °C in screw-cap amber bottles with Teflon liners. Fresh stock standards should be prepared every six months at a minimum. The continuing calibration standard (50 ng) should be prepared weekly and stored at 4 °C (± 2 °C).

7. Decafluorotriphenylphosphine (DFTPP) - prepare DFTPP solution such that a 1-µL injection will contain 50 ng of DFTPP.

8. Nitrogen - ultra high purity (UHP), grade or equivalent (for evaporator).

7.0 PROCEDURES

Tissue samples must be homogenized before pursuing the following steps. See method for tissue sample homogenization (SERAS SOP #1820, Tissue Homogenization Procedure).

7.1 Sample Preparation and Extraction

1. Open the homogenate sample container in a fume hood. Mix the sample thoroughly.

2. Weigh 10 ± 0.01 g aliquot of homogenized tissue sample into a 250-mL beaker, add up to 120 g anhydrous Na_2SO_4 and mix the tissue sample and Na_2SO_4 thoroughly with a stainless steel (SS) spatula or SS spoon. The sample should have a sandy texture at this point.

3. Determine the total percent solid by following the procedure outlined in Section 7.4.

4. Prepare a method blank by using 120 g Na_2SO_4. A method blank must be prepared every 20 samples.

5. Prepare a matrix spike (MS) and a matrix spike duplicate (MSD) by weighing two additional 10 ± 0.01 g aliquots of homogenized tissue sample that was chosen for that purpose. Add 120 g anhydrous Na_2SO_4 to MS and MSD and mix thoroughly with SS spatula. The MS and MSD should have a sandy texture.

6. Transfer the blank, MS and MSD, and tissue samples quantitatively to precleaned soxhlet thimbles for extraction.

7. Place thimbles into soxhlet extractors;

   - add 1-mL of surrogate spike solution to the method blank, the MS and MSD, and all the samples;

   - add 1-mL of BNA matrix spike solution to each of the MS and MSD samples.

8. Add 250-mL dichloromethane (DCM) and two boiling chips to each round bottom flask extractors.

9. Connect water cooled condenser and extract for 17 to 24 hours (ca. 60-90 cycles). After the extraction is complete, concentrate the extract using Step 7.2 (TurboVap II) or Step 7.3 (Kuderna-Danish).

7.2 TurboVap II Concentrator Workstation

1. Allow the system to cool and filter the entire sample extract into a 250-mL Zymark concentration tube through a #541 Whatman filter paper with anhydrous Na_2SO_4 packed funnel. Rinse the round bottom flask with three 10-mL portions of dichloromethane (DCM). Pour the rinsate through the anhydrous Na_2SO_4 packed funnel and combine the rinsate with the extract.

2. Set the TurboVap II water bath temperature to 45 °C. Make sure that the water is at the proper level in the bath. Connect a cylinder of ultra high purity (UHP) nitrogen through a two-stage

regulator set at 20 psig.

3. Place the concentration tubes in the workstation openings and begin evaporating with a gentle stream of UHP nitrogen. As the evaporation process progresses, the nitrogen pressure may be increased, however, any splashing of the extract should be avoided. Periodically (2-3 times) during the concentration, rinse the walls of the tube with DCM.

4. Evaporate the extract to less than 10-mL volume (5-7 mL range). Remove the concentration tube from the TurboVap II.

5. Transfer the extract to a clean (pre-rinsed with DCM) 12-mL culture tube using a clean disposable pipette. Rinse the concentration tube with two 1-mL portions of DCM and transfer to the culture tube. Keep the total volume of the transferred extract to 10 mL or less.

6. Adjust the final volume in the culture tube to 10-mL with DCM. Then proceed with Step 7.4.

7.3 Kuderna-Danish Concentration

1. Allow the system to cool and filter entire sample extract into a 500-mL Erlenmeyer flask through a #541 Whatman filter paper with anhydrous Na_2SO_4 packed funnel. Rinse the round bottom flask with three 10-mL portions of DCM. Pour the rinsate through the anhydrous Na_2SO_4 packed funnel and combine the rinsate with sample extracts. The sample extract is ready for concentration.

2. Assemble a Kuderna-Danish (K-D) apparatus by attaching a 10-mL concentrator tube to a 500-mL evaporative flask.

3. Transfer the extract into a K-D concentrator flask; rinse the Erlenmeyer flask with 60 - 100 mL of methylene chloride to complete the quantitative transfer.

4. Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column.

5. Pre-wet the Snyder column by adding 2 - 3 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (80 – 90^{o}C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor.

6. Concentrate the extract down to less than 10 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

7. Remove the Snyder column and rinse the flask and its lower joints into the concentrator tube with 1 - 2 mL of methylene chloride.

8. Disconnect the concentrator tube and place it on N-Evap with pre-warmed water bath (35 °C). Evaporate the extract to final volume of 10 mL with a gentle stream of clean, dry nitrogen.

