ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN FISH TISSUE USING GC/MS/MS

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1. SCOPE AND APPLICATION

1.1. This method is applicable to the determination of polycyclic aromatic hydrocarbons (PAHs) in fish tissue as listed in Table 3. Method Detection Limits and Practical Quantitation Limits yielded by this method are also provided in Table 3.

1.2. This method is based on EPA 8270E reference 15.1) and the methodology developed by NOAA (references 15.2, 15.3, and 15.4). The Laboratory Information Management System (LIMS) test for the analysis is **T-PAH.**

1.3. This Standard Operation Procedure is restricted to use by an analyst experienced in the use of an Agilent 7010 GC/MS triple quadrupole (GC/MS/MS) mass spectrometer, and in the use of appropriate computer programs. The minimum qualifications required:

- B.S. in Chemistry or Biochemistry with minimum one year of professional chemical or biochemical experience
- Knowledge of principles and practice of gas chromatography/mass spectrometry
- Ability to perform basic laboratory mathematics

Knowledge of the MassHunter GC/MS/MS software and DEP LIMS.

2. SUMMARY OF THE METHOD

Fish tissue samples are extracted using the appropriate procedures listed in the Preparation SOP GC-004 (Ref 15-5). The extract is analyzed using Agilent 7010 series GC/MS/MS triple quadrupole system operating in the Multiple Reaction Monitoring (MRM) mode. Qualitative identification of the parameters in the extract is performed using the retention time and the relative abundance of the characteristic MRM transitions. Quantitative analysis is performed using an internal standard technique.

3. INTERFERENCES

3.1. Raw GC/MS data from all blanks, samples, and spikes must be evaluated for interferences. Determine if the source of interference is in the preparation and/or cleanup of the samples and take corrective action to eliminate the problem.

3.2. Contamination by carryover can occur whenever high-level and low-level samples are analyzed sequentially. To reduce carryover, rinse the syringe several times with solvent between samples. It may be necessary to analyze one or more solvent blanks between samples and even change out the syringe in extreme cases.

3.3 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 gas chromatograms. All of these materials must routinely demonstrate to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 4.10. Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Solvent rinsing should be followed by rinsing with DI water. The glassware should then be drained, dried and heated in a muffle furnace at 550 °C for 8 hours. Some thermally stable materials may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may supplement the muffle furnace heating. Volumetric glassware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil. The use of high purity reagents and solvents helps to minimize

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interference problems. Purification of solvents by distillation in all-glass systems may be required or irradiation with UV light. 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, depending upon the sample characteristics.

3.4 It must be stated here that due to the ubiquitous nature of the Polycyclic Aromatic Hydrocarbons (PAHs) combined with very low detection limits of the GC/MS/MS instruments, some level of observable contamination is always to be expected. Constant efforts have to be made by all the technicians and analysts involved to keep the background contamination levels as low as possible. It is highly recommended to keep all the low-level PAH preparation work physically separated from high level PAH analysis and especially from oil fingerprinting/total hydrocarbons analysis that deal with very high concentrations and even neat petroleum products. Furthermore, special precautions should be taken when using mechanical devices such as grinders during sample preparation. Mechanical devices use petroleum lubricants that easily contaminate tissue samples.

4. DEFINITIONS

4.1. ACCURACY: The closeness of agreement between an observed value and an accepted reference value. When applied to a set of observed values, accuracy will be a combination of a random component and of a common systematic error (or bias) component.

4.2. BATCH: A group of samples, which behave similarly with respect to the sampling or the testing procedures being employed, that are processed as a unit. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch.

4.3. BIAS: The deviation due to matrix effects of the measured value (Xs - Xu) from a known spiked amount. Bias can be assessed by comparing a measured value to an accepted reference value in a sample of known concentration or by determining the recovery of a known amount of contaminant spiked into a sample (matrix spike). Thus, the bias (B) due to matrix effects based on a matrix spike is calculated as:

$$B = (Xs - Xu) - K$$

where:
Xs = measured value for spiked sample,
Xu = measured value for unspiked sample, and
K = known value of the spike in the sample.

The percent recovery (%R) is calculated using the following equation

$$\%R = 100\ (Xs - Xu)/\ K$$

4.4. PRACTICAL QUANTITATION LIMIT (PQL): The lowest concentration that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. The PQL is generally 3 to 5 times the MDL. However, it may be nominally chosen within these guidelines to simplify data reporting. For many analytes the PQL concentration is selected as the lowest non-zero standard in the calibration curve.

4.5. FIELD DUPLICATES: Independent samples which are collected as close as possible to the same point in space and time. They are two separate samples taken from the same

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source, stored in separate containers, and analyzed independently. These duplicates are useful in documenting the precision of the sampling process.

4.6. INTERNAL STANDARD: A pure compound added to a sample extract in known amounts and used to calibrate concentration measurements of other compounds that are sample components. The internal standard must be a compound that is not a sample component.

4.7. LABORATORY FORTIFIED BLANK/LABORATORY CONTROL SAMPLE (LFB/LCS): A known matrix spiked with compound(s) representative of the target analytes. The LFB is analyzed exactly as a sample. This is used to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

4.8. MATRIX: The component or substrate (e.g., surface water, drinking water) which contains the analyte of interest.

4.9. MATRIX SPIKE: An aliquot of sample spiked with a known concentration of target analyte(s). The spiking occurs prior to sample preparation and analysis. A matrix spike is used to document the bias of a method in a given sample matrix.

4.10. MATRIX SPIKE DUPLICATES: A second aliquot of the same sample used for matrix spike spiked with identical concentrations of target analyte(s). The spiking occurs prior to sample preparation and analysis. They are used to document the precision and bias of a method in a given sample matrix.

4.11. METHOD BLANK: An analyte-free matrix to which all reagents are added in the same volumes or proportions as used in sample processing. The method blank must be carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination resulting from the analytical process. For a method blank to be acceptable for use with the accompanying samples, the concentration in the blank of any analyte of concern shall not be higher than the method detection limit.

4.12. METHOD DETECTION LIMIT (MDL): The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix type containing the analyte. For operational purposes, when it is necessary to determine the MDL in the matrix, the MDL should be determined by multiplying the appropriate one-sided 99% t-statistic by the standard deviation obtained from a minimum of seven analyses of a matrix spike containing the analyte of interest at a concentration one to five times the estimated MDL.

4.13. ORGANIC-FREE REAGENT WATER: All references to water in the method refer to water in which interference is not observed at the method detection limit of the compounds of interest. Organic-free reagent water can be generated by passing tap water through a carbon filter bed containing about 1 pound of activated carbon. A water purification system may be used to generate organic-free deionized water.

4.14. PRECISION: The agreement among a set of replicate measurements without assumption of knowledge of the true value. Precision is estimated by means of duplicate analyses. These samples should contain concentrations of analyte above the MDL and utilizes matrix spikes or laboratory fortified blanks. The estimates of precision are based on the relative percent difference (RPD).

