United States Environmental Protection Agency

Office of Research and Development

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

Preparation of Surrogate Recovery Standard and Internal Standard Solutions for Neutral Target Analytes

Battelle
Columbus, OH 43201
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Standard Operating Procedure **CTEPP-SOP-5.25**

Title: Preparation of Surrogate Recovery Standard and Internal Standard Solutions for Neutral Target Analytes

Source: Battelle

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

STANDARD OPERATING PROCEDURE (SOP) FOR THE PREPARATION OF SURROGATE RECOVERY STANDARD AND INTERNAL STANDARD SOLUTIONS FOR NEUTRAL TARGET ANALYTES

Prepared by: _____ Date: _____

Reviewed by: _____ Date: _____

Approved by: _____ Date: _____

Approved by: _____ Date: _____

Approved by: _____ Date: _____

1.0 Scope and Applicability

This standard operating procedure (SOP) describes the method for preparing surrogate recovery standard (SRS), internal standard (IS), and calibration standard solutions for the analysis of neutral target analytes.

2.0 Summary of Method

This SOP describes the method used for preparing SRS and IS solutions for the analysis of neutral target analytes. The method for preparing calibration standard solutions for neutral analytes used for GC/MS analysis is also included.

3.0 Definition

3.1 Surrogate Recovery Standard (SRS): the compounds that are used for QA/QC purposes to assess the extraction and recovery efficiency obtained for individual samples. Known amounts of these compounds are spiked into the sample prior to extraction. The SRSs are quantified at the time of analysis and their recoveries indicate the probable extraction and recovery efficiency for native analytes that are structurally similar. The SRSs are chosen to be as similar as possible to the native analytes of interest, but they must not interfere in the analysis.

3.2 Internal Standard (IS): the compounds that are added to sample extracts just prior to GC/MS analysis. The ratio of the detector signal of the native analyte to the detector signal of the corresponding IS is compared to ratios obtained for calibration curve solutions where the IS level remains fixed and the native analyte levels vary. The IS is used to correct for minor run-to-run differences in GC injection, chromatographic behavior, and MS ionization efficiency.

4.0 Cautions

Standard laboratory protective clothing, gloves, and eye covering are required.

5.0 Responsibilities

5.1 The project staff who prepare the SRS, IS and calibration standard solutions will be responsible for 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 preparation of the standard solutions and ensure that SOPs are followed by all project staff.

6.0 Apparatus and Materials

6.1 Materials

6.1.1 Balance with at least four-place accuracy (x.xxxx g)

6.1.2 Volumetric flasks

6.1.3 Analytical Syringes

6.1.4 Large Kim-wipes (15" x 15")

6.1.5 Latex gloves

6.1.6 4 dram glass vials with Teflon-lined screw caps; muffled

6.1.7 1.8 mL glass GC vials with Teflon-lined screw caps; muffled

6.1.8 Disposable glass pipettes (muffled and stored in clean glass jar)

6.1.9 Vortex mixer (American Scientific Products or equivalent)

6.2 Reagents

6.2.1 Chrysene (ChemService or Supelco)

6.2.2 Benz[a]anthracene (ChemService or Supelco)

6.2.3 Benzo[b]fluoranthene (ChemService or Supelco)

6.2.4 Benzo[k]fluoranthene (ChemService or Supelco)

6.2.5 Benzo[e]pyrene (ChemService or Supelco)

6.2.6 Benzo[a]pyrene (ChemService or Supelco)

6.2.7 Indeno[1,2,3-c,d]pyrene (ChemService or Supelco)

6.2.8 Benzo[g,h,i]perylene (ChemService or Supelco)

6.2.9 Dibenzo[a,h]anthracene (ChemService or Supelco)

6.2.10 Benzylbutylphthalate (ChemService or Supelco)

6.2.11 Di-n-butyl phthalate (ChemService or Supelco)

6.2.12 Bendiocarb (ChemService or Supelco)

6.2.13 2,6-Dichlorobiphenyl (ChemService or Supelco)

6.2.14 4,4'-Dichlorobiphenyl (ChemService or Supelco)

6.2.15 2,4,4'-Trichlorobiphenyl (ChemService or Supelco)

6.2.16 2,2',5,5'-Tetrachlorobiphenyl (ChemService or Supelco)

6.2.17 2,2',3,5'-Tetrachlorobiphenyl (ChemService or Supelco)

6.2.18 2,3',4',5-Tetrachlorobiphenyl (ChemService or Supelco)

6.2.19 3,3',4,4'-Tetrachlorobiphenyl (ChemService or Supelco)

6.2.20 2,2',3,5'6-Pentachlorobiphenyl (ChemService or Supelco)

6.2.21 2,2',4,5,5'-Pentachlorobiphenyl (ChemService or Supelco)

6.2.22 2,2',3,4,5'-Pentachlorobiphenyl (ChemService or Supelco)

6.2.23 2,3,3',4',6-Pentachlorobiphenyl (ChemService or Supelco)

6.2.24 2,3',4,4',5-Pentachlorobiphenyl (ChemService or Supelco)