7.4 Total Solids

Immediately after extracting samples, weigh 3-5 g of the homogenate tissue sample into a tared aluminum dish. Determine the total percent solid by drying in oven placed inside fume hood overnight at 105°C. Before weighing, allow samples to cool in a desiccator.

Concentrations of individual analytes will be reported relative to the dry weight of the homogenate tissue sample. Calculate the total percent solid using the following equation:

$$Percent\ Total\ Solids = \frac{Weight\ of\ Dry\ Sample\ (g)}{Weight\ of\ Sample\ Before\ Drying}\ x\ 100$$

7.5 Lipid Determination

1. Transfer 1 mL of the 10 mL extract (Section 7.2, Step 6 or Section 7.3, Step 8) into a preweighed 7.8 g (2 dram) vial and evaporate to dryness overnight.

2. Calculate the percent lipid content using the following equation:

$$\%\ Lipid = \frac{W_E \times \ _E}{V_S \times \ _S \times \frac{\%\ solids}{100}} \times\ 00$$

where,

W_E = weight of residue after solvent evaporation, in grams
W_S = weight of sample extracted, in grams
V_E = final volume of extract in mL
V_S = volume of extract used to evaporate in mL

3. Use remaining 9-mL extract for GPC cleanup.

7.6 Gel Permeation Chromatography (GPC) Extract Cleanup

GPC clean-up is required to separate the analytes from biological macromolecules (Lipids).

1. Transfer 5-mL extract onto the GPC column using the Zymark.

2. Collect the fraction of extract eluting just after the lipid elution and before the sulfur elution [as determined by injecting a GPC calibration mixture comprised of corn oil, bis-(2-Ethylhexyl)phthalate, methoxychlor, perylene and sulfur] in a 200-mL collecting flask.

3. Transfer the clean extract quantitatively to either the TurboVap II system or K-D device and concentrate to 1-mL final extract volume. Quantitatively transfer the 1-mL extract to a 2-mL vial for analysis.

4. Analyze the 1-mL extract using GC/MS (see Section 7.7).

7.7 GC/MS Condition

The conditions listed below are used for standards and sample analysis.

Column	Restek Rtx-5 (crossbonded SE-54) 30 meter x 0.32 mm ID, 0.50 um film thickness
Injector Temperature	290°C
Transfer Line Temperature	290 °C
Source Temperature	240 °C
Analyzer Temperature	240 °C
Temperature Program	30 °C for 3 min 15 °C /min to 70 °C hold for 0.2 min 8 °C /min to 295 °C hold for 15 min
Splitless Injection	Split time = 60 sec
Injection Volume	1 µL

7.8 Tune (DFTPP)

The instrument must be tuned to meet the ion abundance criteria listed in Appendix D for a 50 ng (1 µl) injection of DFTPP. This criteria must be demonstrated every 12 hours during analysis.

7.9 Initial Calibration

1. Add 20 µL of 2000 µg/mL internal standard solution to each 1-mL aliquot of calibration standards.

2. Inject 1 µL each of the calibration standards after a successful DFTPP injection.

3. Calculate and tabulate the relative response factor (RRF) against the concentration for each compound, including the surrogates, by using the equation listed below. The primary ion from the specific internal standard must be used for quantitation.

$$RRF = \frac{(A_x)(C_{is})}{(A_{is})(C_x)}$$

where,

A_x = Area of the characteristic ion for the compound to be measured
A_{is} = Area of the characteristic ion for the specific internal standard from Appendix B.
C_{is} = Concentration of the internal standard (ng/µL)
C_x = Concentration of the compound to be measured (ng/µL)

The percent relative standard deviation (%RSD) of the RRF for each analyte must be less than or equal to 30%. The average RRF of each compound must not be less than 0.05.

The average RRF and percent relative standard deviation (%RSD) must also be calculated and tabulated on a per compound basis as follows:

$$RRF = \frac{\sum_{=1}^{n} RF_i}{n}$$

where,

RRF_i = relative response factor for each initial calibration level
n = total number of initial calibration levels

$$\%RSD = \frac{SD}{\bar{X}} x\,100$$

where,

$$SD = \sqrt{\frac{\sum_{i=}^{n} (RRF_i - \overline{RRF_i})^2}{n -}}$$

Std. Dev. = standard deviation of n values of RRF (as calculated below)where, RRF_i, RRF and n are defined as above.

7.10 Continuing Calibration

A check of the initial calibration curve must be performed every 12 hours during analysis.

1. Inject 1 µL of a 50 µg/mL standard containing internal standards.

2. Calculate and tabulate the continuing RRF for each compound. All continuing RRF must be equal to or greater than 0.05.

3. Calculate the percent difference (%D) of each continuing RRF compared to the average RRF from the initial calibration curve. The %D for all compounds can be calculated using the equation listed below and must be less than or equal to 25%.

$$\%D = \frac{\left| RRF_C - \overline{RRF} \right|}{RRF_C} \times 100$$

where,

RRF_c = relative response factor of the continuing calibration
RRF = average relative response factor from initial calibration

4. Reanalyze initial calibration standards if any of the following compounds failed the minimum RRF_c (0.05) requirement: N-nitroso-di-n-propylamine, hexachlorocyclopentadiene, 2,4-dinitrophenol, and 4-nitrophenol.