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RPD = 100 [2 * (X1 - X2) / (X1 + X2)]

where: X1 and X2 are the two measurements

4.15. INITIAL CALIBRATION VEIFICATION (ICV): A standard solution from a source, other than normal calibration standards, that is certified and traceable. These standards are used to check the accuracy of a calibration curve immediately after the calibration standards are run.

4.16. SPIKE SOLUTION (SS): A solution of method analytes of known concentrations that is used to fortify an aliquot of laboratory reagent water or sample matrix.

4.17. STANDARD CURVE: A plot of concentrations of known analyte standards versus the instrument response to the analyte. Calibration standards are prepared by successively diluting a standard solution to produce working standards, which cover the working range of the instrument. Standards should be prepared at the frequency specified in section six (6) of this SOP. The calibration standards should be prepared using the same type of solvent as the final sample preparation solvent.

4.18. SURROGATE: An organic compound which is similar to the target analyte(s) in chemical composition and behavior in the analytical process, but which is not normally found in environmental samples. Surrogates are used to monitor the preparation and are added to each field and QC sample prior to extraction.

5. APPARATUS AND EQUIPMENT

5.1 Apparatus.

Agilent 7010 GC/MS/MS triple quadrupole system or equivalent. Must be temperature programmable with an injector port designed for pulsed splitless injection. A Multi-Mode Inlet (MMI) may be used if available. The GC, GC Automatic Sampler and MSD operating parameters are illustrated in Tables 1, 2, and 4. The analytical column is an HP-5MS UI or equivalent, fused silica capillary, 20 m x 0.25 mm x 0.25 um or similar column with Ultimate Union placed after the initial 5 m of the column (5m-15m mid-column backflash configuration).

5.2 Data System

5.2.1 A computer system is interfaced to the GC/MS/MS instrument to acquire and store all chromatographic measurements obtained throughout the duration of the chromatographic run sequence program.

5.2.2 The Agilent 7010 instrument uses the Masshunter software from Agilent Technologies Inc. For reporting data, the instrument files are uploaded into the Laboratory Information Management System (LIMS) using a custom data convertor tool.

5.3 Gases

5.3.1 Helium, UPC Grade, is used as the carrier and collision cell gas. The helium is passed through an oxygen and a hydrocarbon filter before it enters the GC.

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5.3.2 Nitrogen, UPC Grade, is used as a collision cell gas. The N_2 is passed through a hydrocarbon filter before entering the GC.

6. REAGENTS AND CHEMICALS

6.1. Solvents

6.1.1. Methylene Chloride, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

6.1.2. Acetone, Fisher Scientific, Pesticide, HPLC or OPTIMA grade.

6.1.3. Hexane, Fisher Scientific, Pesticide, HPLC or Optima grade

6.2. Standards

6.2.1. For details of standard preparation see APPENDIX

6.3. Stock and Intermediate Standards

6.3.1. Custom made stock standards mixtures are purchased from two different, certified (ISO 9000 or EPA certified) vendors at concentrations ranging from 1000-4000 mg/L. The concentrations and composition of currently purchased custom made standards are stored in the Standard Preparation Tracker module of FDEP LIMS".

6.3.2. Intermediate standard solutions are mixtures of individual standards used to prepare working standards. They are prepared in our lab from stock standards by mixing / diluting with methylene chloride or they can be purchased from a certified manufacturer (ISO 9000 or EPA certified). The concentrations of the components in the intermediate standard are typically 10 mg/L. Intermediate standards are replaced once per 6 months. New standard is checked vs. a second source (QC Standard), after diluting it to one of the working standard levels. The criterion of acceptance is +/- 10% difference. All standard preparations and verifications have to be recorded in the Standard Preparation Tracker located in DEP LIMS. A list containing all standards with their serial numbers used in the analysis must be part of analysis documentation folder.

6.4. Working Standard Solutions (Calibration standards)

6.4.1. Working standard solutions are series of diluted individual or mixture standards in methylene chloride. They are used for calibration and are prepared by diluting intermediate standard solutions with methylene chloride. The internal standard mixture listed below is added to every working standard at 100 ug/L.

6.4.2. Internal Standard Mixture:

1,4-dichlorobenzene-d4	phenanthrene-d10
naphthalene-d8	chrysene-d12
acenaphthene-d10	perylene-d12

6.4.3. The concentrations for each analyte in the working (calibration) standards are 5,10, 20,50, 100, 200 500 and 1000 ug/L. The second source/CCV standard is prepared at 100 ug/L.

6.5. Spiking Solution

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6.5.1. These solutions are used when fortifying sample matrix spikes or lab fortified blanks at extraction. These fortified samples are used to verify the accuracy and precision of the sample preparation and analysis.

6.5.2. Spike solutions are prepared by diluting stock or intermediate standards with acetone. Concentrations of the parameters in these solutions are 1000 ug/L for PAHs and 1000 ug/L for surrogates.

6.5.3. Freshly prepared spike solutions are checked vs. valid working standards. The acceptance criterion is +/- 20 % difference.

6.6. Initial Calibration Verification (ICV). A standard solution from a source, other than normal calibration standards, that is certified and traceable. These standards are used to check the accuracy of a calibration curve immediately after the calibration standards are run. The acceptance criterion is ± 30%.

6.7. Continuous Calibration Verification (CCV). A mid-level calibration standard solution from a source that is certified and traceable. These standards are used to check the accuracy of a calibration curve on daily bases.

6.7.1 The acceptance criterion is ± 20% difference.

6.7.2 The Analyst must ensure that the calibration standards are checked with the corresponding CCV with every initial calibration followed by every 12 hour interval thereafter.

6.8 Analyte protectants.

The use of analyte protectants was proposed by Anastassiades et al. (ref 15-13 and 15-15) and it has become an established practice for pesticides analysis in food products analysis, where triple quad GC/MS/MS technology has found the widest use. The Agilent 7000C GC/MS/MS instrument is very sensitive to matrix induced response enhancements which makes exact quantitation of target analytes problematic. Analyte protectants are generally very polar organic compounds injected at very high concentrations along with every standard and sample in the analytical run sequence. Due to their polarity, they are thought to deactivate all the active sites in the system, and, due to their very high concentration, to render all the other matrices in the samples basically insignificant.

In effect all the samples and standards injected into the GC/MS/MS system have effectively the same matrix – the analyte protectants – and therefore matrix induced response differences are greatly reduced. This method uses a mixture of three specific analyte protectant compounds: Ethoxyglycerin, Gulonolactone and Sorbitol at 25000 ppm, 2500 ppm and 5000 ppm respectively. Due to solubility considerations, the working solution is prepared in MeOH containing 5% DI water. It is injected along with every standard and sample using a “sandwich injection” technique. 0.4 ul of the above analyte protectants mixture is injected, which corresponds to 10 ug, 1 ug and 2 ug of the corresponding analyte protectants on column.

7. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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7.1. Samples must be collected in glass jars with Teflon-lined screw caps.

