

The Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) Study

Detection and Quantification of Target Analytes by Gas Chromatography/Mass Spectrometry (GC/MS)

Battelle
Columbus, OH 43201
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Standard Operating Procedure

CTEPP-SOP-5.24

Title: Detection and Quantification of Target Analytes by Gas Chromatography/Mass Spectrometry (GC/MS)

Source: Battelle

U.S. Environmental Protection Agency
Office of Research and Development
Human Exposure & Atmospheric Sciences Division
Exposure Measurements & Analysis Branch

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STANDARD OPERATING PROCEDURE (SOP)
FOR DETECTION AND QUANTIFICATION OF TARGET ANALYTES
BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

Prepared by: _____ Date: _____

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1.0 Scope and Applicability

This standard operating procedure (SOP) describes the method for detection and quantification of target analytes by gas chromatography/mass spectrometry (GC/MS).

2.0 Summary of Method

This SOP describes the method used for the determination of target analytes in sample extracts and related QA/QC sample extracts generated in CTEPP study.

3.0 Definition

3.1 Extract: The sample extract that contains native target analytes, surrogate recovery standards, and internal standards.

3.2 Equipment: Hewlett Packard gas chromatography/mass selective detector (6890/5973A GC/MSD) equipped with an autosampler.

4.0 Cautions

Standard laboratory protective clothing, gloves, and eye covering is required when aliquoting standard solutions into GC vials for analysis and recapping standard solutions and sample extracts vials after analysis for storage .

5.0 Responsibilities

5.1 The project staff who performs the GC/MS analysis will be responsible for obtaining samples from the sample coordinator, entering relevant information in the extraction/preparation laboratory record books.

5.2 The CTEPP Laboratory Team Leader (LTL), the QA Officer or designee, and Task Order Leader (TOL) will oversee the sample extraction operation and ensure that SOPs are followed by all project staff.

6.0 Apparatus and Materials

6.1 60 m x 0.32 mm id fused silica GC column coated with nonpolar 5% phenyl methylsilicone stationary phase (DB-5 or equivalent).

6.2 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 CTEPP study, the SCAN mode will be employed initially to set up the parameters for the SIM mode, and the extracts will be analyzed in the SIM mode.

- 6.3 Microliter syringe, 10 µL, for injection of liquid standards and sample extracts into GC/MS system.
- 6.4 Perfluorotributylamine, FC-43 (Aldrich or equivalent).

7.0 Procedure

- 7.1 GC/MS Instrument Set-Up.
 - 7.1.1 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.
 - 7.1.2 The column is installed in the GC oven and the column flow is set. The GC column temperature program is set.
 - 7.1.3 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 7.4.
 - 7.1.4 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.

7.2 Daily GC/MS Tuning and Standardization.

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

7.2.2 To tune the GC/MS, Perfluorotributylamine, 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.

7.2.3 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.

7.3 Calibration of the GC/MS system.

7.3.1 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.

7.3.2 The first standard is analyzed in either the SCAN mode or the SIM mode to determine the retention time and the mass of all target analytes, surrogate standard and internal standard. For the GC/MS, the SIM window is typically $RT \pm 5$ seconds for most target analytes and, for a few isomeric analytes, the SIM window range will be manually set up in the first standard analysis, according to the operator's manual.

7.3.3 A multi-point calibration curve will be constructed with calibration standards that typical encompasses a range at least one order of magnitude.

7.3.4 An average response factor (Rf) of each target analyte is generated from the multi-point calibration curve. The percent relative standard deviation (% RSD) of the calculated Rf values in all the calibration solutions should be within $\pm 25\%$. If the % RSD values of some analytes are greater than $\pm 25\%$, the GC/MS system should be checked to determine the sources for this variation. Corrective actions (i.e., cleaning the source) will be taken and another multi-point calibration curve will be generated. A secondary source standard (NIST), if available, will be used at mid-range of the calibration to serve as a calibration check for the accuracy of the calibration standard solutions. The calibration will be deemed acceptable if the secondary standard results in a relative percent difference (%)

RPD) $\leq \pm 30\%$. If the linear regression method is used for the calculation, the correlation coefficient value (r) should be ≥ 0.99 .