5. Reanalyze initial calibration standards if any of the following compounds failed the %D requirement: phenol, 1,4-dichlorobenzene, 2-nitrophenol, 2,4-dichlorophenol, hexachlorobutadiene, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol, acenaphthene, N-nitrosodiphenylamine, pentachlorophenol, fluoranthene, di-n-octylphthalate, and benzo(a)pyrene.

7.11 Sample Analysis

Sample extracts may be analyzed only after the GC/MS system has met the DFTPP, initial calibration, and continuing calibration requirements mentioned above. The same instrument conditions must be employed for the analysis of samples as were used for calibration.

1. Add 20 µL of the 2000 µg/mL internal standard solution into the method blank, the MS/MSD, and all the sample extracts.

2. Inject 1 µL each of the method blank, the MS/MSD, and all the sample extracts.

3. If the analyst has reason to believe that diluting the final extracts will be necessary, an undiluted run may not be required.

4. If analytes are detected at a level greater than the highest calibration standard, sample extracts

must be diluted so that the analyte response is within the linear range established during calibration.

5. If dilutions of sample extracts are made, additional internal standards must be added to maintain the required concentration (40 ng/µL) of each internal standard in the extract.

7.12 Identification of Target Compounds

Target compound identification will be conducted by comparison of the sample mass spectrum to the mass spectrum of a standard of the suspected compound. Two criteria must be satisfied to verify the identifications:

- Elution of the sample component at the GC relative retention time as the standard component
- Correspondence of the sample component and standard component mass spectra

1. For establishing correspondence of the GC relative retention time (RRT), the sample component RRT must compare within ± 0.06 RRT units of the RRT of the standard component. For reference, the standard must be run on the same shift as the sample. If coelution of interfering components prohibits accurate assignment of the sample component RRT from the total ion chromatogram, the RRT should be assigned by using extracted ion current profiles for ions unique to the component of interest.

2. For comparison of standard and sample component mass spectra, reference mass spectra must be obtained from the 50 µg/mL continuing calibration. These standard spectra may be obtained from the run used to obtain reference RRTs. In the case of co-elution of standard spectra component, reference spectra from the National Bureau of Standards (NBS) Mass Spectral Library should be used to establish the presence of compounds of interest.

3. The requirements for qualitative verification by comparison of mass spectra are as follows:

   a. All ions present in the standard mass spectra at a relative intensity greater than 10% (most abundant ion in the spectrum equals 100%) *must* be present in the sample spectrum.

   b. The relative intensities of ions specified in (a) must agree within ± 20% between the standard and sample spectra. (For example: for an ion with an abundance of 50% in the standard spectra, the corresponding sample ion abundance must be between 30 and 70 percent.)

   c. Ions greater than 10% in the *sample* spectrum but not present in the *standard* spectrum must be considered and accounted for by the analyst making the comparison.

All compounds meeting the identification criteria must be reported with their spectra. For all compounds below the quantitation limit, report the actual value followed by "J", e.g., "3J".

4. If a compound cannot be verified by all of the criteria in step 3, but in the technical judgment of the mass spectral interpretation specialist, the identification is correct, then the analyst shall report that identification and proceed with the calculation in Section 8.0. The analyst should note in the case narrative that technical judgment was utilized.

7.13 Library Search

A library search shall be executed for non-target compounds present in the method blank and the sample for the purpose of tentative identification. For this purpose, the 1985 release of the National Bureau of Standards (NBS) Mass Spectral Library (or more recent release), containing 42,261 spectra, will be used.

1. Any non-surrogate organic compounds not listed in Appendix A for the combined base/neutral/acid fraction shall be tentatively identified via a forward search of the NBS mass spectral library. (Substances with responses less than 10% of the nearest internal standard are not required to be searched in this fashion.) Only after visual comparison of sample spectra with the nearest library searches will the mass spectral interpretation specialist assign a tentative identification. Analyst should be careful not to report a volatile (VOA) target compound in such practice if the VOA analysis is also requested.

   NOTE: Computer generated library search routines must not use normalization routines that would misrepresent the library or unknown spectra when compared to each other.

2. Guidelines for making tentative identification:

   - Relative intensities of major ions in the reference spectrum (ions greater than 10% of the most abundant ion) should be present in the sample spectrum.
   - The relative intensities of the major ions should agree within ± 20%. (For example: for an ion with an abundance of 50% in the standard spectra, the corresponding sample ion abundance must be between 30 and 70 percent.)
   - Molecular ions present in reference spectrum should be present in sample spectrum.
   - Ions present in the sample spectrum but not in the reference spectrum should be reviewed for possible background contamination or presence of co-eluting compounds.
   - Ions present in the reference spectrum but not in the sample spectrum should be

reviewed for possible subtraction from the sample spectrum because of background contamination or co-eluting compounds.