7.2. All samples must be refrigerated at <-10 degrees C. Please ref to the DEP SOP GC-004-1 for details (Ref. 15.5).

7.3. All samples must be extracted within 365 days of collection and completely analyzed within 40 days of extraction.

8. SAMPLE PREPARATION PROCEDURE

8.1. See Preparation SOP GC-004-1: "EXTRACTION OF ORGANOHALIDE PESTICIDES, PAHs AND PCBs FROM FISH TISSUE USING PRESSURIZED FLUID EXTRCATION TECHNIQUE (REF. 15.5)

9. GENERAL DESCRIPTION OF GC/MS ANALYSIS

9.1. GC/MS/MS Maintenance and Tuning

9.1.1 DFTPP tune recommended by EPA 8270E is not applicable for Agilent 7010 GC/MS/MS. Instead, as recommended by 8270E paragraph 11.3.1, this SOP modifies the method to follow the manufacturer's tuning instructions. GC/MS/MS tuning includes periodic autotunes as well as a daily tune check.

9.1.2 Initial checking and maintenance of the instrument consists mainly of the following steps:

9.1.2.1 Check the vacuum operation and periodically run an air/water leak test. Oxygen, nitrogen and water have to be found at below 5% of mass 69 level. Air/water leak test must be run each time the vacuum system is vented. It may take several hours (even overnight) of pumping down to bring the air/water content below 5%!

CAUTION: In order to properly perform the air/water leak test nitrogen gas flow to the collision cell has to be turned manually off at the source! After the test, nitrogen gas has to be turned back on!

9.1.2.2 Perform the triple quadrupole GC/MS system verification (tune check) before each run sequence. Print the tune check report and include it with the documentation. The tune check requirements are listed in Appendix 3. Periodically perform the EI high sensitivity autotune of the GC/MS/MS triple quadrupole system and save the current settings in the autotune file.

The autotune has to be performed every time the tune check fails. The report is printed automatically; it is placed in the autotune report log. If the autotune criteria are not met, repeat the autotune. If it still fails, run the autotune using the default values. If that too fails, the ion source may need to be cleaned. **Manual tuning of the instrument without the assistance of an Agilent Technologies' triple quad trained specialist is NOT RECOMMENDED!**

9.1.2.3 Replace the injector liner (Agilent 4 mm single taper splitless glasswool liner part no: 5190-3167), cut 1-3 inch of the column after running

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contaminated samples or encountering unusually heavy matrix. Occasional trimming of the column is still necessary even if backflush technology is used.

9.1.2.4 Run an Instrument Blank and look for instrument contamination.

9.1.2.5 In case the instrument is contaminated raise the furnace temperature to 260 ºC and leave it overnight.

9.1.2.6 To perform maintenance of the MMI inlet replace the liner and septum and clean the inside of the inlet body with cotton swabs soaked with methanol and methylene chloride. **Do not use a harsh brush to clean the inside of the inlet body!** To complete the MMI inlet cleaning perform an inlet bake out procedure. Set the inlet mode to split (split ratio 200:1) and let the gas flow for a couple of minutes. Then raise the inlet temperature to 330 ºC and the oven temperature to 300 ºc. Bake for 1-2 hrs. It is beneficial and recommended to bake the ion source at 350 ºC at the same time.

9.1.2.7 The autosampler syringe has to be frequently and thoroughly cleaned to avoid injection repeatability problems. A dirty plunger will cause reproducibility problems, bad injections occurring randomly, and poor peak shapes and calibration problems. Replacing the syringe on regular, frequent basis is highly recommended.

9.1.2.8. Re-check for instrument contamination and if needed repeat the procedure until the instrument is contamination free.

9.2. GC/MS/MS Initial Calibration

9.2.1. Analyze the calibration standards at the following levels: 5, 10, 20, 50, 100, 200, 500 and 1000 ug/L. At each level, the internal standards should be present at 100µg/L. The linearity of the calibration curve is defined by an RSD that is less than or equal to 20% of the Response Factors of the calibration curve, or by a correlation coefficient greater than or equal to 0.995. If a higher order curve is to be used, the correlation coefficient has to be at least 0.995. When a new calibration is inspected, a linear fit is usually the first choice, followed by the average response factor (%RSD which is equivalent to a weighted linear fit forced through origin), then followed by a quadratic fit and finally higher order curves.

When the calibration curve fit is selected in the Masshunter software the, percent calibration drift has to be monitored in order to have all the calibration levels within 70-130% of the expected value. Special care has to be taken to carefully fit the curves at the lower end of the calibration range where the vast majority of problems occur. All calibration curves should be weighted curves (1/x or 1/x2); do not use equal weighing. It is permissible to remove individual analyte calibration levels from either the lowest and/or highest levels of the curve (the PQL may have to be adjusted). Removal of interior levels is forbidden. An entire single standard calibration level may be removed from the interior of the curve whenever the standard was not correctly injected or a wrong standard was injected. Do not use the “include origin” (include “0”) option in the software. If more than 10% of the total number of analytes fail the calibration fit criteria, or more than 20% of analytes fail the percent calibration drift criteria, then the instrument system must be corrected. Some possible

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problems include standard mixture degradation, injection port inlet contamination, analytical column contamination, and active sites in the column or chromatographic system.

9.2.2. Each time the column length is trimmed due to contamination, or a new column is installed the retention times for every compound has to be re-determined. The MRM mode time segments (acquisition windows) have to be then adjusted accordingly. A mid-level standard should be run to confirm that all the peaks are fully within the acquisition windows. Both the analysis end of the method as well as the data-gathering end of the method must be adjusted. Alternatively, RT locking procedures can be used to keep the retention times unchanged after the column is trimmed. Approximate retention times, the primary quantitation transitions and confirmatory, secondary transitions (if applicable) are given along with MRM acquisition time segments start/stop times in Table 5. The primary and secondary ions should all be present, and the relative intensities should agree within ±30% (absolute) of the reference mass spectrum after background subtraction is performed.

9.2.3. The Initial calibration curve is always checked immediately against the second source initial calibration verification standard (ICV). The acceptance criterion is ± 30%. If the Initial Calibration is not run with the batch, the CCV acceptance criterion is ± 20% (see 9.3.2)

9.3. Daily GC/MS Performance and Calibration Check

The initial calibration curve and instrument performance must be verified on each day of analysis by the measurement of one or more check standards.

9.3.1 Degradation/tailing factor check
A modified GC/MS tuning standard is used to perform this task. The standard contains 5 mg/L each of DDT-p, p' and Pentachlorophenol. Benzidine, which is also recommended by method 8270E, is usually not detectable in the presence of analyte protectants. Since the absence of a benzidine peak in the chromatograms has no bearing on the chromatographic behavior of the target analytes and surrogates, only pentachlorophenol is used for tailing factor determinations. The degradation check/tailing factor standard is run at the beginning of the analytical run sequence to verify injection port inertness and GC column performance. A full scan version of the acquisition method is used for this purpose. Degradation of DDT to DDD and DDE should not exceed 20%.

