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The University of Arizona
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Standard Operating Procedure **SOP-BCO-L-21.1**

Title: Preparation of Calibration and Surrogate Recovery Solutions for GC/MS Analysis of Pesticides

Source: The University of Arizona

U.S. Environmental Protection Agency
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Human Exposure & Atmospheric Sciences Division
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Preparation of Calibration and Surrogate Recovery Solutions for GC/MS Analysis of Pesticides

1.0 Purpose and Applicability

This standard operating procedure (SOP) describes procedures for preparing calibration curve solutions used for GC/MS analysis of chlorpyrifos, diazinon, malathion, DDT, DDE, DDD, and γ-chlordane in dust, soil, air, and handwipe sample extracts. It also covers preparation of the surrogate recovery standard (SRS) spiking solution, the internal standard (IS) spiking solution, and the field spiking solution.

2.0 Definitions

2.1 Surrogate Recovery Standard (Surrogate or SRS): The compound that is used for QA/QC purposes to assess the extraction and recovery efficiency obtained for individual samples. A known amount of this compound is spiked into the collected sample (dust, soil, dermal wipe, PUF, etc.) prior to extraction. The "surrogate" is quantified at the time of analysis and its recovery indicates the probable extraction and recovery efficiency for native analytes that are structurally similar. The surrogate recovery standard is chosen to be as similar as possible to the native analytes of interest. It must not, however, interfere in the analysis.

2.2 Internal Standard (IS): The compound that is added to sample extracts just prior to GC/MS or GC/ECD analysis. The ratio of the detection signal of the native analyte to the detection signal of the 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, detector efficiency, and final extract volume.

2.3 Calibration Curve Solutions: Those solutions used to calibrate the detector response and quantify analytes in sample extracts. Five solutions are prepared; each solution contains native analytes, surrogate recovery standard and internal standard. The concentrations of the native analytes and the surrogate vary in these solutions; the concentration of the IS remains fixed.

2.4 Field Spike Solution: The solution of native analytes (chlorpyrifos, diazinon, malathion, DDT, DDE, DDD, and γ-chlordane) that is prepared in the laboratory and sent to the field for use. In the field, a small aliquot of this solution is spiked with a syringe onto various field blank sampling media (PUF, hand wipe. For

details see SOPs UA-F-8.1, UA-F-9.1, and UA-F-3.1). These spiked samples are returned for analysis with the other field samples and blanks. The measurement of analyte recovery in these samples is used to assess the effects of sample handling and shipping on the recovery of analytes.

3.0 References

3.1 R.W. Whitmore, F.W. Immermann, D.E. Camann, A.E. Bond, R.G. Lewis, and J.L. Schaum, "Non-occupational Exposures to Pesticides for Residents of Two U.S. Cities," Arch. Environ. Contam. Toxicol., **26**, 47-59 (1994).

3.2 R.G. Lewis, R.C. Fortmann, and D.E. Camann, "Evaluation of Methods for the Potential Exposure of Small Children to Pesticides in the Residential Environment," Arch. Environ. Contam. Toxicol., **26**, 37-46 (1994).

4.0 Discussion

4.1 The calibration solutions described here provide: 1) one surrogate recovery compound that is structurally similar to chlorpyrifos, diazinon, and malathion; 2) one surrogate recovery compound specifically for DDT; 3) one surrogate recovery compound for the remaining organochlorine pesticides; 4) an internal standard for quantification; 5) a calibration range that will encompass the expected air, soil, and dust concentrations as determined from US EPA residential pesticide exposure studies (Refs. 3.1 and 3.2).

4.2 The calibration curve standards will cover a 100X concentration range of 0.01-1 μg/mL. Concentrations at the low end will cover primarily air and soil samples, while the higher levels bracket sample concentrations in dust samples.

5.0 Responsibilities

5.1 An analyst in the pesticide extraction laboratory will be responsible for the preparation of the solutions defined here. The analyst will be responsible for assuring that this SOP is followed correctly, and that deviations and changes are reported to the pesticides extraction supervisory scientist as quickly as possible.

5.2 The analyst will be responsible for maintaining accurate and complete records of all solutions prepared, and for preparing new solutions at the appropriate intervals.

6.0 Materials and Reagents

6.1 Materials

6.1.1 Balance with 4 place accuracy (x.xxxx g).

6.1.2 Volumetric flasks of various sizes.

6.1.3 Syringes of various sizes.

6.1.4 Assorted sizes of muffled/silanized glass vials with Teflon-lined screw caps.

6.1.5 1.5 mL V-Vials with Mininert cap (Wheaton and Supelco)

6.2 Reagents

6.2.1 Chlorpyrifos (>99% pure) (ChemService).

6.2.2 Diazinon (>99% pure) (ChemService).

6.2.3 4,4'-Dibromophenyl (DBB) (IS) (>99% pure) (ChemService).

6.2.4 Fenchlorphos (SRS) (>99% pure) (ChemService).

6.2.5 High purity methyl-t-butyl ether (ChemService).

6.2.6 Malathion (>95% purity).

6.2.7. DDT (>95% purity).

6.2.8. DDE (>95% purity).

6.2.9. DDD (>95% purity).

6.2.10. γ-Chlordane (>95% purity).

6.2.11. High-purity dichloromethane (DCM).

6.2.12. $^{13}C_{12}$-DDT (CIL).

6.2.13. $^{13}C_{12}$-DDE (CIL).

