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Sample Preparation for the Analysis of Organophosphate and Brominated Flame Retardants in Consumer Products Using Sonication

1. SCOPE AND APPLICABILITY

This standard operating procedure (SOP) describes the procedure involved in the preparation of foam and textile samples for the analysis of select organophosphate flame retardants (OPFRs), polybrominated diphenyl ethers (PBDEs) and two additional brominated flame retardants (BFR). The SOP describes how foam and textile samples are cryomilled, solvent extracted using sonication and prepared for Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis.

2. DEFINITIONS

- BFR – Brominated Flame Retardant
- CCV – Continuous Calibration Verification
- DCM – Dichloromethane
- GC-MS/MS – Gas Chromatography-Tandem Mass Spectrometry
- LCS – Laboratory Control Sample
- LCSD – Laboratory Control Sample Duplicate
- MB – Method Blank
- OPFR – Organophosphate Flame Retardant
- PBDE – Polybrominated Diphenyl Ether
- RM – In House Reference Material
- RPD – Relative Percent Difference
- SDS – Safety Data Sheet

3. PRINCIPLE

Foam, batting and textile samples are either cut into small pieces or cryomilled. Additive organic flame retardants are extracted from samples by vortexing in toluene, followed by sonication. An

aliquot of the sample extract is then prepared for Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis by the addition of deuterated and carbon-13 labelled internal standards.

4. INTERFERENCES

Foam, batting and textile samples may contain high levels of flame retardants. Therefore, subsampling should be performed in designated laboratory area to prevent contamination. Samples must be carried and stored in enclosed vessels or sealed bags. Sample preparation and extraction must be performed in designated fume hood. Fume hoods and benchtops must be thoroughly cleaned to minimize background or cross contamination. Laboratory work surface should be lined with clean aluminum foil when cutting subsamples. Aluminum foil, surgical razor blades and nitrile gloves are to be replaced after each subsampling event. Scissors, when reused shall be solvent rinsed after each sample.

Trace-level analysis requires careful and clean preparation and analysis techniques along with high-purity standards and solutions. Solvents, reagents, glassware, and other items used during sample preparation may introduce unexpected interferences or contamination to the sample. These materials must be demonstrated to be free from interferences and contamination by analyzing a method blank with each sample batch.

5. PRESERVATION AND HOLDING TIMES

5.1. Storage

Keep samples in the packaging they arrived in and store at ≤6°C. Samples are stored in sealed glass jars or sealed plastic bags with the sample fully enclosed in aluminum foil. Long term storage should be at ≤6°C in the aforementioned containment vessels.

Cryomilled samples should be stored in sealed glass vials and in the dark at ≤6°C when not in use.

Sample extracts should be stored in sealed glass vials and in the dark at ≤6°C when not in use. Septa on autosampler vials containing sample extracts should be replaced as soon as possible after puncture, and the vials returned promptly to dark ≤6°C storage.

5.2. Holding Times

There is no holding time limit on OPFRs, PBDEs and BFRs in foam, batting or textile samples stored at ≤6°C in the aforementioned containment vessels.

6. EQUIPMENT AND SETUP

For details on the recommended equipment see Table 1.

7. GLASSWARE/EQUIPMENT CLEANING

7.1. Reusable Glassware

Reusable glassware must be properly washed after use to prevent interference in subsequent analysis due to sample carryover.

Recommended procedures for cleaning glassware are described in SOP 02.0011.00, "Procedure for Washing Glassware for the Analysis of PCDDs/PCDFs, PCBs, OCPs, PBDEs, BFRs and Other Persistent Organic Pollutants in Environmental Samples".

7.2. Disposable Glassware

Glassware in this classification includes new and unused GC vials, Pasteur pipettes and test tubes.

Disposable glassware should be cleaned prior to use by wrapping glassware in aluminum foil and baking in the muffle furnace at approximately 500°C for 3 hours.

7.3. Reusable Metal

Equipment in this classification includes steel grinding vial sets (impactor, end caps and center cylinder) and scissors.

Recommended procedure for cleaning metal parts are described in SOP 02.0011.00, "Procedure for Washing Glassware for the Analysis of PCDDs/PCDFs, PCBs, OCPs, PBDEs, BFRs and Other Persistent Organic Pollutants in Environmental Samples". It is recommended to perform the washing of the grinding vial sets two times with liquid detergent. Grinding vial sets are immediately dried after washing.