The percent breakdown calculation is performed using the following formula (EPA method 8081)

$$\%\text{Decomp. DDT} = \frac{\text{Sum of Peak Areas (DDE + DDD)}}{\text{Sum of Peak Areas (DDE + DDD + DDT)}} \times 100\ \%$$

The following are the Relative Retention Times for DDD-p,p' and DDE-p, p' (relative to DDT-p, p'):

DDE-p, p' RRT = 0.885
DDD-p, p' RRT = 0.949

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Usually it is necessary to perform spectral deconvolution of the chromatograms to remove the analyte protectants "hump" and obtain correct integration values and clean spectra for target analytes. Deconvolution for GC/MS data is the process of computationally separating co-eluting components and creating a pure spectrum for each component. Specifically, for each observed EIC that results from two or more components, deconvolution calculates the contribution of each component to EIC.

As illustrated in Figure 1 below, the tailing factor for pentachlorophenol should not be higher than 2 according to the following equation:

Tailing Factor = BC / AB

where the peak is defined as follows: AC is the width at 10% height; DE is the height of peak and B is the height at 10% of DE. This equation compares the width of the back half of the peak to the width of the front half of the peak at 10% of the height. If DDT-p, p' degradation and or tailing factors are excessive the injection port maintenance has to be performed until proper chromatographic performance is restored.

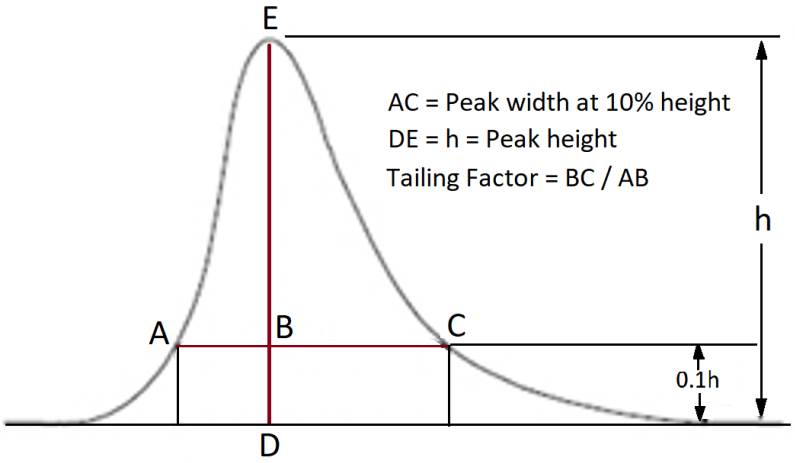


Figure 1: Peak Tailing Factor

9.3.2 Analyze midpoint level standards as continuing calibration check (CCV) standards. These calibration check standards must be analyzed at the start of each run and at 12-hour intervals thereafter. The acceptance criteria for all components are 80-120% recovery. If multiple CCV failures are observed corrective action must be taken prior to the analysis of samples. When compounds fail, they may still be reported as non-detects (with a "J" qualifier) if it can be demonstrated that there was adequate sensitivity to detect the compounds at the applicable quantitation limit. For situations when the failed compound is present, the concentrations in the affected samples must be reported as estimated.

9.3.3 Calibration verification standard must also meet the following criteria:

9.3.3.1 Internal standard responses: the peak area for each of the internal standards may not change beyond the factor of two (-50% to +100%)

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from that of the average internal standard response of all the levels in the most recent initial calibration.

9.3.3.2 Internal standards retention times- the retention times of the internal standards in the calibration verification standard must not drift by more than 30seconds from that of the mid-point standard level of the most recent initial calibration.

9.3.3.3 If any of the requirements mentioned in 9.3.3 are not met, the system must be evaluated, and corrective action must be taken before sample analysis begins. Some possible problems are standard mixture degradation, injection port inlet contamination, contamination at the front end of the analytical column, active sites in the column or chromatographic system, and a contaminated MS source.

9.4 GC/MS Sample Analysis.

9.4.1 Quantitative analysis of PAHs is accomplished by the internal calibration method. All target component positives found have to be quantified using at least a five-point calibration curve (for linear fit), at least six points for quadratic and at least seven points for higher order curves. Each sample extract MUST contain 100 µg/L of internal standard. The extracts should be diluted as deemed necessary. **The concentration of the internal standard must remain at 100 µg/L when diluting samples by adding additional internal standard to the extract.**

9.4.2 Analysis Run Sequence.

The analyst will set up an analysis run sequence by placing the GC vials containing the sample extracts and standards on the autosampler tray in the following order or similar:

- Tissue matrix sample (to saturate the system)
- GC/MS degradation/performance check
- Instrument Blank (containing internal standards at 100 ug/L)
- PAH calibration set (when initial calibration is run, levels 1-8)
- PAH ICV initial calibration verification check (when initial calibration is run)
- PAH CCV continuing calibration check
- extraction blank or instrument blank
- GPC blank 1
- GPC-blank 2
- laboratory fortified blank (LFB)
- laboratory fortified blank (LFB) duplicate (if prepared)
- sample matrix spike,
- sample matrix spike duplicate
- Samples (up to 12 hours)
- PAH CCV continuing calibration check
- Instrument blank
- samples (up to 12 hours)

9.4.3 Print a hard copy of the run sequence and place it in the final data folder.

9.5 Sample Analysis using the Masshunter Software.

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9.5.1 Using the Qual software examine the GC/MS degradation/performance check sample. Calculate DDT decomposition and the tailing factor for pentachlorophenol.

9.5.2 Examine the quantitation reports soon after the samples have been analyzed. Check all of the calibration check standards analyzed during the run sequence and insure that they met the minimum criteria given above.

9.5.3 The concentration reported for an identified hit in the sample extract, expressed in ug/L, is quantitated using the initial calibration curve. However, the analyst must ensure that all identification criteria are fulfilled, and the peak area integration was correctly done. If not, the integration has to be re-done by choosing a new set of integration parameters or by manual integration (see SOP CM-018.1 for laboratory's manual integration policy, ref 15.10).

9.5.4 If the response for any of the compounds exceeds the calibration curve, the extract must be diluted so that the response will be within the limits of the curve, and then reanalyzed. Make sure, when performing dilutions, to use methylene chloride spiked with the appropriate levels of internal standard so that their response will be the same in the dilution as it was in the original extract.

9.5.5 The internal standard responses and retention times in the calibration check standard must be evaluated immediately after data acquisition. If the retention time for any internal standard changes by more than 0.05 minutes from the last check calibration, then the chromatographic system must be evaluated for either a) chromatographic matrix induced shift or inspected for malfunctions (such as leaks) and corrections must be made. The m/z abundances for all of the internal standards must be within a factor of two (50% to 200%) from the abundance in the daily calibration standard check. Corrective action is indicated if the m/z abundance is outside of these limits. If the holding time for a sample has expired and the internal standard areas are out of the acceptance range, then the final report must be commented and any positives detected must be qualified with a "J" to indicate that the result is an estimate.

9.5.6 Analysis of surrogates.

9.5.6.1 The analyst should monitor the performance of the extraction, cleanup, analytical system, and effectiveness of the method by spiking each sample, QC sample and Method Blank with surrogates. Surrogate compounds are expected at 200 µg/L in the sample extracts, Blanks, LFBs, and Matrix Spikes.