NOTE: Data system library reduction programs can sometimes create these discrepancies.

3. If in the technical judgment of the mass spectral interpretation specialist, no valid tentative identification can be made, the compound should be reported as *unknown*. The mass spectral specialist should give additional classification of the unknown compound, if possible (i.e., unknown phthalate, unknown hydrocarbon, unknown acid type and unknown chlorinated compound). If probable molecular weights can be distinguished, include them.

8.0 CALCULATIONS

8.1 Target Compound

Identified target compounds must be quantitated by the internal standard method. The internal standard used must be the one nearest the retention time to that of a given analyte (see Appendix C). The extracted ion current profile (EICP) area of characteristic ions of analytes listed in Appendix B is used for quantitation.

Calculate the concentration in the sample using the relative response factor (RRF) obtained from the continuing calibration standard as determined in Section 7.10 and the equation listed below. If samples are analyzed under the initial calibration curve, the average RRF must be used.

$$Concentration(\mu g/kg) = \frac{(A_x)(I_s)(V_t)(DF)}{(A_{is})(RRF)(W)(V_i)(S)}$$

where,

A_x = Area of the characteristic ion for the compound to be measured
A_{is} = Area of the characteristic ion for the internal standard
I_s = Amount of internal standard injected (ng)
W = Weight of soil/sediment extracted (kg)
V_i = Volume of extract injected (µL)
V_t = Volume of the concentrated extract (mL)
DF = Dilution Factor
S = Decimal percent solid

When the target compound concentrations are below the quantitation limits but the spectrum meets the identification criteria, report the concentration as estimated by flagging the results with a "J".

8.2 Tentatively Identified Compounds (TICs)

An estimated concentration for tentatively identified compounds (TICs) must be calculated by the internal standard method. The nearest internal standard free of interferences must be used. The equation for calculating concentration is the same as in Section 8.1. Total area counts or peak heights from the total ion chromatograms are to be used for both the compound to be measured and the internal standard. An RRF of one (1) is to be assumed.

8.3 Surrogate Spike Recoveries

Calculate surrogate standard recovery on all samples, blanks, and spikes by using the equation listed below.

$$Percent\ Recovery\ (\%R) = \frac{Q_D}{Q_A}\ x\ 100$$

where,
Q_d = Quantity determined by analysis
Q_a = Quantity added to sample

8.4 Matrix Spike Recoveries

The percent recoveries and the relative percent difference (RPD) between the recoveries of each of the 11 compounds in the matrix spike samples will be calculated and reported by using the following equations:

$$Matrix\ Spike Recovery(\%R) = \frac{SSR - SR}{SA}\ x\ 100$$

where,
SSR = Spike sample result
SR = Sample result
SA = Spike added

$$RPD = \frac{|MSR - MSDR|}{(MSR + MSDR)/2}\ x\ 100$$

where,
RPD = Relative percent difference
MSR = Matrix spike recovery
MSDR = Matrix spike duplicate recovery

The vertical bars in the formula above indicate the absolute value of the difference; hence RPD is always expressed as a positive value.

9.0 QUALITY ASSURANCE/ QUALITY CONTROL

9.1 Tune (DFTPP)

Prior to initiating any data collection activities involving samples, blanks, or standards, it is necessary to establish that a given GC/MS system meets the instrument tune criteria specified in Appendix D. The purpose of this instrument check is to assure correct mass calibration, mass resolution, and mass transmission. This is accomplished through the analysis of DFTPP.

1. The analysis of DFTPP must be performed every 12 hours during the analysis.

2. The key ions produced during the analysis of DFTPP and their respective ion abundance criteria are given in Appendix D.

9.2 Initial Calibration for Target Compounds and Surrogates

Prior to the analysis of samples and required blanks, and after instrument performance criteria have been met, the GC/MS system must be initially calibrated at a minimum of six concentrations to determine the linearity of response utilizing target compound and surrogate standards.

1. The levels of the initial calibration standards for semi-volatile target compounds and surrogates are 10, 20, 50, 80, 120, and 160 µg/mL. Eight compounds: 2,4-dinitrophenol, 2,4,5-trichlorophenol, 2-nitroaniline, 3-nitroaniline, 4-nitroaniline, 4-nitrophenol, 4,6-dinitro-2-methylphenol, and pentachlorophenol will only require a four-point initial calibration at 50, 80, 120, and 160 µg/mL since detection at less than or equal to 20 µg/mL is difficult.

2. The calibration of the GC/MS is evaluated on the basis of the magnitude and stability of the relative response factors of each target compound and surrogate. The minimum RRF of each compound at each concentration level in the initial calibration across all five points must be equal to or greater than 0.05; the %RSD must not exceed 30%.

9.3 Continuing Calibration for Target Compounds and Surrogates

Once the GC/MS system has been calibrated, the calibration must be verified each 12-hour time period for each GC/MS system during the analysis.