7.4. Reusable Plastics

Equipment in this classification includes pipette bulbs and O-rings.

Soak plastics in liquid detergent and gently scrub using nylon brush as is described in SOP 02.0011.00. Plastics may not be compatible with specific organic solvents. Follow the supplier's and/or manufacturer's recommended directions for solvent compatibility.

8. CONSUMABLES

For details on the recommended consumables and supplies, see Table 2.

9. STANDARDS AND REAGENTS USED

9.1. Solvents

For details on the recommended solvents, see Table 3.

9.2. Cryogenic Fluid

For details on the recommended cryogenic fluid, see Table 3.

Always use a low-pressure liquid nitrogen supply, with a delivery pressure of 22 psi.

9.3. Surrogate Standard, Laboratory Control Spike Standard and Internal Standard

For details on the recommended composition and recommended preparation of the Surrogate Standard, Laboratory Control Spike Standard and Internal Standard see Tables 4-6. When available, it is recommended to use certified analyte concentrations corrected for purity.

9.4. Method Blank

The method utilizes analyte-free foam designated as Blank Foam A. Blank Foam A has been confirmed to be analyte-free by three independent analytical techniques: X-Ray Fluorescence (XRF), Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and GC-MS. Blank Foam A is used for the Method Blank (MB) and Laboratory Control Sample (LCS & LCSD) analyte-free matrix.

To prepare Blank Foam A, sonicate 1-3 g of Foam A in DCM for 5 minutes. Discard DCM and repeat sonication process with fresh DCM two additional times. Let cleaned Foam A dry in the hood.

10. PROCEDURE

10.1. Batch QC Requirements

The following Quality Control (QC) analyses must be performed for each sample preparation batch: Method Blank (MB), Laboratory Control Sample (LCS), Laboratory Control Sample Duplicate (LCSD), Reference Material and Sample Duplicate. A sample preparation batch is defined as up to 24 samples, including QC samples prepared together. QC samples must be carried through the complete sample preparation process along with the samples. The purpose and criteria for each QC sample are listed below. See Table 7 for method QC requirements.

10.1.1. Method blank (MB)

The MB is analyzed at a minimum frequency of one per batch and is subjected to the entire analytical process.

The MB is prepared as follows; 50 mg (±5 mg) of analyte-free foam (Foam A) is weighed and spiked with Surrogate Standard (see Table 4).

10.1.2. Laboratory Control Sample (LCS) and Laboratory Control Sample Duplicate (LCSD)

The LCS and LCSD are analyzed at a minimum frequency of one pair per batch and are subjected to the entire analytical process.

The LCS and a LCSD are prepared as follows; 50 mg (±5 mg) of analyte-free foam (Foam A) is weighed and spiked with Surrogate Standard and Laboratory Control Spike Standard (Tables 4 and 5). The percent recovery of the target analytes in the LCS must be within 50-150%.

The LCS and LCSD must have a relative percent difference (RPD) of ≤30% for each target analyte.

10.1.3. Duplicates

A duplicate sample is analyzed at a minimum frequency of one per batch and is subjected to the entire analytical process. A duplicate sample is used to determine the precision of the analysis.

Cryomilled duplicate samples must have a relative percent difference (RPD) of ≤30% for each target analyte. Failure to meet the acceptance criteria, due to the inability of cryomilling to completely homogenize the sample must be noted in the case narrative of the final report.

Un-milled duplicate samples must have a relative percent difference (RPD) of ≤50%. Failure to meet the acceptance criteria due to an unavoidable lack of sample homogeneity must be noted in the case narrative of the final report.

10.1.4. Reference Material

In-house reference material (RM) sample is analyzed at a minimum frequency of one per batch and is subjected to the entire analytical process. The RM monitors the accuracy of the sample preparation process variability.

The RM is prepared as follows; 50 mg (±5 mg) of RM foam is weighed and spiked with Surrogate Standard (see Table 4). Cryomilled RM results must be within 70-130% of defined value (see Table 7). Cryomilled RM is stored in a sealed glass vial and in the dark at ≤6°C.

Un-milled RM results must be within 50-150% of defined value. Un-milled RM is stored in a sealed glass vial and in the dark at ≤6°C.