6.2.25 2,3,3',4,4'-Pentachlorobiphenyl (ChemService or Supelco)

6.2.26 3,3',4,4',5-Pentachlorobiphenyl (ChemService or Supelco)

6.2.27 2,2',4,4',5,5'-Hexachlorobiphenyl (ChemService or Supelco)

6.2.28 2,2',3,4,4',5'-Hexachlorobiphenyl (ChemService or Supelco)

6.2.29 3,3',4,4',5,5'-Hexachlorobiphenyl (ChemService or Supelco)

6.2.30 2,2',3,4,4',5,5'-Heptachlorobiphenyl (ChemService or Supelco)

6.2.31 Lindane (ChemService or Supelco)

6.2.32 Heptachlor (ChemService or Supelco)

6.2.33 Aldrin (ChemService or Supelco)

6.2.34 gamma-Chlordane (ChemService or Supelco)

6.2.35 alpha-Chlordane (ChemService or Supelco)

6.2.36 p,p'-DDE (ChemService or Supelco)

6.2.37 Dieldrin (ChemService or Supelco)

6.2.38 Endrin (ChemService or Supelco)

6.2.39 p,p'-DDT (ChemService or Supelco)

6.2.40 cis-Permethrin (ChemService or Supelco)

6.2.41 trans-Permethrin (ChemService or Supelco)

6.2.42 Atrazine (ChemService or Supelco)

6.2.43 Propoxur (ChemService or Supelco)

6.2.44 Diazinon (ChemService or Supelco)

6.2.45 Chlorpyrifos (ChemService or Supelco)

6.2.46 Pentachloronitrobenzene (ChemService or Supelco)

6.2.47 Phenanthrene-d10, IS (ChemService or Supelco)

6.2.48 Dibromobiphenyl, IS (ChemService or Supelco)

6.2.49 Benzo[e]pyrene-d12, IS (CIL or Supelco)

6.2.50 Dibenzo[a,h]anthracene-d14, SRS (CIL or Supelco)

6.2.51 Atrazine-d5 (Merck), SRS or IS (CIL)

6.2.52 p,p'-DDE-d4, SRS (CIL)

6.2.53 Butyl benzyl phthalate-d4, SRS (CIL)

6.2.54 Cyfluthrin (ChemService or Supeclo)

6.2.55 Diazinon-d10, IS (CIL or Supelco)

6.2.56 2,2',4,5,5'-Pentachlorobiphenyl-C13

6.2.57 Bisphenol-A (ChemService or Supeclo)

6.2.58 Bisphenol-A-d6, SRS (CIL)

6.2.59 n-Nonylphenol (ChemService or Supeclo)

6.2.60 Dichloromethane (DCM); distilled in glass

7.0 Procedures

7.1 Prepare Stock Solutions

7.1.1 Prepare a stock solution at 1 mg/mL for those target analytes that cannot be purchased as stock solutions. Using a four-place balance, weigh approximately 0.0100 g (e.g. 9.9 mg) of the analyte directly into a clean vial. Record the weight in the laboratory record book (LRB). *Note that bendiocarb, propoxur, and atrazine were removed from the target analyte list during the CTEPP study because of interferences present in the sample extracts.*

7.1.2 Add approximately 8 mL of dichloromethane (DCM) to the vial; mix well. Transfer the mixture to a 10 mL volumetric flask. Add approximately 1 mL of the DCM to the vial; mix well; and transfer to the 10 mL volumatric flask. Continue add the rinse to the 10 mL volumetric flask to the 10 mL mark and record the exact concentration in the LRB.

7.1.3 Label with the LRB number (nine-digit unique code: 5 digit lab notebook number-2 digit page number-2 digit line number on which entered), analyte, concentration, solvent used, and expiration date. Mark the volume with a felt-tip pen on the outside of the vial. Enter the same data in the laboratory notebook on the page where the preparation is described, together with the lot number and manufacturer of the standard.

7.1.4 If the concentrations of the purchased stock solutions are higher than 1 mg/mL, dilute the stock solutions to 1 mg/mL accordingly. Label the solutions following step 7.1.3.

7.1.5 Store the stock solutions at -10˚C or below.

7.2 Prepare SRS and IS Spiking Solutions

7.2.1 For all sample matrices except water, prepare the IS solution in DCM containing phenanthrene-d10 (10 g/mL), dibromobiphenyl (10 g/mL), diazinon-d10 (10 g/mL), and benzo[e]pyrene-d12 (5 g/mL). Remove 100 L of phenanthrene-d10 (1 mg/mL), 100 L of dibromobiphenyl (1 mg/mL), 100 L of diazinon-d10, and 50 L of benzo[e]-pyrene-d12 (1 mg/mL) and place into a 10 mL volumetric flask. Add DCM to the 10 mL mark. Mix well and transfer the solution to a clean glass vial. For each sample extract and calibration standard solution, 10 L of this internal standard solution spiked into each 1 mL extract will give final concentrations at 0.1 g/mL of phenanthrene-d10, dibromobiphenyl, diazinon-d10, and at 0.05 g/mL of benzo[e]pyrene-d12.

