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Quality Systems and Implementation Plan for Human Exposure Assessment

The University of Arizona
Tucson, Arizona 85721

Cooperative Agreement CR 824719

Standard Operating Procedure **SOP-BCO-L-30.0**

Title: Analysis of Samples for PAH by GC/MS

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Analysis of Samples for PAH by GC/MS

1.0 Purpose and Applicability

This standard operating procedure (SOP) describes the methods used for the detection and quantification by GC/MS of PAH in a variety of matrices, including air, house dust, and soil. Other SOPs (BCO-L-27.0 and BCO-L-28.0) detail the extraction of these samples. This analysis involves automated GC/MS analysis using a high-resolution capillary column, electron impact ionization, and selected ion monitoring.

2.0 Definitions

2.1 Extract: the 1.0-mL sample volume that contains native analytes, surrogate recovery standards, and internal standards. Each sample (dust, soil, air) is reduced to this type of extract for analysis.

3.0 References

3.1 Hewlett Packard 5973 Mass Selective Detector Hardware Manual

3.2 Hewlett Packard 6890 series GC Operator's Manual, HP Part No G1530-90310.

3.3 Hewlett Packard 6890 series GC Maintenance and Troubleshooting, HP Part No G1530-90320

3.4 On-Line Hewlett Packard ChemStation Software Manual B.02.05.

4.0 Discussion

4.1 For analysis, a small aliquot of the extract (2 μL) is injected splitless into a heated GC injector (290°C). Analytes and solvent vapors are swept onto the GC column by the helium carrier gas. The GC column temperature is then increased (temperature-programmed) and the components eluting from the column are identified and quantified by mass spectrometry (MS). Component identification is normally accomplished on the basis of the GC retention time and mass spectral fragmentation characteristics.

4.2 Both the range and limit of detection depend strongly on the properties of the individual compounds of interest. Absolute limits of detection may vary from a few picograms (pg) to a few nanograms (ng), depending on the multiplier voltage

used. Precision also depends greatly on the chemical; for GC introduction, repeatability is typically ± 20% at a 0.1-ng level.

5.0 Responsibilities

5.1 The GC/MS analyst will have been trained in the use and maintenance of the GC/MS instrument, and this will be verified either through the use of training records or through years of service at Battelle with known participation in programs where GC/MS operating skill was required.

5.2 It will be the responsibility of the Mass Spectrometry Laboratory Director to certify that GC/MS operators are fully trained.

5.3 It will be the responsibility of the analyst to verify the correct operation of the instrument, through mass calibration and standard calibration curve analyses, prior to analysis of samples. It will be his/her responsibility to promptly report any deviations in instrument performance to the Laboratory Director, to seek guidance in the correction of the problems that go beyond routine repairs and maintenance, and to re-analyze samples which were acquired under unacceptable instrument operating conditions.

5.4 The Laboratory Director will review data before submission, and will review especially high end and low-end sample analyses for correct interpretation of the data (e.g., was signal saturated, was signal-to-noise ratio sufficient for quantification, etc.).

6.0 Materials and Reagents

6.1 Materials

6.1.1 60 m x 0.32 mm id fused silica GC column coated with nonpolar 5% phenyl methylsilicone stationary phase (DB-5 or equivalent).

6.1.2 Automated Hewlett-Packard gas chromatograph/mass selective detector (6890/5973A GC/MSD) equipped with an autosampler. The instrument can be operated in either the full-scan (SCAN) or selected ion monitoring (SIM) mode. In the SCAN mode, the detector scans all masses repeatedly during the GC run between a lower and an upper mass limit. This mode is best suited for analyzing unknown compounds because it provides a complete mass spectrum for each GC peak. The mass spectrum may then be used to identify the compound using a computer-based compilation of standard spectra along with a suitable library search algorithm. In the SIM

mode, the mass spectrometer monitors only pre-selected ions, rather than scanning all masses continuously between two mass limits. This results in increased sensitivity. With the GC/MSD system in the SIM mode, detection limits of ~0.001 µg/mL in the extract are attainable. For the present purposes, the SCAN mode will be employed initially to set up the parameters for the SIM mode, and the extracts will be analyze in the SIM mode.

6.1.3 Microliter syringes, 10 µL, for injection of liquid standards and sample extracts into GC/MS system.

6.2 Reagents

6.2.1 Helium carrier gas (purity >99.995%).

6.2.2 Perfluorotributylamine (FC-43) for MS standardization.

6.2.3 Calibration solutions, as detailed in SOP BCO-L-26.0.

6.2.4 Methyl-t-butyl ether (MTBE); high purity.

6.2.5 Dichloromethane (DCM); high purity.

7.0 Procedure

7.1 Initial Preparations

7.1.1 GC/MS Instrument Set-Up

7.1.1.1. The helium sweep flow (across the GC injector septum) and carrier gas flow are set at 3-5 mL/min and 1-2 mL/min, respectively.

