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

Extracting and Preparing Liquid Food Samples for Analysis of Polar Organic Pollutants

Battelle
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Standard Operating Procedure **CTEPP-SOP-5.29**

Title: Extracting and Preparing Liquid Food Samples for Analysis of Polar Organic Pollutants

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 EXTRACTING AND PREPARING LIQUID FOOD SAMPLES FOR ANALYSIS OF POLAR ORGANIC POLLUTANTS

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1.0 Scope and Applicability

This standard operating procedure (SOP) describes the method for extracting and preparing liquid food samples for analysis of acidic persistent organic pollutants.

2.0 Summary of Method

The method for extracting and preparing a liquid food sample for analysis of acidic persistent organic pollutants is summarized in this SOP. It covers the extraction, concentration, and derivatization of samples that are to be analyzed by gas chromatography/mass spectrometry (GC/MS).

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 food 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 perform the sample extractions will be responsible for obtaining samples from the sample coordinator, entering relevant information in the extraction/preparation laboratory record books, and sending final extracts for analyses.

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

6.0 Apparatus, Materials, and Reagents

6.1 Apparatus and Materials

6.1.1 Dionex Accelerated Solvent Extraction (ASE) instrument (Model #200)

6.1.2 Collection vial with open top caps up to 60 ml capacity

6.1.3 Cellulose fiber filter B 1.93 cm (Dionex B grade D28, part number 049458)

6.1.4 Clean glass wool (silyated)

6.1.5 Microliter syringes in volumes of 1000, 100, 50, and 20 microliters

6.1.6 Glass funnels (muffled)

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

6.1.8 Latex gloves

6.1.9 1.8 mL glass GC vials with Teflon-lined screw caps; muffled and silyated

6.1.10 Kuderna-Danish concentrators (large 24/40 3-ball Snyder condenser, 125 mL or 250 mL reservoir flask and 25 mL tube); (Kontes 570000)

6.1.11 Small 19/22 3-ball Snyder condensers

6.1.12 Pasteur glass pipettes (muffled and stored in clean glass jar)

6.1.13 Vortex mixer (American Scientific Products)

6.1.14 Graduated cylinders

6.1.15 Heated water bath

6.1.16 Clean glass vials

6.1.17 22-mm stainless steel extraction cell

6.1.18 Collection vial septa, Teflon/silicone (I-Chem - 288-72-22)

6.1.19 Weighing boats

6.1.20 Spatulas

6.1.21 Balance (4-place xxx.x g capacity)

6.1.22 Quartz fiber filters

6.1.23 Eppendorf Reference adjustable pipettes in range of 10 to 100 and 100 to 1000 microliters

6.1.24 Beakers (muffled)

6.2 Reagents

6.2.1 Extrelute (EM Science)

6.2.2 Methanol (distilled-in-glass)

6.2.3 Boiling chips (Hengar crystals)

6.2.4 Surrogate Recovery Standard Spiking Solution

6.2.5 Internal Standard Spiking Solution

6.2.6 Matrix Standard Spiking Solution

6.2.7 MilliQ water

6.2.8 Diethyl ether (EE); distilled-in-glass

6.2.9 Reagent grade Ottawa Sand (muffled)

6.2.10 Sodium sulfate anhydride (J. T. Baker analytical reagent grade or equivalent)

6.2.11 Diazald

6.2.12 Carbitol (analytical grade)

6.2.13 N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), Pierce Chemical company

6.2.14 Potassium hydroxide (KOH) aqueous solution

6.2.15 Hydrochloric acid, 36-38 percent

6.2.16 Dichloromethane (DCM); distilled-in-glass

6.2.17 Alumina 80 to 200 mesh (Fisher)

6.2.18 Acetone (distilled-in-glass)

7.0 Procedure

7.1 Extraction, Concentration, Methylation, and Silylation.

7.1.1 To the extent possible, retrieve up to 24 samples from the same materials batch from the freezer. Extract and analyze these samples as a batch.