1. The level of the continuing calibration standard for target compounds and surrogates is 50 µg/mL.

2. The standard is to be analyzed every 12 hours after an acceptable DFTPP analysis.

3. The continuing calibration of the GC/MS system is evaluated on the basis of the magnitude of the relative response factors and the percent difference between the *average* RRF of each

compound from the initial calibration and the RRF of that compound in the continuing calibration standard. The minimum RRF of each compound in the continuing calibration must be greater than or equal to 0.05. The %D must not exceed 25%.

4. If any of the requirements listed in Item 3 are not met, a new initial calibration must be analyzed.

9.4 Internal Standard Responses and Retention Times

The response of each of the internal standards in all calibration standards, samples, and blanks is crucial to the provision of reliable analytical results because the quantitative determination of semi-volatile compounds by these procedures is based on the use of internal standards added immediately prior to analysis.

1. The specific compounds used as internal standards are given in Section 6.0, paragraph 5. The amount of each internal standard in the injection volume (1 µL) of the sample extract analyzed by GC/MS must be 40 ng (40µg/mL).

2. The area response of each internal standard from the EICP and the retention time of the internal standard are evaluated for stability. The area of the internal standard in a sample must not vary by more than a factor of 2 (i.e., -50% to +100%) from the area of the same internal standard in the associated continuing calibration standard. Likewise, the retention time of an internal standard must be within ± 0.50 minutes (30 seconds) of its retention time in the continuing calibration standard.

3. If samples are analyzed under the initial calibration, the area of the 50 µg/mL standard must be used for monitoring.

4. The area response of each internal standard in all samples, blanks and spikes must be tabulated. If it is outside the QC limits, no action needs to be taken at this point. However, all internal standards must be present to avoid the reanalysis.

9.5 Method Blank Analysis

A method blank is a weight of a clean reference matrix (pure anhydrous Na_2SO_4) that is carried through the entire analytical procedure. The weight of the reference matrix must be approximately equal to the weight of samples associated with the blank. The purpose of a method blank is to determine the levels of contamination associated with the processing and analysis of samples.

1. The method blank must be prepared for each batch not exceeding 20 samples.

2. A method blank must contain less than or equal to five times (5x) the QL of the phthalate esters listed in Appendix A. For all other target compounds, the method blank must contain less than

or equal to the QL of any single target compound.

3. If a method blank exceeds the limits for contamination above, the analyst must consider the analytical system out of control. The source of the contamination must be investigated and appropriate corrective actions taken and documented before further sample analysis proceeds. All samples processed with a contaminated method blank must be re-extracted and reanalyzed.

9.6 Surrogate Recoveries

The recoveries of the six surrogates are calculated from the analysis of each sample, blank, matrix spike and matrix spike duplicate. The purpose of the surrogates is to evaluate the preparation and analysis of samples.

1. The surrogates are added to each sample, blank, matrix spike, and matrix spike duplicate prior to extraction, at the concentrations described in Sections 6.0 and 7.1.

2. The recoveries of the surrogates are calculated according to the equation in Section 8.3.

3. The recoveries must be within the quality control limits given below.

Compound	% Recovery
Nitrobenzene-d_5	23 - 120
2-Fluorobiphenyl	30 - 115
Terphenyl-d_{14}	18 - 137
Phenol-d_6	24 - 113
2-Fluorophenol	5 - 121
2,4,6-Tribromophenol	19 - 122

4. If two base/neutral or two acid surrogates are out of QC limits *OR* if recovery of any *one* base/neutral or acid surrogate is below 10%, the following actions are required:

   a. Check to be sure that there are no errors in calculations, surrogate solutions, and internal standards. Also check that the quantitation ions of internal standards and surrogates are properly integrated.

   b. Reanalyze the sample if none of the above reveal a problem. If a blank does not meet the specification, it may be reanalyzed alone.

   c. Do not reanalyze dilutions if surrogate recoveries are outside the limits.

   d. If the sample associated with the matrix spike and matrix spike duplicate does *not*

meet specifications, it should be reanalyzed only if the MS/MSD surrogate recoveries are within the limits. If the sample and associated MS/MSD show the same pattern (i.e., outside the limits), then the sample does *not* require reanalysis and a reanalysis must not be submitted. Document in the narrative the similarity in surrogate recoveries.

5. If the reanalysis of the sample solves the problem, then the problem was within the laboratory's control. Therefore, submit *only* data from the analysis with surrogate spike recoveries *within* the QC limits. This shall be considered the *initial* analysis and shall be reported as such on all data deliverables. If the reanalysis is outside the analysis holding time, provide the data from both analyses.