7.1.1.2 The column is placed in the GC oven and the column flow is set. The column is conditioned initially at 70°C, then increased to 300°C at 2°/min, and held at 300°C for at least 1 hour.

7.1.1.3 The MS and data systems are set according to the manufacturer's instructions. Electron ionization (70 eV) and an electron multiplier gain of about 10^5 should be used. Once the entire GC/MS system has been set up, the system is calibrated as described in Section 7.1.3.

7.1.1.4 The injector module of the HP 6890/5973 autosampler is positioned on the injection port of the GC. It houses the syringe holder and a "turret"-type sample tray with space for 5 vials (one to three samples, one wash and one waste bottle), and associated electronics to perform the injection sequence. A tray module, which can position any one of 100 vials (sample extracts) in the injector, is located adjacent to the injector module. The turret tray rotates the 5 vials into position directly below the syringe. Settings for the sample volume (1 - 5 μL), number of injections per sample (1 - 4), number of sample pre-washes (0 - 10), and number of solvent post-washes (0 - 10) are selected through the front panel of the controller unit. Samples are loaded in the tray module in the order in which they are to be analyzed.

7.1.2 Daily GC/MS Tuning and Standardization

7.1.2.1 Once daily, the GC/MS system must be tuned according to manufacturer's instructions, to verify that acceptable performance criteria are achieved.

7.1.2.2 To tune the GC/MS, FC-43 is introduced directly into the ion source via the molecular leak. The instrumental parameters (i.e., lens voltages, resolution, etc.) are adjusted to give documented, standard relative abundance as well as acceptable resolution (i.e. baseline mass resolution) and Gaussian peak shape. If the instrument fails to tune under autotune conditions, then the ion source will require cleaning as per the manufacturer's instructions, or other corrective issues must be considered and carried out.

7.1.2.3 After tuning is complete, output one spectrum of FC-43 from the calibration analyses and store this spectrum in the instrument calibration file folder in the MS laboratory.

7.1.3 Initial Calibration of the GC/MS System

7.1.3.1 Before analyzing a sample set on a new column, or after the instrument has been vented for cleaning or maintenance, calibration runs are performed with the Calibration Standards, under the same conditions used to analyze the field samples.

7.1.3.2 The first standard is analyzed in the SCAN mode to determine the retention time of all target analytes, surrogate standard and internal standard. For the GC/MSD, the SIM window is typically RT ± 5 s for most target PAH and, for a few isomeric PAH, the SIM window range will be manually set up in the first standard analysis, according to the operator's manual.

7.1.3.3 Calibration standards encompass four levels, plus a zero level, that bracket the expected concentration range of interest.

7.1.3.4 A linear response corresponds to a correlation coefficient >0.98 for a linear least squares fit of the concentration versus relative response data.

7.1.3.5 Once response linearity has been demonstrated, samples can be analyzed as described below.

7.2 Preparation of Samples for Analysis

7.2.1 Arrange the sample vials in sets of 20 samples in the following order: one standard, five samples, one standard, five samples, one standard, etc., until 20 samples have been analyzed

7.2.2 Inspect each vial. If any defects are noted, such as low volume with respect to the marked volume line, record them in the "Comments" column of the Data Sheet.

7.2.3 For samples with low volume, dilute to the indicated line with DCM. Recap and analyze.

7.3 Sample Extract Analysis

7.3.1 Sample analysis is accomplished using a 60 m x 0.32 mm id, 0.25 µm film thickness, DB-5 fused silica capillary column. Optimum analytical results are achieved with this column by temperature-programming the GC oven from 70°C for 2 min to 290°C at 8°/min, then holding at 290°C for 20 min. The injection port is held at 290°C.

7.3.2 The data acquisition time and total analysis time (including column bake out after each run) will be set according to the target PAH eluting time and the sample matrices. A specific "method" will be established for each type of sample matrix.

7.3.3 Data processing involves: (1) generating a calibration curve for each target PAH from the results of the standard analyses, (2) calculating the concentrations of target PAH in the sample extracts and in standards with the update calibration curves using HP Chem software, and (3) manually reviewing each data file to ensure that the identified and quantified target peaks are the correct peaks.

7.3.4. The characteristic ions for each analyte include the "target ion", which is used for quantification, and the "qualifier ion", which is used to verify detection on the basis of correct intensity (± 30%) relative to the target ion intensity.

7.3.5 For sample extracts where PAH levels exceed the calibration range of standards, prepare dilutions at 1:10 and 1:100 for re-analysis.

7.3.5.1 For 1:10 dilutions, add 890 µL of DCM to a muffled/silylated GC vial, add 9 µL of the IS spiking solution, and 100 µL of the sample extract. Cap the vial, invert several times to mix, label, and analyze as per steps 7.3.1-7.3.6.