7.1.2 Put on clean gloves. Place 10 mL of a sample or blank in a clean beaker and spike with L of the surrogate recovery standard spiking solution using a 50 or 100 L syringe. Spike a L aliquot of the matrix spike standard spiking solution to a designated matrix spiked sample **(<u>not all</u>** the samples). Note that the spiked levels may be changed and the exact spiked amounts will be recorded in the laboratory record book (LRB).

7.1.3 Add 10 mL of milliQ water and 10 mL of acetone to the sample, swirl to mix the sample.

7.1.4 Filter the sample through a quartz fiber filter pre-wet with MilliQ water into a separatory funnel, rinse the beaker with about 2 mL of MilliQ water and follow steps 7.16 to 7.2. If the sample does not filter, follow the steps from 7.1.5 through 7.2.

7.1.5 For each sample or blank, screw one end cap of the steel cell onto the chamber and place one cellulose fiber filter onto the top of the end cap. The filter should cover the frit on the end cap. Place 0.5 g of muffled Alumina (80 to 200 mesh) on top of the filter.

7.1.6 Mix a known amount (10 mL) of liquid food with a known amount (~4 g) of Extrelute. Record exact volume and weights in the LRB. Place the mixture in the cell and spike the mixture with 50 L of the surrogate recovery standard spiking solution, using a 50 or 100 L syringe. Note that the spiked levels may be changed and the exact spiked amounts will be recorded in the LRB. Place the remaining mixture in the cell..

7.1.7 Fill the void in the cell with muffled Ottawa sand and tighten the other end cap of the cell.

7.1.8 The 22-mm extraction cells should be used with the 60 mL capacity collection vials. Place the open top caps with the septa teflon side down onto the collection vials.

7.1.9 Place the cells and the collection vials on the ASE unit.

7.1.10 Set the ASE parameters as follows:

Pressure:	2000 psi
Solvent:	Methanol
Temperature:	110C
Heat:	5 minutes
Static:	5 minutes
Flush:	100 %
Purge:	60 seconds
Cycles:	2

7.1.11 A total of 24 cells can be extracted as a set. After extraction is complete, remove cells and collection vials from the ASE unit.

7.1.12 Using a wide-neck funnel with a clean quartz fiber filter, pour the extract from the collection vial into a Kuderna-Danish concentrator (KD) which has a 125 mL reservoir flask. Rinse the ASE cell vial twice with ~5 mL methanol; vortex each rinse; adding the rinse to the KD flask through the filter. Rinse the filter twice with an additional ~5 mL of methanol and add the rinses to the KD flask.

7.1.13 Add 2-3 boiling chips to the KD tube/flask. Attach the large Snyder condenser to the flask if using 250 mL adaptor. Attach the small Snyder condenser to the flask if using

125 mL adaptor.

7.1.14 Concentrate the extract in a heated (~95°C) water bath to ~15 mL. Rinse the adaptor with methanol; remove the adaptor; and replace the large Snyder column with a small Snyder column if necessary. Continue concentrating the sample to ~10 mL. This is an allowable stopping point; the sample extracts can be stored in –20°C freezer for further processing next day.

7.1.15 Transfer the concentrated methanol extract into a separatory funnel; rinse the tube with ~ 5 mL of MilliQ water; vortex the rinse; add rinse to the funnel. Finally, add 15 mL of MilliQ water to the funnel.

7.1.16 Adjust the pH to >12 with 40% KOH (approximately 9 drops). Check the pH value with pH paper to make sure that the pH value is greater than 12. Extract the mixture with hexane three times (3 x 20 mL). Transfer the aqueous layer (bottom layer) to a clean vial and discard the hexane layer each time.

7.1.17 Acidify the aqueous layer to pH < 2 with concentrated HCl (approximately 9 drops) and check the pH value with pH paper and make sure that the pH value is less than 2. Extract the acidified aqueous layer with dichloromethane (DCM) three times (3 x 20 mL). Transfer the DCM layer to the KD tube through a quartz fiber filter or glass wool containing sodium sulfate anhydride. Discard the aqueous layer after the last extraction.