10.2. Instrument/Equipment QC requirements

All measuring instruments (e.g., analytical balance, calibration weights, pipettes) must not be used past their calibration due date. Verify this by checking the calibration sticker on equipment prior to use. Measuring instruments shall be calibrated according to the established calibration program.

10.3. Sample Preparation Procedure

10.3.1. Preliminary Steps

Prepare QC and sample labels with ECL Authorization Number, Sample ID, date and initials of analyst.

For QC samples prepare the following labels, MB, LCS, LCSD, RM and sample duplicate.

10.3.2. Cryomilling

When sufficient sample material is available it is recommended to cryogenically mill the samples by following the steps below. Analyte-free foam (Foam A) is cryomilled and apportioned for the MB, LCS and LCSD. Cryomilled RM is stored in a glass vial at ≤6°C. Effective cryomilling is dependent on material type. The inability of cryomilling to completely homogenize the sample must be noted in the case narrative of the final report.

(1) Prepare grinding vial sets by installing one end plug to each center cylinder and placing impactor in the assembly.

(2) Using a laboratory marker or equivalent, label each grinding vial with corresponding ECL Sample ID number.

(3) Using a clean blade or scissors, cut 1-2 cm^3 pieces of sample and transfer to corresponding grinding vial. Install remaining end plug to center cylinder.

It is recommended to loosely fill the grinding vial to 50-75% of volume. Overfilling and/or packing the sample material will prevent grinding.

It is important to line the laboratory hood work surface with aluminum foil to prevent work surface contamination and sample cross contamination. High levels of flame retardants may be present within a sample. Aluminum foil, surgical razor blades and nitrile gloves are to be replaced after processing each sample.

(4) Turn Freezer Mill power on at rear of instrument. Load two grinding vials into the lower grinding chambers and load two additional samples into the precooling chambers (upper chambers).

(5) Close Freezer Mill lid and secure latch. Open Dewar liquid nitrogen valve.

Warning: Open and Close Freezer Mill Lid Slowly to Avoid Splashing Liquid Nitrogen. Use appropriate personnel protective equipment when handling liquid nitrogen and or cryogenically cooled grinding vials.

(6) Load Freezer Mill program ‘Foam/Fabric’ and select ‘run’ to start milling. The recommended program settings are shown in Figure 2.

(7) After the Foam/Fabric program completes, remove the grinding vials from the lower grinding chambers and wrap with aluminum foil. Move the two grinding vials from the precool chambers to the lower grinding chambers and load additional grinding vials into the precool chambers. Repeat process for remaining grinding vials.

(8) Bring grinding vials to room temperature in laboratory hood before opening grinding vials and transferring samples.

(9) Open vials by using the vial extractor or by hand and transfer contents into corresponding 40 mL amber glass vial.

10.3.3. Un-milled and Cryomilled Sample Weighing

(1) Verify calibration of the analytical balance using calibration weights and record CCV in corresponding Analytical Balance Log Book.

(2) For weighing un-milled QC foam and samples, use the following procedure.

Using a clean blade or scissors, cut small pieces of foam and or fabric subsample from the primary sample material. Tare a plastic weighing boat on the analytical balance and weigh 50 mg (± 5 mg) sample material. Record weight on Sample Preparation for Flame Retardants in Consumer Products worksheet (DCN 07.0183.00) and transfer sample to corresponding 8 mL sample tube. Seal sample tube with tube screw top.

(3) For weighing cryomilled QC foam and samples, use the following procedure.

Gently shake the vial containing the cryomilled sample to mix settled contents. Tare a sample tube (8 mL) on the analytical balance. Using a disposable polypropylene spatula transfer 50 mg (± 5 mg) of cryomilled sample to each corresponding tube and seal sample tube with tube screw top. Record weight on Sample Preparation for Flame Retardants in Consumer Products worksheet (DCN 07.0183.00).

10.3.4. Extract Collection Tubes

Label and cap extract collection tubes (10 mL) and weigh using analytical balance. Collection tubes and caps must be weighed and labelled as pairs. Record weights in laboratory notebook.

10.3.5. Quality Control Standard Spikes

(1) Remove the surrogate standard and laboratory control spike standard from the refrigerator and allow them to reach room temperature. Vortex standards before use.

(2) Add surrogate standard (see Table 4) to all QC (MB, LCS, LCSD and RM) and all samples. Addition of standard should be directly to the surface of the sample material.