7.4 Sample Analysis Sequence:

- 7.4.1 A sample analysis sequence is set up that consists of standard solutions and sample extracts.
- 7.4.2 Typically standard solutions and the sample extracts are bracket in a analysis sequence, the standard solutions are analyzed after approximately every five samples.
- 7.4.3 A FC-43 tuning is checked approximately every 24 hours. After this sequence run is completed, the multi-range calibration curves are generated based on the analyses of the standard solutions. The Rf value of each analyte is checked to ensure that the % RSD is within $\pm 25\%$. The calculated values of the standard solutions are checked to ensure that the % RPD is within $\pm 30\%$ of the expected values. If the calibration check is outside of the 25% RSD or 30% RPD, corrective actions will be taken (i.e., cleaning the source) will be taken and another multi-point calibration curve will be generated

7.5 Sample Extract Analysis.

- 7.5.1 Sample analysis is accomplished using a 60 m x 0.32 mm id, 0.25 μm film thickness, DB-5 fused silica capillary column (or equivalent GC column). A typical GC temperature program is to set the initial GC temperature at 70° C for 2 minutes and then to 150° C at 15° C/minute; then to 290° C at 6° C/minute and at 290° C for 28 minutes. The GC temperature program may change to obtain better analyte resolution, depending upon the compound classes that are analyzed.
- 7.5.2 The data acquisition time and total analysis time (including column bake out after each run) will be set according to the target analyte's elution time and sample matrices.
- 7.5.3 Data processing involves: (1) generating a calibration curve for each target analyte from the results of the standard analyses, (2) calculating the concentrations of target analytes in the sample extracts and in standards with 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.
- 7.5.4 The characteristic ions for each analyte include the "primary ion", which is used to quantification and the "secondary ion", which is used for confirmation.

7.6 Calculations

- 7.6.1 The Rf value is generated using $Rf = (A_s/A_{IS}) * (C_{IS}/C_s)$ from the analyses of standard solutions used in the multi-point calibration curve. A_s is the analyte area count; A_{IS} is the internal standard area count; C_{IS} is the internal standard concentration; and C_s is the analyte concentration. The C_s value is obtained from $(A_s/A_{IS}) * (C_{IS}/Rf)$, where Rf values are the average Rf values generated from the multi-point calibration curve.
- 7.6.2 The HP Chem software will process these calculations and a QUAN report will be generated for each sample or standard. The reporting unit is pg/ μ L or ng/mL.
- 7.6.3 The data generated from HP Chem software will be electronically input into an EXCEL spreadsheet for calculation of target analytes in the samples.

8.0 Records

- 8.1 All operations, maintenance, and performance calibration data are stored in each instrument's logbook.
- 8.2 All analytical results are logged in specific study folders.
- 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 QUAN report lists the file name and sample name together with the calculated concentration. These hardcopy files are kept in the individual study folder for reference and comparison of instrument performance until the end of the program. All records will be archived for three years after the completion of the study.
- 8.4 All data files are stored on ZIP disks or CD for permanent facility records. 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 CTEPP analytical database.

9.0 Quality Control and Quality Assurance

- 9.1 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 ~1 m of the column should be removed.

9.2 Those samples that were analyzed during the period when:

- (1) % RSD of Rf values for the calibration standard solutions is $> \pm 25\%$ or;
- (2) % RPD of the NIST standard solution (if available) against certified values is $> \pm 30\%$ or;
- (3) % RPD of the calibration standard solutions is $> \pm 30\%$, will be reanalyzed.

10.0 Reference

- 10.1 J. C. Chuang, C. Lyu, Y-L Chou, P. J. Callahan, M. Nishioka, K. Andrews, M. A. Pollard, L. Brackney, C. Hines, D. B. Davis, and R. Menton, "Evaluation and Application of Methods for Estimating Children's Exposure to Persistent Organic Pollutants in Multiple Media." EPA/600/R-98/164a, EPA/600/R-98/164b, and EPA/600/R-98/164c (Volume I, II, and III), 1999.
- 10.2 Hewlett Packard 5973 Mass Selective Detector Hardware Manual.
- 10.3 Hewlett Packard 6890 Series GC Operator's Manual, HP Part No. G1530-90310.
- 10.4 Hewlett Packard 6890 Series GC Maintenance and Troubleshooting, HP Part No. G1530-90320.
- 10.5 On-Line Hewlett Packard ChemStation Software Manual B.02.05.