7.1.18 Concentrate the DCM extract to 2 mL in a heated (~ 65°C) water bath and spike the extract with 10 L of the Internal Standard spiking solution, and vortex for 3 seconds to mix. Split the sample extract into two portions (I and II). Portion I is to be used for methylation (see steps 7.1.19, 7.1.20, and 7.2) and portion II is to be used for silylation (see steps 7.1.21 to 7.1.23, and 7.2).

7.1.19 Transfer portion I of the extract, using a muffled disposable glass Pasteur pipette, to a clean 1.8 mL GC vial. Mark the volume on the side of the vial and label the sample with the LRB number and field sample ID. The target analytes for the portion I sample extract are dicamba, 2,4-D, pentachlorophenol, and 2,4,5-T.

7.1.20 Add 50 µL of methanol to the portion I of the sample extract. Methylate this portion with etheral diazomethane generated in situ from Diazald, carbitol, and 37% aqueous KOH. The methylated sample is ready for GC/MS analysis.

7.1.21 Spike 100 μL of MTBSTFA using a 100 μL syringe or a calibrated Eppendorf into

portion II of the sample extract. Vortex for 3 seconds to mix. The target analyte for the portion II sample extract from North Carolina field study is 3,5,6-trichloro-2-pyridinol (3,5,6-TCP). The target analytes for the portion II sample extract from Ohio field study are 3,5,6-TCP and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMP).

7.1.22 Seal the vial with a Teflon septum and placed the vial in one well of the tube-heater sand bath or oven at 65° to 75°C. Record the exact temperature and starting time in the LRB.

7.1.23 Remove the GC vial from the heater after one hour. Record the exact temperature and ending time in the LRB.

7.2 Store portions I and II of the extracts in a freezer ($\leq$ -10°C) until GC/MS analysis.

8.0 Records

8.1 Records of the preparation of field samples, blanks, and matrix spikes will be retained in a LRB that is kept in the extraction laboratory. This LRB will record all sample preparation activities. These samples will be recorded in the LRB by field sample ID and the laboratory generated QA/QC sample will be assigned with a laboratory sample number (a unique number that combines the 5 digit LRB number-2 digit page number-2 digit line number). The date of extraction, the lot number of solvents used for extraction, and the spike level of the surrogate recovery standards and internal standards will be recorded in the LRB.

8.2 The LRB 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 QC/QA samples will include a field blank and laboratory method blank consisting of a clean wipe material that will be extracted together with the field samples. The field blank analyses are performed to verify that minimal contamination occurs through sample handling during shipping and field operations. The laboratory method blank analyses are performed to verify that minimal contamination occurs through sample preparation. In addition, duplicate food samples and matrix spiked food samples will also prepared and analyzed for target analytes.

9.2 Field crews will be reminded to wear clean clothing and shoes, to remove all pesticide products from their residences that may contain these analytes, and to refrain from using these materials while part of the field crew. Field crews will also be reminded to obtain clean clothing after visiting a home where they know or suspect that target pesticides have been applied within the previous week. Cigarette smoking is not permitted during the field sampling. Field crews should store the samples in a clean environment away from any known combustion sources.

9.3 Surrogate recovery values of 50-150% in blanks, and actual samples will be deemed acceptable, and no correction to the data will be made. The data will be flagged for recoveries less than 50% or greater than 150%. The concentration of the surrogate spiking solution will be checked against a calibration curve for recoveries greater than 150%, to determine whether inadvertent solvent loss has resulted in higher spike levels. If this has occurred, the surrogate spiking solution will be re-prepared and re-analyzed.

9.4 One laboratory method blank that is analyzed as a sample concurrently with a field sample set will be analyzed for typically every 50 samples processed. If significant target analyte levels (>0.1 µg) are found in the field blanks, the source of contamination must be identified and more laboratory blanks, together with additional field blanks, trip blanks, and storage blanks, will be analyzed.

10.0 Reference

10.1 J. C. Chuang, K. Hart, D. B. Davis, and J. K. Finegold, "Development and Evaluation of Immunochemical Methods for Environmental Monitoring and Human Exposure Assessment", Final Report, EPA Contract Number 68-D4-0023 Work Assignment 4-01, 1998.