6. If none of the steps mentioned above solves the problem, then, except as noted below, *re-extract* and *reanalyze* the sample. If the re-extraction and reanalysis of the sample solves the problem, submit *only* data from the analysis with surrogate recoveries *within* the QC limits. This shall be considered the *initial* analysis and shall be reported as such on all data deliverables. If the re-extraction is outside the holding time, provide the data from both analyses.

   a. If surrogate recoveries in a blank do not meet specifications even after reanalysis, *all* of the samples associated with that blank must be re-extracted along with the blank. The blank is intended to detect contamination in samples processed *at the same time*.

   b. Do not re-extract diluted samples if surrogate recoveries are outside the limits.

   c. Never re-extract the MS/MSD, even if surrogate recoveries are outside the limits.

   d. If the sample associated with the MS/MSD does not meet specifications after reanalysis, it should be re-extracted only if the reanalysis surrogate recoveries are not within the limits and MS/MSD surrogate recoveries are within the limits. If the sample and associated MS/MSD show the same pattern (i.e., outside the limits), then the sample does not require reanalysis and a reanalysis must not be submitted. Document in the narrative the similarity in surrogate recoveries.

7. If the re-extraction and reanalysis of the sample does not solve the problem (i.e., the surrogate recoveries are outside the QC limits for both analyses), then submit the surrogate recovery data and sample analysis data from the initial analysis of *both* sample extracts (e.g., the first analysis of both extracts of the sample). Distinguish between the initial analysis and the analysis of the re-extracted sample on all data deliverables.

9.7 Matrix Spike and Matrix Spike Duplicate Analysis

The purpose of spiking target compounds into two aliquots of a sample is to evaluate the effects of the

sample matrix on the methods used.

1. The MS/MSD must be prepared every 10 samples per matrix within each project.

2. The mixture of the spike solution specified in Section 6.0 must be used to result in the concentration specified.

3. The recoveries of the matrix spike compounds are calculated according to the equation in Section 8.4, The relative percent difference between the results for each spiked analyte of the matrix spike and the matrix spike duplicate is calculated according to the equation in Section 8.4.

4. The quality control limits for recovery and relative percent difference are given below. These limits are only advisory at this time, and no further action is required when the limits are exceeded.

Compound	% Recovery	RPD
Phenol	26 - 90	35
2-Chlorophenol	25 - 102	50
1,4-Dichlorobenzene	28 - 104	27
N-Nitroso-di-n-propylamine	41 - 126	38
1,2,4-Trichlorobenzene	38 - 107	23
4-Chloro-3-methylphenol	26 - 103	33
Acenaphthene	31 - 137	19
4-Nitrophenol	11 - 114	50
2,4-Dinitrotoluene	28 - 89	47
Pentachlorophenol	17 - 109	47
Pyrene	35 - 142	36

9.8 Dilution Analysis

If the concentration of any sample extract exceeds the initial calibration range, that sample extract must be diluted and reanalyzed as described in Section 7.11, steps 4 and 5.

1. Use the results of the original analysis to determine the approximate dilution factor required to get the largest analyte peak within the initial calibration range.

2. The dilution factor chosen should keep the response of the largest analyte peak for a *target compound* in the upper half of the initial calibration range of the instrument.

3. Do *not* submit data for more than two analyses, i.e., the original sample and *one* dilution, or, if

the semi-volatile screening procedure was employed, from the most concentrated dilution analyzed and one further dilution.

10.0 DATA VALIDATION

Data validation will be performed by the Data Validation and Report Writing Group and therefore it is not applicable to this method. However, data is considered satisfactory for submission purposes when *ALL* the requirements mentioned below are met.

1. All samples must be analyzed under an acceptable tune, initial calibration, and continuing calibration check at the required frequency.

2. All the QC requirements described in Section 9.0 must be met at all times.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, refer to U.S. EPA, OSHA and corporate health and safety practices. More specifically, refer to ERT/SERAS SOP #3013, SERAS Laboratory Safety Program.

12.0 REFERENCES

Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Test Methods for Evaluating Solid Waste, Third Edition, SW-846, September 1986.

U.S. EPA Contract Laboratory Program (CLP), Statement of Work for Organic Analysis, Revision 2/88.

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U.S. EPA Bioaccumulation Monitoring Guidance: Analytical Methods for USEPA Priority Pollutants and 301(h) Pesticides in Tissues from Estuarine and Marine Organisms (May, 1988). Prepared by Tetra Tech, Inc.

APPENDIX A
Target Compound List and Quantitation Limits
SOP #1821
November 1994

SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

Target Compound List and Quantitation Limits[1]

COMPOUND	QL[2] (mg/kg)
Phenol	1.0
bis(2-Chloroethyl)ether	1.0
2-Chlorophenol	1.0
1,3-Dichlorobenzene	1.0
1,4-Dichlorobenzene	1.0
Benzyl alcohol	1.0
1,2-Dichlorobenzene	1.0
2-Methylphenol	1.0
bis(2-Chloroisopropyl)ether	1.0
4-Methylphenol	1.0
N-Nitroso-Di-n-propylamine	1.0
Hexachloroethane	1.0
Nitrobenzene	1.0
Isophorone	1.0
2-Nitrophenol	1.0
2,4-Dimethylphenol	1.0
bis(2-Chloroethoxy)methane	1.0
2,4-Dichlorophenol	1.0
1,2,4-Trichlorobenzene	1.0
Naphthalene	1.0
4-Chloroaniline	1.0
Hexachlorobutadiene	1.0
4-Chloro-3-methylphenol	1.0
2-Methylnaphthalene	1.0
Hexachlorocyclopentadiene	1.0
2,4,6-Trichlorophenol	1.0
2,4,5-Trichlorophenol	2.0
2-Chloronaphthalene	1.0
2-Nitroanaline	2.0
Dimethylphthalate	1.0
Acenaphthylene	1.0
3-Nitroanaline	2.0
Acenaphthene	1.0
2,4-Dinitrophenol	2.0

