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 Polar Target Analytes

Battelle
Columbus, OH 43201
Contract No. 68-D-99-011

Standard Operating Procedure **CTEPP-SOP-5.26**

Title: Preparation of Surrogate Recovery Standard and Internal Standard Solutions for Polar 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 POLAR TARGET ANALYTES

Prepared by: _____ Date: _____

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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 polar target analytes.

2.0 Summary of Method

This SOP describes the method used for preparing SRS and IS solutions for the analysis of polar target analytes. The method for preparing calibration standard solutions for polar 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 Silylated 4 dram glass vials with Teflon-lined screw caps; muffled

6.1.7 Silylated 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 Pentachlorophenol (ChemService or Supelco)

6.2.2 2,4-D (ChemService or Supelco)

6.2.3 Dicamba (ChemService or Supelco)

6.2.4 2,4,5-T (ChemService or Supelco)

6.2.5 3,5,6-Trichloro-2-pyridinol (3,5,6-TCP), ChemService or Supelco

6.2.6 Labeled TCP, Carbon-13 and Nitrogen-15 isotopes ($^{13}C^{15}N$-TCP), IS (synthesized by Dow-Elanco, reference number B-844-167A)

6.2.7 Dicamba-d3, IS (ChemService or Supelco)

6.2.8 C13 2,4-D, SRS (ChemService or Supelco)

6.2.9 Dichloromethane (DCM)

6.2.10 Acetonitrile (ACN)

6.2.11 2-Isopropyl-4-methyl-6-hydroxypyrimidine (IMP)

6.2.12 1-hydroxypyrene

6.2.13 1-Hydroxy benz[a]anthracene

6.2.14 6-hydroxy chrysene

6.2.25 3-Hydroxy chrysene

6.2.26 3-Hydroxy benz[a]anthracene

6.2.27 3-Phenoxy benzoic acid (3-PBA)

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.

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 laboratory notebook 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 and 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 Prepare the IS solution in DCM containing dicamba-d3, and $^{13}C^{15}N$-TCP at 10 g/mL. Remove 100 L of dicamba-d3 (1 mg/mL), and 100 L of $^{13}C^{15}N$-TCP (1 mg/mL) and inject 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 of 0.1 g/mL of dicamba-d3, and $^{13}C^{15}N$-TCP.

7.2.2 Label the IS solution following step 7.1.3.

7.2.3 Prepare the SRS spiking solution containing C13 2,4-D at 1.0 g/mL in DCM. Remove 100 L of C13 2,4-D (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. Dilute the 10 g/mL SRS solution with ACN to 1.0 g/mL for acid SRS spiking solution.

7.2.4 Label the SRS spiking solution following step 7.1.3.

7.2.5 Store the spiking solutions at -10ºC or below.

7.3 Prepare Calibration Standard Solutions

7.3.1 For all sample matrices except urine, prepare a calibration matrix spike solution at 1 g/mL in DCM for all target analytes (compounds listed in sections 6.2.1 to 6.2.5, and 6.2.11). Add 10 L of each 1 mg/mL stock solution to a 10 mL volumetric flask and dilute to volume. For urine sample, prepare a calibration matrix spike solution at 1 g/mL in DCM for all target analytes (compounds listed in sections 6.2.1, 6.2.2, 6.2.5, and 6.2.11 to 6.2.27). Add 10 L of each 1 mg/mL stock solution to a 10 mL volumetric flask and dilute to volume.

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 mixed solutions from 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 zero-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.

Table 1. GC/MS Calubration 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
0.02/0.02/0.1	200	200	100
0.05/0.05/0.1	500	500	100
0.1/0.1/0.1	1000	1000	100

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

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 zero-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 handling. 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 and analyzed, 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.