The following compounds are used as surrogates in this method:

<u>Compound</u>
Nitrobenzene-d_5
2-Fluorobiphenyl
Terphenyl-d_{14}

9.5.6.2 To determine acceptance accuracy limits for surrogate the following procedure should be used (See 15.14): (1) for each sample analyzed, calculate the percent recovery of each surrogate in the sample, (2) once a minimum of twenty samples of the same matrix has been analyzed,

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calculate the average percent recovery (p), and standard deviation of the percent recoveries (s), for each of the surrogates, (3) for a given matrix, calculate the upper and lower control limit for method performance for each surrogate standard as follows:

Upper Control Limit (UCL) = p+ ts
Lower Control Limit (LCL) = p- ts
Where "t" stands for Student's coefficient for 99% confidence level for n-1 degrees of freedom (n = number of measurements). "p" stands for average recovery.

The control limits are supposed to be checked once per year if enough measurements have been conducted. Temporary acceptance limits of 30-150 % will be used until enough statistical data is acquired to perform the necessary calculations.

9.5.6.3 If a surrogate recovery fails, the sample has to be re-extracted and re-analyzed. If the sample exceeded the holding time for extraction, and the recovery is biased high, the re-extraction is not required and the reported hits have to be qualified as estimated (J), with the following comment entered in the Sample Report: "Estimated values for "parameter" are due to surrogate recovery out of control limits". If the recovery is biased low the sample must be re-extracted and re-analyzed.

9.5.7. Analysis of Matrix Spikes and LFBs

Check the Matrix Spike and Lab Fortified Blank recoveries. They must be within the current acceptance ranges. A comment must be added to the final report explaining any deviation from the routine limits whenever the spikes or surrogates are out of range. Matrix spikes and LFBs recovery and precision limits should be calculated based on available in-house data once enough data points are collected. See ref 15.12, chapter 5.3 for details. LFBs and Matrix spikes recovery limits are calculated independently; see Table 6 for details. In case LFB failures are observed the LFBs may be rerun and reanalyzed. All the associated samples that have positives (above PQL) for the analytes failing in the LFBs must be re-run and reanalyzed as well. Similarly, if the number of matrix spikes failure is deemed excessive matrix spikes (along with the background sample) may be rerun and reanalyzed. Refer to ref 15.12 chapter 5.3 for details on disposition of QC failures.

9.5.8. Blank Contamination

In the event a positive identification is found in both the sample(s) and the instrument blank, the instrument must be cleaned and the sample(s) rerun/reanalyzed.

In the event a positive identification is found in both the sample(s) and the extraction blank then the unexpired samples have to be re-extracted and re-analyzed. If samples passed the holding time, the results will be reported with a "V" qualifier up to ten times the amount found in the blank. The re-extraction is not necessary when the analytes in question are non-detects in the samples or whenever the values found in the samples are >10x the value detected in the method blank.

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In the event a positive identification is found in a field blank, but method blank(s) pass, the field blank detection only affects the samples in the same job. If those samples do not show any detections for the analyte found in the field blank, the field blank does not need to be re-extracted. If the detections are found up to 10x the level in the field blank, all samples with such detections and the field blank must be re-extracted. If the detections are above the 10x field blank level, no re-extractions are necessary.

9.5.9 Continuing Calibration

9.5.9.1 After initial calibration, the GC/MS/MS response is checked immediately by running an Initial Calibration Verification standard (ICV), and then, on a continuing basis (every 12 hours), by using a continuing calibration check (CCV), middle range PAH standard. Criterion of acceptance for continuing calibration check is +/-20 % of initial calibration. If a CCV fails, all the samples after by this standard, have to be re-run for all positive hits found at concentrations higher than PQL level. If the samples have exceeded the analysis holding time then all hits must be flagged as estimated.

9.5.9.2 If CCVs do not meet method requirements but the associated analytical results are below the detection limit or below the quantifiable threshold (PQL) and no systematic failure is observed (i.e., the failure is deduced to be due to a statistical outlier in a multi-component analysis where results from other CCV components and LFB results do not indicate a systematic bias in the analysis or detector response), an appropriate test comment and result qualifier (J) shall be provided to indicate the CCVs are outside control limits. However, when a systematic or system failure is detected or the affected analytical data are above the quantifiable threshold, the analysis shall be re-run if possible. If re-analysis is not an option, the analytical results shall be qualified as estimates (see 15.11 for more details).

9.5.10 Quantitation of positives.

Positives are quantified based on their peak area. The concentration reported for an identified hit in the sample extract, expressed in ug/L, is based on calibration curve of the initial calibration. The Masshunter software used to process data will do the interpolation for the sample extract. However, the analyst must ensure that all identification criteria are fulfilled, and the peak area integration was correctly done. If not, the integration has to be corrected by choosing a new set of integration parameters or by manual integration (for manual integration procedure see 15.10). Only extract concentrations are calculated by the processing software. The final analyte concentrations in sample are calculated in the QC Manager during the data upload process software. This is done as follows:

- C_{sample} (ug/kg) = (C_x) × ($V_{extract}$) × (DF)/(W_{sample}) for tissue samples
- C_x is the concentration, in ug/L, of the analyte in the sample extract determined by the processing software.
- $V_{extract}$ is the volume of the extract, in mLs (usually 0.5 mL) recorded in the preparation report.

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- DF is the dilution factor of the sample extract recorded in the dilution report.
- W_{sample} is the weight of the sample taken for extraction. The weights are expressed in grams and are recorded in the preparation report.

10. REPORTING AND DOCUMENTATION

10.1 When analysis is complete, generate a .txt file in the Masshunter software for all the reported samples, QC samples and CCVs. The QC Manager is then used to download the final sample results into the Laboratory Information Management System (LIMS). If a sample has been diluted then make sure that the information was entered properly into the QC Manager. The following convention should be used in QC manager:
PAH_CCV for CCV
SURRPAH_CCV for surrogate in CCV
SURRPAH_1234567 for surrogate in sample

10.2 Add the appropriate qualifiers and comments to the sample data in the QC Manager after performing a complete review of the sample data and QC data. Follow ref 15.12) for details of data review and reporting. See (ref 15.11 for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option. Definitions of the available qualifying codes are listed in DEP SOP LB-027 (ref 15.11).

10.3 All quantitation reports for samples and QC should be placed in a results folder labeled with the job number, the sample numbers, and the type of analysis.

10.4 Enter the results for each sample in the analysis results form. Print out QC results from the QC Manager. Include a copy of dilutions page if any. Also include a printout of sample positives from the data review (scrollable report) and file it in the data folder.

10.5 Submit the result folder to the GC Pesticides section supervisor. The supervisor will review results and then authorize the sample. The supervisor will then file the folders in the GC pesticides filing cabinets.

11. QUALITY CONTROL

11.1 See DEP SOP GC-001-2, ref 15.12 for detailed information on quality control, definitions and interferences. See DEP SOP LB-027, ref. 15.11 for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option.