[1]On a wet-weight basis
[2]QL denotes Quantitation Limits

SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

Target Compound List and Quantitation Limits[1] (cont)

COMPOUND	QL[2] (mg/kg)
4-Nitrophenol	2.0
Dibenzofuran	1.0
2,6-Dinitrotoluene	1.0
2,4-Dinitrotoluene	1.0
Diethylphthalate	1.0
4-Chlorophenyl-phenylether	1.0
Fluorene	1.0
4-Nitroanaline	2.0
4,6-Dinitro-2-methylphenol	2.0
N-Nitrosodiphenylamine	1.0
4-Bromophenyl-phenylether	1.0
Hexachlorobenzene	1.0
Pentachlorophenol	2.0
Phenanthrene	1.0
Anthracene	1.0
Carbazole	1.0
Di-n-butylphthalate	1.0
Fluoranthene	1.0
Pyrene	1.0
Butylbenzylphthalate	1.0
3,3'-Dichlorobenzidine	13.5
Benzo(a)anthracene	1.0
Bis(2-Ethylhexyl)phthalate	1.0
Chrysene	1.0
Di-n-octylphthalate	1.0
Benzo(b)fluoranthene	1.0
Benzo(k)fluoranthene	1.0
Benzo(a)pyrene	1.0
Indeno(1,2,3-cd)pyrene	1.0
Dibenzo(a,h)anthracene	1.0
Benzo(g,h,i)perylene	1.0

[1]On a wet-weight basis
[2]QL denotes Quantitation Limits

APPENDIX B
Characteristic Ions for Target Compounds and Surrogates
SOP #1821
November 1994

SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

Characteristic Ions for Target Compounds and Surrogates

Parameter	Primary Ion	Secondary Ion(s)
1,4-Dichlorobenzene-d_4 (ISTD)[(1)]	152	115
Phenol	94	65, 66
bis(2-Chloroethyl)ether	93	63, 95
2-Chlorophenol	128	64, 130
1,3-Dichlorobenzene	146	148, 113
1,4-Dichlorobenzene	146	148, 113
1,2-Dichlorobenzene	146	148, 113
2-Methylphenol	108	107
Benzyl alcohol	79	77, 108
bis(2-Chloroisopropyl)ether	45	39, 121
4-Methylphenol	108	107
N-Nitroso-di-n-propylamine	70	42, 101, 130
Hexachloroethane	117	201, 199
Naphthalene-d_8 (ISTD)	136	68
Nitrobenzene	77	123, 65
Isophorone	82	95, 138
2-Nitrophenol	139	65, 109
2,4-Dimethylphenol	107	121, 122
bis(2-Chloroethoxy)methane	93	95, 123
2,4-Dichlorophenol	162	164, 98
1,2,4-Trichlorobenzene	180	182, 145
Naphthalene	128	129, 127
4-Chloroaniline	127	129
Hexachlorobutadiene	225	223, 227
4-Chloro-3-methylphenol	107	144, 142
2-Methylnaphthalene	142	141
Acenaphthene-d_{10} (ISTD)	164	160, 162
Hexachlorocyclopentadiene	237	235, 272
2,4,6-Trichlorophenol	196	198, 200
2,4,5-Trichlorophenol	196	198, 200
2-Chloronaphthalene	162	164, 127
2-Nitroanaline	65	92, 138
Dimethylphthalate	163	194, 164
Acenaphthylene	152	151, 153
3-Nitroanaline	138	108, 92
Acenaphthene	153	152, 154

[(1)]ISTD denotes Internal Standard

Characteristic Ions for Target Compounds and Surrogates (cont)

Parameter	Primary Ion	Secondary Ion(s)
2,4-Dinitrophenol	184	63, 154
4-Nitrophenol	109	139, 65
Dibenzofuran	168	139
2,4-Dinitrotoluene	165	63, 182
2,6-Dinitrotoluene	165	89, 121
Diethylphthalate	149	177, 150
4-Chlorophenyl-phenylether	204	206, 141
Fluorene	166	165, 167
4-Nitroanaline	138	92, 108
Phenanthrene-d_{10} (ISTD)	188	94, 80
4,6-Dinitro-2-methylphenol	198	182, 77
N-Nitrosodiphenylamine	169	168, 167
4-Bromophenyl-phenylether	248	250, 141
Hexachlorobenzene	284	142, 249
Pentachlorophenol	266	164, 268
Phenanthrene	178	179, 176
Anthracene	178	179, 176
Carbazole	167	166, 139
Di-n-butylphthalate	49	150, 104
Fluoranthene	202	101, 100
Chrysene-d_{12} (ISTD)	240	120, 236
Pyrene	202	101, 100
Butylbenzylphthalate	149	91, 206
3,3'-Dichlorobenzidine	252	254, 126
Benzo(a)anthracene	228	229, 226
Bis(2-Ethylhexyl)phthalate	149	167, 279
Chrysene	228	226, 229
Perylene-d_{12} (ISTD)	264	260, 265
Di-n-octylphthalate	149	
Benzo(b)fluoranthene	252	253, 125
Benzo(k)fluoranthene	252	253, 125
Benzo(a)pyrene	252	253, 125
Indeno(1,2,3-cd)pyrene	276	138, 227
Dibenzo(a,h)anthracene	278	139, 279
Benzo(g,h,i)perylene	276	138, 277