7.2.2 For water sample, prepare the IS solution in DCM containing atrazine-d5 (10 g/mL). Remove 100 L of atrazine-d5 (1 mg/mL) and place into a 10 mL volumetric flask. Add DCM to the 10 mL mark. Mix well and transfer the solution to a clean glass vial. Remove 1 mL of the atrazine-d5 (10 g/mL) and place into another 10 mL volumetric flask. Add DCM to the 10 mL mark and the final concentration is 1.0 g/mL. For each sample extract and calibration standard solution, 50 L of this internal standard solution (1.0 g/mL) spiked into each 1 mL extract will give final concentrations at 0.05 g/mL of atrazine-d5

7.2.3 For all sample matrices except water, prepare the SRS spiking solution for containing dibenzo[a,h]anthracene-d14, atrazine-d5, p,p'-DDE-d4, butyl benzyl phthalate-d4, and

bisphenol-d6 at 10 g/mL in DCM. Remove 100 L of each target SRS (1 mg/mL) and inject into a 10 mL volumetric flask. Dilute with DCM to the 10 mL mark. Mix well and transfer the solution to a clean glass vial. Prepare 2,2',4,5,5'-Pentachlorobiphenyl-C13 SRS solution at 10 g/mL in DCM using the same procedures. Remove 1 mL of the SRS solution (10 g/mL) and place into another 10 mL volumetric flask. Add DCM to the 10 mL mark and the final concentration is 1.0 g/mL. The concentrations of the individual SRS compounds may vary using the similar procedures in different sample media. *Note that this SRS solution is used for all but drinking water. There is no SRS used in the drinking water samples. Atrazine-d5 was remove from the SRS spiking solution during the CTEPP study because atrazine was removed from the target analyte list.*

7.2.4 Label the SRS spiking solution following step 7.1.3.

7.2.5 Store the spiking solutions at -10°C or below.

7.3 Prepare Calibration Standard Solutions

7.3.1 Prepare three calibration matrix spike solutions at 1 g/mL in DCM: one for target PCB (compounds listed in sections 6.2.13 to 6.2.30), one for target PAH, neutral pesticides, phthalates, and phenols (compounds listed in sections 6.2.1 to 6.2.12 and 6.2.31 to 6.2.46, 6.2.53, 6.2.56, and 6,2,58), and one for atrazine (6.2.42). Add 10 L of each 1 mg/mL stock solution to a 10 mL volumetric flask and dilute to volume. *Note that bendiocarb, propoxur, and atrazine were removed from the calibration matrix spike solution because these compounds were removed from the target analyte list during the CTEPP study.*

7.3.2 Pour the solutions into clean screw-cap vials, cap, and mix. Label the solutions following step 7.1.3.

7.3.3 Prepare calibration solutions from each of the calibration matrix spike, SRSs and ISs standard solutions from 7.2.2, 7.2.3 and 7.3.1 to be used for GC/MS analysis. The concentration ranges for the calibration standard solutions will depend upon the sample matrix. A typical example is given in Table 1. Aliquot each solution according to Table 1 to a 10 mL volumetric flask and add DCM to the mark of 10 mL. Note that the 0-level standard solution is used as a QA/QC standard for the instrument blank and the solvent blank, but not for the calibration curve. Concentrations of the calibration standard solutions may varied among different sample matrices.

7.3.4 Store the calibration standard solutions at -10°C or below.

Table 1. GC/MS Calibration Standard Solutions

Concentration, g/mL analyte/SRS/IS	L of 1 g/mL of analyte aliquoted	L of 1.0 g/mL of SRS aliquoted	L of 10 g/mL of IS aliquoted
0.0/0.0/0.0	0	0	0
0.005/0.005/0.1	50	50	100
0.01/0.01/0.1	100	100	100
0.03/0.03/0.1	300	300	100
0.1/0.1/0.1	1000	1000	100
0.2/0.2/0.1	2000	2000	100

8.0 Records

8.1 Records of the preparation of standard solutions will be retained in a project laboratory record book that is kept in the extraction laboratory. This record book will serve as a continuing file for reference on expected performance of the methods. These standard solutions will be identified in the laboratory record book by the assigned laboratory analysis number (a unique number that combines the 5 digit laboratory book number-2 digit page number-2 digit line number), the lot number of DCM used for preparation, the date of preparation, and the expiration date.

8.2 The record of the preparation of the standard solutions will be maintained in a project laboratory notebook that is kept in the extraction laboratory. The record book will be retained in the laboratory where these operations are performed until the conclusion of the study and will be archived in a secure room for three years after completion of the study.

9.0 Quality Control and Quality Assurance

9.1 The 0-level standard solution serves as the QA/QC standard for these calibration solutions. The presence of native analytes or a surrogate in these solutions will indicate either carryover from the previous GC/MS run or contamination in the laboratory; either condition requires appropriate corrective actions. For carryover, indicated by proportionately equivalent amounts of all analytes from the previous run, the time that the split valve is closed during injection can be lengthened. For laboratory contamination,

indicated by random amounts of analytes in the "zero" standard, the standards must be prepared again, with greater caution used in cleaning syringes and glassware.

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.