(3) Add laboratory control spike standard (see Table 5) to LCS and LCSD. Addition of standard should be directly to the surface of the sample material.

10.3.6. Foam and Textile Sonication Extraction (see Figure 1)

(1) Add 4 mL toluene to each QC and sample tube and ensure tube caps are fastened.

(2) Vortex each QC and sample tube for 3 minutes at maximum speed setting (~2500 RPM).

(3) Place all QC and sample tubes in sonicator rack. Verify sonicator water level is above sample extract level. Sonicate samples for 10 minutes at high setting. When complete, remove each sample tube and wipe exterior water dry with a paper towel.

(4) Centrifuge QC and sample tubes containing the foam or textile matter for 5 minutes at maximum RMP setting (~3500 RPM).

(5) Using a clean Pasteur pipette remove the liquid extract from sample tubes and filter particulate matter using a 3 mL Bond Elut cartridge fitted with a 10 µm pore size frit and a 20 µm pore size frit (10 µm frit is positioned below 20 µm frit). Avoid removal of solid sample particulate material. Liquid extract is collected in pre-weighed extract collection tubes (see 10.3.4).

(6) Repeat extraction two additional times with 3 mL of fresh toluene. Vortex for 3 minutes, sonicate for 10 minutes, centrifuge for 5 minutes and remove liquid extract with clean Pasteur pipette and filter as described above. Pool the three extracts in collection tube.

(7) Transfer any remaining liquid extract to filter cartridge and apply vacuum to cartridge manifold to draw out extract remaining in flow control liners and/or bound to frits. Be careful not to splash the collected extract when applying vacuum.

(8) Cap and vortex extract collection tubes and weigh using analytical balance. Record weights in laboratory notebook and calculate extract volume for each tube using the density of toluene (0.866 g/mL at 20°C).

(9) Mark extract level, seal with parafilm and refrigerate at ≤6°C to prevent solvent evaporation.

10.3.7. Preparation of Autosampler Vials for GC-MS/MS Analysis

(1) Remove the Internal Standard vial and sample extracts from the refrigerator and allow to reach room temperature. Vortex to mix contents.

(2) Prepare appropriate number of 300 µL GC autosampler vials by labeling with QC and sample ID labels.

(3) Transfer 80 µL of each QC and sample extract to corresponding autosampler vial.

(4) Transfer 20 µL of Internal Standard solution (see Table 6) to each autosampler vial.

(5) Vortex each GC autosampler vial to mix contents. The samples are now ready to be analyzed by GC-MS/MS.

10.3.8. Dilutions of Primary Extract for Analytes Measured at High Levels

Dilutions of the pooled sample extracts may be required if high concentrations of analytes are detected by GC-MS/MS. Analytes measured at or above the highest calibration point will require dilution. It is recommended to prescreen samples by preparing a 1:25 dilution of each sample extract in toluene and determining dilution factors in reference to calibration standard 7 response. Final dilutions must be recorded in laboratory notebook. After each dilution, thoroughly mix the diluted sample extracts by hand or by vortexing. Example dilutions steps are shown below.

- To make a 1:10 dilution; transfer 1.25 mL of sample extract to clean tube containing 8.75 mL of toluene. This will yield a 1:8 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 10 fold dilution in the GC vial.
- To make a 1:25 dilution; transfer 500 µL of sample extract to clean tube containing 9.50 mL of toluene. This will yield a 1:20 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 25 fold dilution in the GC vial.
- To make a 1:50 dilution; transfer 250 µL of sample extract to clean tube containing 9.75 mL of toluene. This will yield a 1:40 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 50 fold dilution in the GC vial.
- To make a 1:100 dilution; transfer 125 µL of sample extract to clean tube containing 9.875 mL of toluene. This will yield a 1:80 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 100 fold dilution in the GC vial.
- To make a 1:250 dilution; transfer 50 µL of sample extract to clean tube containing 9.950 mL of toluene. This will yield a 1:200 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 250 fold dilution in the GC vial.
- To make a 1:500 dilution; transfer 25 µL of sample extract to clean tube containing 9.975 mL of toluene. This will yield a 1:400 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 500 fold dilution in the GC vial.
- To make a 1:1000 dilution; transfer 100 µL of sample extract to clean tube containing 9.9 mL of toluene. This will yield a 1:100 dilution of the sample extract. The diluted extracted is further diluted by transferring 1.25 mL of the primary dilution to clean tube containing 8.75 mL of toluene. This will yield a 1:800 dilution of the sample extract. Following steps 1-5 in section 10.3.7 will yield a final 1000 fold dilution in the GC vial.