(1)ISTD denotes Internal Standard

Characteristic Ions for Target Compounds and Surrogates (cont)

Parameter	Primary Ion	Secondary Ion(s)
SURROGATES		
Phenol-d_6	99	42, 71
2-Fluorophenol	112	64
2,4,6-Tribromophenol	330	332, 141
Nitrobenzene-d_5	82	128, 54
2-Fluorobiphenyl	172	171
Terphenyl-d_{14}	244	122, 212

SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

APPENDIX C
Internal Standards with Corresponding Target Compounds
and Surrogates Assigned for Quantitation
SOP #1821
November 1994

SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

Internal Standards with Corresponding Target Compounds
and Surrogates Assigned for Quantitation

1,4-Dichorobenzene-d_4	Naphthalene-d_8	Acenaphthene-d_{10}	Phenanthrene-d_{10}	Chrysene-d_{12}	Perylene-d_{12}
Phenol	Nitrobenzene	Hexachlorocyclo-pentadiene	4,6-Dinitro-2-methylphenol	Butylbenzylphthalate	Di-n-octylphthalate
bis(2-Chloroethyl) ether	isophorone	2,4,6-Trichlorophenol	N-nitrosodiphenylamine	3,3'-Dichlorobenzidine	Benzo(b)fluoranthene
2-Chlorophenol	2-Nitrophenol	2,4,5-Trichlorophenol	4-Bromophenyl phenyl ether	Benzo(a)anthracene	Benzo(k)fluoranthene
1,3-Dichlorobenzene	2,4-Dimethylphenol	2-Chloronaphthalene	Hexachlorobenzene	bis(2-Ethylhexyl) phthalate	Benzo(a)pyrene
1,4-Dichlorobenzene	bis(2-Chloroethoxy) methane	2-Nitroanaline	Pentachlorophenol	Chrysene	Indeno(1,2,3-cd) pyrene
1,2-Dichlorobenzene	2,4-Dichlorophenol	Dimethyl Phthalate	Phenanthrene	Terphenyl-d_{14} (surr)	Dibenz(a,h)anthracene
2-Methylphenol	1,2,4-Trichlorobenzene	Acenaphthylene	Carbazole		Benzo(g,h,i)perylene
Benzyl alcohol	Naphthalene	3-Nitroaniline	Anthracene		
bis(2-Chloro-isopropyl)ether	4-Chloroaniline	Acenaphthene	Di-n-butylphthalate		
4-Methylphenol	Hexachlorobutadiene	2,4-Dinitrophenol	Fluoranthene		
N-Nitroso-Di-n-propylamine	4-Chloro-3-methylphenol	4-Nitrophenol	Pyrene		
Hexachloroethane	2-Methylnaphthalene	Dibenzofuran			
2-Fluorophenol (surr)	Nitobenzene-d_5 (surr)	2,4-Dinitrotoluene			
Phenol-d_6 (surr)		2,6-Dinitrotoluene			
		Diethyl phthalate			
		4-Chlorophenyl phenyl ether			
		Fluorene			
		4-Nitroaniline			
		2-Fluorobiphenyl (surr)			
		2,4,6-Tribromophenol (surr)			

surr = surrogate compound

APPENDIX D
Ion Abundance Criteria for Tune (DFTPP)
SOP #1821
November 1994

SEMIVOLATILES ANALYSIS OF TISSUE SAMPLES BY GC/MS

Ion Abundance Criteria for Tune (DFTPP)

Mass	Ion Abundance Criteria
51	30.0 - 80.0 percent of mass 198
68	Less than 2.0 percent of mass 69
69	Present
70	Less than 2.0 percent of mass 69
127	25.0 - 75.0 percent of mass 198
197	Less than 1.0 percent of mass 198
198	Base peak, 100 percent relative abundance (see note)
199	5.0 - 9.0 percent of mass 198
275	10.0 - 30.0 percent of mass 198
365	Greater than 0.75 percent of mass 198
441	Present but less than mass 443
442	40.0 - 110.0 percent of mass 198
443	15.0 - 24.0 percent of mass 442

NOTE: All ion abundances MUST be normalized to m/z 198, the nominal base peak, even though the ion abundances of m/z 442 may be up to 110 percent that of m/z 198.