7.3.5.2 For 1:100 dilutions, add 980 µL of DCM to a muffled/silylated GC vial, add 10 µL of the IS spiking solution and 10 µL of the sample extract. Cap the vial, invert to mix, label, and analyze as per steps 7.3.1-7.3.6

7.4 Calculations

7.4.1 Extract Concentrations

7.4.1.1 The relative area (A_S/A_{IS}) of the target ions and analyte concentration values (C_s) are analyzed using a standard linear regression analysis routine (e.g., HP Chem) to calculate the slope (m) and the intercept (b) from the equation $y = mx+b$, where y is the relative area (A_S/A_{IS}), and x is the analyte concentration in µg/mL. These data are taken from the standards that are analyzed concurrently with a given sample set.

7.4.1.2 Once the slope and intercept have been established for each analyte, then these values can be input into the same equation ($y = mx+b$), together with the A_S/A_{IS} (y) value from a sample, to obtain the corresponding concentration value (x).

7.4.1.3 For diluted samples, multiply the solution concentration obtained by the appropriate factor (10 or 100) to obtain the concentration of the original sample extract.

7.4.2 Calculation of Target Compound Content of Field Samples

7.4.2.3 For dust or soil samples, multiply the analyte concentration (µg/mL) by the volume (1.0 mL); then divide by the amount of material extracted (g). This gives the dust or soil concentration (µg/g or ppm). To obtain the surface loading of dust samples (µg/m^2), multiply the analyte concentration in dust (µg/g or ppm) with the <62 µm dust loading (g/m^2).

7.4.2.4 For air concentrations (µg/m^3), multiply the analyte concentration (µg/mL) by the extract volume (1.0 mL) and divide by the air volume sampled (typically 11.52 m^3 for a sampling rate of 4 L/min for 48 h). Multiply this air concentration by 1,000 to obtain the air concentration in ng/m^3.

7.4.2.5 To determine the recovery of the surrogate recovery compound, multiply its concentration in the extract (µg/mL) by the volume of the extract (1.0 mL); divide this value by the amount spiked (µg) and multiply by 100%.

7.4.3 Limit of Detection

7.4.3.1 The limit of detection LOD for a target compound is obtained from the above data. It is defined as:

$$LOD = A + 3.3\sigma \qquad (1)$$

where A = intercept (coefficient) from the least squares fit to the calibration curve; and s = standard deviation of the lowest concentration measurements.

7.5 Quality Control

7.5.1 Instrument Tuning and Standardization

7.5.1.1 Refer to Section 7.1.2. These procedures provide a means of monitoring MS performance characteristics over time, and permanent records of the information are kept in the laboratory.

7.5.2 Calibration for Quantitative Analysis

7.5.2.1 Refer to the quantitative analysis calibration procedures in Sections 7.1.3 and 7.4. If quantitative responses (in area counts) of the lowest level standard mixture fall below the detection limits, the instrument and/or GC column and injector must be checked for performance degradation. The injector and/or ion source should be cleaned or the first 0.5 m of the column should be removed and replaced with a new retention gap.

7.5.2.2 Those samples which were analyzed during the period when low level standards were not detected will be reanalyzed.

8.0 Records

8.1 All operations, maintenance and performance calibration data are stored in each instrument's log book.

8.2 All analytical results are logged in specific project books.

8.3 Hardcopy output and disk copy of QUAN reports will be generated after the data are reviewed by the qualified analyst. For each analysis set, one file folder will be used to hold/archive the hardcopy output of QUAN reports (samples and standards), and the calibration curve. The calibration curve sheet lists the slope, intercept, and fit (r^2) for the data. The QUAN report lists the file name and sample name together with the calculated concentration. The sample name includes the 9 digit laboratory notebook number (for internal tracking) and the UA field ID number. These hardcopy files are kept in the pesticide/PAH extraction laboratory for reference and comparison of instrument performance until the end of the program. At the completion of the program, they will be stored in the office of the Battelle PI.

8.4 All data files are stored on ZIP disks for permanent record. The disks are stored permanently in the GC/MS laboratory as part of the GC/MS laboratory records

8.5 Final calculations of the data are performed in Excel spreadsheets and stored on removable disks. All data are entered into the project database and sent to UA after one-over-one review of the data. The final data results are stored on removable disks in the office of the Battelle co-PI.

8.6 A Laboratory Data Sheet is shown in Figure 1. One sheet is filled out and filed with the hard copy output for the sample set.

Laboratory Data Sheet – Project PAH Analyses

Date _____

Analyst _____

Standards LRB #s _____

Helium Gas Cylinder ID # _____

Date Received _____

GC Column ID _____

Serial # _____

Date Installed _____

GC Carrier Gas Flow _____

GC Temperature Program _____

_____

_____

_____

Autosampler Settings

injections/sample _____

injection volume (µL) _____

syringe pre-rinse _____

syringe post-rinse _____

Comments:

Figure 1. Example of laboratory data sheet for analysis of PAH by GC/MS.