10.4. Data Analysis

Refer to SOP "Determination of Organophosphate and Brominated Flame Retardants in House Dust and Consumer Products by Gas Chromatography – Tandem Mass Spectrometry", DCN 05.0029.00. A list of target analytes (compound name, abbreviation and CAS No.) is included in Table 8.

10.5. Data Reporting

Record data relevant to sample preparation on the Sample Preparation for Flame Retardants in Consumer Products worksheet (DCN 07.0183.00) and or laboratory notebook.

10.6. Method Performance

Method Performance parameters are found in SOP "Determination of Organophosphate and Brominated Flame Retardants in House Dust and Consumer Products by Gas Chromatography – Tandem Mass Spectrometry ", DCN 05.0029.00.

11. MAINTENANCE AND TROUBLE SHOOTING

11.1. Sonicator

Refill the water bath in the Branson sonicator prior to each use, ensure complete coverage of sample extract. Drain and refill the water bath periodically.

11.2. Analytical Balance

Verify that the analytical balance and calibration weight set are within their calibration date window. Check the performance of the analytical balance prior to each batch by performing a CCV.

11.3. Manual and Electronic Pipettes

Verify that the manual and electronic pipettes are within their calibration date window.

11.4. Contamination

Sample preparation practices must be followed as described in section 4, Interferences.

12. REFERENCES

13.1. ECL SOP 02.0011.00: "Washing Glassware for the Analysis of PCDDs/PCDFs, PCBs, OCPs, PBDEs, BFRs and Other Persistent Organic Pollutants in Environmental Samples."

13.2. SPEX Handbook of Sample Preparation and Handling, 13th Edition, spexsampleprep.com.

13.3. SPEX SamplePrep 6875D Large Freezer/Mill Operating Manual, spexsamleprep.com.

13.4. Fisher Scientific Digital Multi-Tube Vortexer Instruction Manual, fishersci.com.

13.5. Branson Ultrasonic Bath Model 3800 Operation's Manual 100-214-294, bransonic.com.

13.6. Drucker Centrifuge Model 755VES Operator's Manual, druckerdiagnostics.com.

13.7. ECL SOP 05.0029.00: "Determination of Organophosphate and Brominated Flame Retardants in House Dust and Consumer Products by Gas Chromatography – Tandem Mass Spectrometry."

APPENDIX

Table 1: Foam and Textile Sample Preparation List of Recommended Equipment

Item	Description	Supplier	Part #
Pipette 20 µL	E4 Electronic Pipette, LTS E4-20XLS+, 2-20 µL	Mettler-Toledo[1]	17014487
Pipette 100 µL	E4 Electronic Pipette, LTS E4-100XLS+, 10-100 µL	Mettler-Toledo	17014483
Pipette 300 µL	E4 Electronic Pipette, LTS E4-300XLS+, 20-300 µL	Mettler-Toledo	17014488
Pipette 1 mL	E4 Electronic Pipette, LTS E4-1000XLS+, 100-1000 µL	Mettler-Toledo	17014482
Pipette 5 mL	E4 Electronic Pipette, LTS E4-5000XLS, 500-5000 µL	Mettler-Toledo	17012312
Analytical Balance	Mettler Toledo XS104, Max Capacity 120 g, Readability 0.1 mg	Mettler Toledo	11106012
Vacuum Manifold	Supelco Visiprep-DL 24 Port Vacuum Manifold	Sigma-Aldrich[2]	57265
Vortexer	Digital Multi-Tube Vortexer	Fisher Scientific[3]	02-215-452
Sonicator	Branson Bransonic Ultrasonic Bath Model 3800	VWR[4]	89375-434
Centrifuge	Variable Speed Horizontal Centrifuge, 2200g/4000rpm	VWR	95037-282
Freezer Mill	6875D Freezer Mill	SPEX[5]	6875D
Grinding Vial Set	Large Stainless Steel Grinding Vial Set	SPEX	6803
Extractor	Large Vial Extractor	SPEX	6808
Volumetric flask – 3 mL	Volumetric Cylindrical Flask, ±0.015 mL	VWR	14209-628
Volumetric flask – 5 mL	Volumetric Cylindrical Flask, ±0.02 mL	VWR	14204-538
Volumetric flask – 10 mL	Volumetric Cylindrical Flask, ±0.02 mL	VWR	14203-258