11.2 All data generated by a chemistry analyst are subjected to supervisory review and authorization. The supervisor is assessing the validity of data generated by assuring that all QA/QC criteria are met by checking the following review/authorization checklist:

- Check sample preparation report
  - procedure
  - surrogate and spiking solutions
  - preparation holding time
- Check project log-in folder for special requests
- Check initial calibration for linearity and fit

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- Check continuing calibration check standards
- Check surrogate recoveries
- Check accuracy and precision for LFB and Matrix Spikes
- Check internal standard recoveries
- Check retention time drift
- Check for blanks contamination
- Check for positives:
  - Proper integration of the peaks (baseline)
  - Retention time confirmation
  - Amount confirmation
  - If response is within the calibration range
  - Calculations
  - Qualifiers
- Check information in the MassHunter Report Header:
  - SOP #
  - Instrument ID
  - Analyst name
- Evaluate the data against any field information
- Check field preservation record
- Check LIMS report:
  - Preparation and analysis dates
  - MDLs
  - Amounts reported for positives
  - Qualifiers
  - Comments

12. DATA ARCHIVAL

12.1 All raw data are retained in electronic or hard copy format. All paper formats are maintained either in the laboratory office or in a State warehouse for at least ten years. Electronic chromatographic raw data are archived by analysis file ID and instrument ID on dual CDs by the Laboratory Support Section.

12.2 Move the valid data files (tune, ICALS, CCVs, QC and sample data files) soon after collection from the hard disk of the instrument computers to the network drive. For the Agilent 7010 instrument copy the files to \\TLHLAB3\GC\BACKUP\GC-TQ-2_. Raw data files on the hard disk of each instrument may be deleted after 3 to 4 months or they are no longer in need.

12.3 Copy the processed data files and quantitation reports into the network directory for the instrument. For the Mass Hunter data files use the directory \\TLHLAB3\GC\UPLOAD\GC-TQ-2 to convert in QC Manager for reporting in LIMS. These files may be deleted after all results are authorized.

13. DETERMINATION OF METHOD DETECTION LIMITS

13.1 The MDLs were established using procedures given in EPA Chapter 40 Part 136, Appendix B of the Federal Register, Ref 15.9.

13.2 Seven replicate samples are analyzed near the MDL. The MDL is the concentration of the analyte at which the analyte is present with 99% confidence.

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13.3 The results of the seven replicate data sets are tabulated and the statistical calculations are performed electronically to establish or update the MDLs and PQLs for each analyte and matrix.

14. SAFETY/HAZARDOUS WASTE MANAGEMENT

See DEP SOP OG-001, Ref. 15.8 for specific details on safety and hazardous waste management.

15. REFERENCES

15.1 Test Methods for Evaluating Solid Wastes, Third Edition, SW-846, Method 8270E, Semi-volatile Organic Compounds By Gas Chromatography/Mass Spectrometry (GC/MS), revision 6, June 2018

15.2 "Extraction, clean-up and gas chromatography/mass spectrometry analysis of sediments and tissues for organic contaminants" NOAA technical Memorandum NMFS-NWFSC-59, March 2004.

15.3 "Analytical Quality Assurance Plan – Mississippi Canyon 252 (Deepwater Horizon) Natural Resource Damage Assessment" Version 1.0 NOAA, June 7, 2010.

15.4 Extraction, clean-up end gas Chromatography/Mass spectrometry Analysis of edible seafood for parent and Alkyl polycyclic aromatic Hydrocarbons in response to the Deepwater horizon MC252 Oil spill Event. NOAA method, July 2010.

15.5 DEP SOP GC-004-1, Extraction of Organohalide PesticidesPAHs and PCBs from Fish Tissue Using Pressurized Fluid Extraction Technique.

15.6 Florida Department of Environmental Protection, Standard Operating Procedure LB-007 *Procedure and Policy for Demonstration of Capability for Methods, Instruments and laboratory Staff.*

15.7 Florida Department of Environmental Protection, Standard Operating Procedure LB-031 *Method Detection Limit Verification.*

15.8 DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories).

15.9 Code of Federal Regulations, Title 40, Part 136, Appendix B.

15.10 DEP SOP CM-018-1 Laboratory Policy Regarding Manual Chromatographic Peak Integration.

15.11 DEP SOP LB-027. Reporting Qualified Data and Correcting Quality Control Problems.

15.12 DEP SOP GC-001-2. Quality Assurance/Quality Control and Data Review Authorization in the GC-Pesticide Laboratory.

15.13 Mastovska, K., Lehotay, S.J., Anastassiades, M., "Combination of analyte protectants to overcome matrix effects in routine GC analysis of pesticides residues in food matrices" anal. Chem. **2005**, 77, 8129-813715.14 EPA 8000D Determinative Chromatographic Separation; Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods, SW 846; 3rd Edition, Update 5, Revision 4, July 2014.

15.15 Anastassiades, M., Mastovska, K., Lehotay, s.J., "Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides" Journal of Chromatography A, 1015 (2003) 163-184.

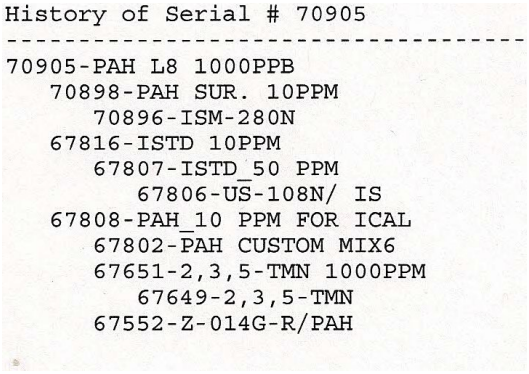
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16. APPENDICES

APPENDIX 1
Standard Preparation Procedure

Stock solutions usually come in 1mL glass vials. Take the vials out of the freezer at least 30min before preparation. Open vials and transfer the entire solution into 1mL GC vials (using Pasteur pipettes). Record the transfer date and keep the vial label.
The Intermediate Standard is prepared by diluting the Stock solution. The Working Level Standards are prepared by diluting the Intermediate Standard.
All standard preparations are recorded in the Standard/Reagent Preparation Tracker. In order to prepare fresh standards go to LIMS-Technical-Standard/Reagent Preparation Tracker. Select GC from the pull down list. Hit "Reports" and then "History". When prompted enter the standard tracking number for the standard you are going to replace. Examine the "History Tree". An example of a "History Tree" is shown below:



In order to get exact information on how the standard was prepared look at "history data sheets" that will show exact information on standards preparations.

Before preparing new standards consult with the GC group supervisor whether any changes to standards preparation procedures should be made. Those may include different prepared volumes, concentrations, new, additional concentration levels, etc.

All freshly prepared standards need to be entered in the Standard Preparation Tracker.

APPENDIX 2
Analyte protectants

Follow the instructions in appendix 1 to re-prepare analytes protectants solutions.

Notes:
Neat D-sorbitol is hydroscopic and should be stored in a desiccator-Sorbitol and, especially, gluconolactone are difficult to dissolve in MeOH. To achieve complete dissolution of these components, 1 – 3 hours of hot sonication and repeated vigorous shaking (vortexing) may be needed. To avoid extensive

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sonication, required quantities of analyte protectants may be initially dissolved in 5 mL of water. The resulting solution may then be brought up to the final volume of 100 mL by adding methanol. The resulting solution is saturated with gluconolactone and therefore should be kept at room temperature to avoid excessive precipitation. Some precipitation, however, appears to be unavoidable.