6.2.14. High-purity hexane.

7.0 Procedure

7.1 Prepare Stock Solutions

7.1.1 Prepare a stock solution at 1 mg/mL for each of the seven analytes (chlorpyrifos, diazinon, malathion, DDT, DDE, DDD, and γ-chlordane), the IS (DBB), and the SRS (fenchlorphos, $^{13}C_{12}$-DDT, $^{13}C_{12}$-DDE). Using a 4-place balance, weigh directly into a clean 20 mL vial approximately 0.0100 g (e.g. 9.8 mg) of the analyte. Record the weight in the project pesticide extraction laboratory notebook.

7.1.2. Calculate the volume of solvent needed to produce a 1 mg/mL solution using the equation below:

solvent vol, mL= x mg of analyte / 1 mg mL^{-1}.

7.1.3 Add this volume of methyl-t-butyl ether using a 10 mL analytical syringe calibrated in 0.1 mL increments. Add the cap and shake gently to mix. Label with laboratory notebook number (9 digit unique code: 5 digit lab notebook number-2 digit page number-2 digit line number on which entered), analyte, concentration, solvent used, preparer's initials, and date of preparation. Mark the volume with a felt-tip pen on the outside of the vial. Enter this same data in the laboratory notebook where the preparation is described, together with the lot number and manufacturer of the standard.

7.1.4. Store the solutions at 0°C or below.

7.2. Prepare Spiking Solutions

7.2.1 To prepare the IS spiking solution for pesticides (and PAH), see SOP BCO-L-26.0.

7.2.2 Pour this solution into a muffled/silylanized screw-cap vial, cap, and invert several times to mix.

7.2.3 Label with the laboratory notebook number (9 digit unique code: 5 digit lab notebook number-2 digit page number-2 digit line number on which entered), analyte, concentration, solvent used, preparer's initials and date

of preparation. Mark the volume with a felt-tip pen on the outside of the vial. Enter this same data in the laboratory notebook where the preparation is described.

7.2.4 To prepare the pesticide SRS spiking solution at 25 µg/mL in mtbe, (10 µL of 25 µg/mL spiked to each matrix for 0.25 µg/mL final concentration), add 250 µL of the 1 mg/mL fenchlorphos, $^{13}C_{12}$-DDT, and $^{13}C_{12}$-DDE stocks to 10 mL volumetric of mtbe.

7.2.5 Repeat steps 7.2.2 and 7.2.3.

7.2.6 To prepare the field spiking solution at 5 µg/mL in mtbe of diazinon, chlorpyrifos, malathion, DDT, DDE, DDD, and γ-chlordane (50 µL of 5 µg/mL spiked to each matrix in the field), add 50 µL of each 1 mg/mL stock to 10 mL volumetric of mtbe.

7.2.7 Repeat step 7.2.2.

7.2.8 Aliquot ~1 mL volumes of the Field Spiking solution into 6 prelabeled V-Vials with Mininert caps. Wrap these vials in a Zip-lock bag and ship them to UA on dry ice.

7.2.9 Hold the remainder of the Field Spiking solution at Battelle in a -20°C freezer.

7.3. Prepare Dust/Soil Calibration Standards

7.3.1 Prepare a mixed solution at 25 µg/mL in mtbe of the 7 analytes. Add 250 µL of each 1 mg/mL stock to a 10 mL volumetric and dilute to volume.

7.3.2 Pour this solution into a muffled/silylanized screw-cap vial, cap, and invert several times to mix.

7.3.3 Label with laboratory notebook number (9 digit unique code: 5 digit lab notebook number-2 digit page number-2 digit line number on which entered), analyte, concentration, solvent used, preparer's initials and date of preparation.

7.3.4 Mark the volume with a felt-tip pen on the outside of the vial. Enter this same data in the laboratory notebook where the preparation is described.

Table 1. GC/MS Pesticide Calibration Standards.

Concentration, µg/mL analyte/SRS/IS	µL of 25 µg/mL analyte mix	µL of 25 µg/mL SRS solution	µL of 25 µg/mL IS solution
0/0/0.10	0	0	100
0.010/0.025/0.10	4	10	100
0.025/0.05/0.10	10	20	100
0.10/0.10/0.10	40	40	100
0.25/0.15/0.10	100	60	100
0.50/0.20/0.10	200	80	100
1.00/0.25/0.10	400	100	100

7.3.5 Prepare 7 solutions to be used for GC/MS calibration curves, according to the matrix in Table 1, with each solution prepared and diluted in 10 mL volumetric with DCM. Label as AIR STDS.

7.3.6 Prepare 7 solutions to be used for GC/MS calibration curves of soil, dust, and handwipe extracts, according to the matrix in Table 1, with each solution prepared and diluted in 10 mL volumetrics with DCM. Label as SOIL/DUST STDS.

7.3.7 For each solution, dilute to volume as indicated. Pour into a muffled/silylated vial, and label as per Step 7.3.3.

7.3.8 Aliquot with a clean Pasteur pipette ~1 mL volumes of these solutions to individual 1.8 mL screw-cap vials that are also so labeled. Label and store as per 7.3.3.

7.3.9 Store the vials in a tray in rows according to concentration.

7.4 Calculations

None. The use of these calibration solutions for quantification of samples is discussed in SOP BCO-L-15.1.

7.5 Quality Control

7.5.1 The 0/0/1 µg/mL solution will serve 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 of which must be dealt with appropriately. 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 re-prepared, with greater caution used in cleaning syringes and glassware.

8.0 Records

8.1 The preparation of all standards and solutions will be recorded in the project pesticide extraction laboratory notebook. Each solution will be labeled with a unique 9 digit laboratory record book number (5 digit book number-2 digit page number-2 digit line number; e.g. 46680-45-12) corresponding to the record of its preparation.

8.2 All entries will be made in ink, and will be signed and dated in the notebook.

8.3 This notebook will be retained in the laboratory where it is used, and will be transferred to the office of the Battelle co-PI at the conclusion of the program.