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APPENDIX 3
Tune check criteria

Triple Quadrupole GC/MS System Verification - Tune

Instrument Name	GC_TQ-2 / US12166802	**MS Model**	7000C
Tune Date & Time	3/30/2016 8:18:46 AM	**Source**	EI Extractor
Tune File	D:\MassHunter\GCMS\1\7000\atunes.eiexPAH.tune.xml		

Instrument Actuals

Ionization mode	EI+	Rough Vacuum	1.38E+2 mTorr
Source Temperature	330 °C	High Vacuum	7.73E-5 Torr
Quad. 1 Temperature	180 °C	Turbo Speed	100.0 %
Quad. 2 Temperature	180 °C	Turbo Power	37.011 W
Emission Current	35.0 µA		

MS1 Checktune Results	Value	Limit	Result
Low mass assignment (target 69.00, actual 69.00)	0.00	<= 0.20	OK
Mid mass assignment (target 264.00, actual 264.00)	0.00	<= 0.20	OK
High mass assignment (target 502.00, actual 502.10)	0.10	<= 0.20	OK
Low mass isotope position (target 70.00, actual 70.10)	0.10	<= 0.20	OK
Mid mass isotope position (target 265.00, actual 265.00)	0.00	<= 0.20	OK
High mass isotope position (target 503.00, actual 503.10)	0.10	<= 0.20	OK
Low mass isotope ratio	1.07%	>= 0.5% and <= 1.6%	OK
Mid mass isotope ratio	5.63%	>= 4.2% and <= 6.9%	OK
High mass isotope ratio	10.04%	>= 7.9% and <= 12.3%	OK
Ratio of mid mass to low mass	20.36%	>= 5.0%	OK
Ratio of high mass to low mass	1.80%	>= 0.8%	OK
Low mass precursor ratio	0.08%	<= 3.00%	OK
Mid mass precursor ratio	0.04%	<= 6.00%	OK
High mass precursor ratio	0.04%	<= 12.00%	OK

MS2 Checktune Results			
Low mass assignment (target 69.00, actual 69.00)	0.00	<= 0.20	OK
Mid mass assignment (target 264.00, actual 264.00)	0.00	<= 0.20	OK
High mass assignment (target 502.00, actual 502.00)	0.00	<= 0.20	OK
Low mass isotope position (target 70.00, actual 70.05)	0.05	<= 0.20	OK
Mid mass isotope position (target 265.00, actual 265.10)	0.10	<= 0.20	OK
High mass isotope position (target 503.00, actual 503.07)	0.07	<= 0.20	OK
Low mass isotope ratio	1.08%	>= 0.5% and <= 1.6%	OK
Mid mass isotope ratio	5.52%	>= 4.2% and <= 6.9%	OK
High mass isotope ratio	9.41%	>= 7.9% and <= 12.3%	OK
Low mass precursor ratio	0.01%	<= 3.00%	OK
Mid mass precursor ratio	0.02%	<= 6.00%	OK
High mass precursor ratio	0.14%	<= 12.00%	OK

Detector			
EMV	1654	<= 2900	OK
Maximum gain factor	1010	>= 100	OK

Air and Water Check	Abundance	Relative Abundance	Limit	Result
PFTBA (69.00)	14917805			
Water	66363	0.44%	<= 20.00%	OK
Oxygen	62295	0.42%	<= 2.50%	OK
Nitrogen	232173	1.56%	<= 10.00%	OK

* Nitrogen values are calculated from oxygen abundance

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17. TABLES

TABLE 1: GC/MS/MS Operating Conditions: (METHOD PAH-MRM-yymmdd)

Parameter	Setting
Mass spec:	70 eV. Peak width 0.7s. detector setting-gain. Source temp. 330 C. Quads 180 C. Collision cell He: 2.25 mL/min, Nitrogen 1.5 mL/min.
Scan Time:	About 3.3 cycles/s. MRM mode dwell times variable, summing up to about 300 ms per time segment. Gain 10 for each transition monitored.
GC Oven Temperature Program:	40 °C for 1.5 min, to 200 °C at 25 C °/min, hold 0 min, to 310 C at 8 C/min hold at 310 °C for 4.0 min. This program is the same for full scan (used for GC/MS decomposition check) or MRM.
Injector operating conditions:	Temp: 320 °C; Mode: Pulsed splitless; Pressure: Constant Flow ON, 16.000 psi at 45 °C (1.5 mL/min);
MS interface temperature:	300 °C
Injection Type:	Pulsed splitless. Pulse pressure 30 psi for 1.4 min; purge to split vent 50 mL/min at 1.5 min. Gas saver ON. Gas saver 20 mL/min after 3 min.
Injection Volume:	1 µL
Column 1 configuration	HP-5MS UI, 5m-0.25mm-0.25um; in: front MMI inlet, out aux EPC 6
Column 1 flow	1.1 ml/min for 12 min, then at 100 mL/min to 1.2 mL/min for 9.7 min, then at 100 mL/min to -5 mL/min for until end run
Column 2 configuration	HP-5MS UI, 15m-0.25mm-0.25um; in: t aux EPC 6 ; out: vacuum
Column 2 flow	1.2 ml/min for 12 min, then at 100 mL/min to 1.3 mL/min for 9.7 min then at 100 mL/min t0 2.4 mL/min until end run
Backflush:	end run; see column flows conditions
Total Run Time:	25.7 min.

TABLE 2: GC AUTOSAMPLER OPERATING PARAMETERS FOR GCMS ANALYSIS

Autosampler Parameter	Setting
Syringe size	10 ul
Injection volume	2 ul
Last Bottle	150
Solvent A washes	3 pre- 15 post (DI Water)
Solvent A volume	8 ul
Solvent B washes	3 pre- 15 post (Acetone)
Solvent B volume	8 ul
Sample washes	1
Sample wash volume	4 ul
Sample pumps	7
Dwell time pre-inj	0

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Autosampler Parameter	Setting
Dwell time post-inj.	0
Solvent wash draw speed	300 ul/min
Solvent wash dispense speed	6000 ul/min
Sample wash draw speed	50 ul/min
Sample wash dispense speed	6000 ul/min
Injection dispense speed	68ul/min
Viscosity delay	7 sec
Sample depth	disabled
Injection type	3-layer Sandwich
L1 airgap	0.0 ul
L2 Volume	0.2 ul MeOH
L2 airgap	0.0 ul
L3 Volume	0.4 ul analyte protectants
L3 airgap	0.0 ul
Sample overlap	Not enabled

TABLE 3: Internal standard and surrogate associations, MDLs, PQLs and RTs for PAHs in fish tissue

Analyte	Approximate Retention Time (min)	Associated Internal Standard	MDL ug/L	MDL ug/kg	PQL ug/L	PQL ug/kg
			extract	sample	extract	sample
Nitrobenzene –d5 surrogate	4.36	Naphthalene-d8				
Naphthalene	5.24	Naphthalene-d8	10	1.0	40	4.0
2-Fluorobiphenyl surrogate	6.55	Acenaphthene-d10				
2-Methylnaphthalene	6.10	Naphthalene-d8	10	1.0	40	4.0
1-Methylnaphthalene	6.20	Naphthalene-d8	10	1.0	40	4.0
Biphenyl	6.65	Acenaphthene-d10	10	1.0	40	4.0
2,6 Dimethylnaphthalene	6.78	Acenaphthene-d10	10	1.0	40	4.0
Acenaphthylene	7.04	Acenaphthene-d10	5.0	0.50	20	2.0
Acenaphthene	7.21	Acenaphthene-d10	5.0	0.50	20	2.0
2,3,5-Trimethylnaphthalene	7.60	Naphthalene-d8	10	1.0	40	4.0
Fluorene	7.72	Acenaphthene-d10	5.0	0.50	20	2.0
Terphenyl-d14 surrogate	11.34	Chrysene-d12				
Dibenzothiophene	8.58	Phenanthrene-d10	5.0	0.50	20	2.0
Phenanthrene	8.72	Phenanthrene-d10	10	1.0	40	4.0
Anthracene	8.78	Phenanthrene-d10	6.0	0.60	24	2.4
1-Methylphenanthrene	9.56	Phenanthrene-d10	6.0	0.60	24	2.4
Fluoranthene	10.48	Phenanthrene-d10	6.0	0.60	24	2.4
Pyrene	10.87	Chrysene-d12	5.0	0.50	20	2.0
Benzo(a)anthracene	13.49	Chrysene-d12	5.0	0.50	20	2.0
Chrysene	13.58	Chrysene-d12	6.0	0.60	24	2.4
Benzo(b)fluoranthene	16.08	Perylene-d12	7.0	0.70	28	2.8

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Analyte	Approximate Retention Time (min)	Associated Internal Standard	MDL ug/L	MDL ug/kg	PQL ug/L	PQL ug/kg
Benzo(k)fluoranthene	16.11	Perylene-d12	5.0	0.50	20	2.0
Benzo(a)pyrene	16.81	Perylene-d12	5.0	0.50	20	2.0
Indeno(1,2,3-cd)pyrene	19.30	Perylene-d12	5.0	0.50	20	2.0
Dibenzo(a,h)anthracene	19.50	Perylene-d12	5.0	0.50	20	2.0
Benzo(g,h,i)perylene	19.69	Perylene-d12	5.0	0.50	20	2.0

TABLE 4. MRM method acquisition parameters

Analyte	Time segment/time segment start time	Quantifier transition ions	Collision energy (V) quant	Qualifier transition ions	Collision energy (V) qual
Nitrobenzene –d5	TS1/3.51	128-82.1	14	82.1-54.2	15
Naphthalene-d8	TS2/4.70	136-108.1	25		
Naphthalene	TS2/4.70	128.1-102.1	30	128.1-127.1	22
2-Methylnaphthalene	TS3/5.60	142-115	30	142-141	30
1-Methylnaphthalene	TS3/5.60	142-115	30	142-141	30
2-Fluorobiphenyl	TS4/6.40	172-171.1	15	172-172	15
Biphenyl	TS4/6.40	154-153	25	154-152	25
2,6 Dimethylnaphthalene	TS4/6.40	156-141	30	156-115	30
Acenaphthylene	TS4/6.40	152-150	40	152-151	40
Acenaphthene-d10	TS4/6.40	164-162.2	20		
Acenaphthene	TS4/6.40	154-153	40	153-152	40
2,3,5-Trimethylnaphthalene	TS5/7.40	170-155	25	170-153	25
Fluorene	TS5/7.40	166-165.1	35	166-139.1	45
Dibenzothiophene	TS6/8.20	184-139	40	184-152	40
Phenanthrene-d10	TS6/8.20	188-160.2	28		
Phenanthrene	TS6/8.20	178.1-152.1	26	178.1-151.1	38
Anthracene	TS6/8.20	178.1-152.1	26	178.1-151.1	38
1-Methylphenanthrene	TS7/9.15	192-191	25	192-165	30
Fluoranthene	TS8/21.51	202.1-200.2	40	202.1-202.1	10
Pyrene	TS8/22.68	202.1-200.2	45	202.1-202.1	10
Terphenyl-d14	TS8/23.20	244.2-240.2	30	244.2-242.2	18
Benzo(a)anthracene	TS9/12.60	228-226.2	35	228-202.2	35
Chrysene-d12	TS9/12.60	240-236.2	42		
Chrysene	TS9/12.60	228-226.2	35	228-202.2	35
Benzo(b)fluoranthene	TS10/15.40	252-250	42	250-248	40
Benzo(k)fluoranthene	TS10/15.40	252-250	42	250-248	40
Perylene-d12	TS10/15.40	264-260.1	40		
Benzo(a)pyrene	TS10/15.40	251.9-250.2	40	251.9-226.2	50
Indeno(1,2,3-cd)pyrene	TS11/30	276-274	42	276-276	35
Dibenzo(a,h)anthracene	TS11/30	278-276	38	278-278	35
Benzo(g,h,i)perylene	TS11/30	276-274	42	276-276	35

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TABLE 5: QC MEASUREMENTS AND ACCEPTACE CRITERIA

QC MEASUREMENT	ACCEPTANCE CRITERION	CORRECTIVE ACTION
LFB, Matrix Spike Precision	≤30%	Re-analyze/Re-extract/Flag
LFB, Matrix Spike Recoveries	40-140%	Re-analyze/Re-extract/Flag
ICV	±30%	Rerun/recalibrate/flag
CCV	±20%	Re-run and recalibrate/Flag
Surrogate Recovery	30-150%	Reanalyze/Re-extract/Flag
Internal std recovery	50-200%	Reanalyze/recalibrate/flag
Instrument Performance check	< 20% DDT degradation, tailing factor more than 2.0 for pentachlorophenol	Perform instrument maintenance, rerun the performance check sample
Method Blank	<MDL	Rerun/re-extract; qualify samples with “V” if up to 10X the value in method blank.
% cal drift	± 30% of expected value	Change fit/ recalibrate/flag
Linearity Criterion	20% RSD 0.995 CC	Use appropriate cal. fit / Re-run/ Recalibrate

Statistical recovery limits for LFB/MS and surrogate recoveries are typically based on QC Manager statistics for the last two to five calendar years (if available). The limits are recalculated at least annually. The results of statistical calculations are saved at FLDEP1\DEAR\LABS\LAB5\CHEMISTRY\ORGANIC\QC LIMITS STATS. The statistical recovery limits are then uploaded into QC Manager limits tables. The initial QC limits are replaced with statistical ones whenever enough data points are accumulated. For more details refer to ref 15.12 chapter 5.3.

Appendix of Significant Changes

04/06/2016 This SOP replaces two former SOPs, SOP-017-1.2 and SOP-019-1.2.

02/09/2022 Calibration chapter updated. Corrections to references. Re-extraction rules for field blanks added. Statistical limits calculations updated. Updated to method 8270E. Updated table 5.

01/17/2023 Clarification of re-extraction rules in case of MBLK contamination.

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