[1]mt.com, [2]sigmaaldrich.com, [3]fishersci.com, [4]vwr.com, [5]spexsampleprep.com

Table 2: Foam and Textile Sample Preparation List of Recommended Consumables and Supplies

Item	Description	Supplier	Part #
Scalpel Blade	Bard-Parker Safety Lock Rib-Back Scalpel Blades, Sterile, Surgical	VWR	89176-354
Weighing Dish	Plastic Micro Boat, Anti-Static EMS, 5 mL Capacity	VWR	100502-714
8 mL Glass Tube	Disposable Screw-Cap Finish, Borosilicate Glass, 13x100 mm, 8 ml	VWR	53283-800
Tube Cap 13-415	Black Phenolic Cap PTFE/Rubber Liner, 13-415 GPI Thread Finish	Kimble[6]	45066C-13
12 mL Glass Tube	Disposable Screw-Cap Finish, Borosilicate Glass, 16x100 mm, 12 ml	VWR	53283-802
Tube Cap 15-415	Black Phenolic Cap PTFE/Rubber Liner, 15-415 GPI Thread Finish	Kimble	45066C-15
Pasteur Pipette	Durex Borosilicate Glass pipette, 9"	VWR	14673-043
Pipette Bulbs	Small Latex Bulb, 57 mm	VWR	82024-554
Spatula 3.5 mm	Disposable Polypropylene Spatula, 3.5 mm diameter, 140 mm length	Fisher Scientific	18-001-019
Spatula 7 mm	Disposable Polypropylene Spatula, 7 mm diameter, 210 mm length	Fisher Scientific	18-001-018
Vial 40 mL	40 mL Vial Amber Borosilicate Teflon-Lined Closure	VWR	89126-544
GC Vials	12 x 32 amber vial with 300 µL capacity fused insert, Borosilicate	Wheaton[7]	225328
GC Vial Caps	ABC cap with PTFE/Silicone Septum	Wheaton	W225332-0204
ParaFilm	ParaFilm M (Pechiney Plastic Packaging) Laboratory Film 2” width	VWR	52858-076
Bond Elut Cartridge	3 mL Bond Elut cartridge Reservoir, 2 - 20 um filter	Agilent[8]	12131014
Flow Control Liners	Disposable Flow Control Valve Liners for VISIPREP-DL	Sigma-Aldrich	57059
Frit 10 um	ISOLUTE Frits, 3 mL (9 mm) 10 um PE	Biotage[9]	120-1062-B
20 µL pipet tips	ART Barrier, Low Retention 20 µL Barrier (2749-05-HR)	VWR	89031-352
200 µL pipet tips	ART Barrier, Low Retention 200 µL Barrier (2769-05-HR)	VWR	89031-374
300 µL pipet tips	ART Barrier, Low Retention 300 µL Barrier (2739-05-HR)	VWR	89031-394
1000 µL pipet tips	LTS 1 ml Tips, RT-LTS-A-L1000µL-/F	Mettler-Toledo	30389212
5000 µL pipet tips	LTS 5 mL Tips, RT-LTS-A-5000µL	Mettler-Toledo	30389256

[6]kimble-chase.com, [7]wheaton.com, [8]agilent.com,

Table 3: Recommended Solvents and Cryogenic Fluid

Solvent/Reagent	Description	Supplier	Part #
Toluene	JT Baker, Ultra Resi-Analyzed, [CAS No. 108-88-3]	Avantor[10]	9336-02
Methylene Chloride	JT Baker, Ultra Resi-Analyzed, [CAS No. 75-09-2]	Avantor	9264-02
Liquid Nitrogen	Cryogenic Fluid, 180LTRS 22 PSI [CAS No. 7727-37-9]	Airgas[11]	NI 180LT22

[10]avantormaterials.com, [11]airgas.com

Table 4. Surrogate Standard Composition

Component	Supplier Conc. [ng/µL]	Volume [µL]	Final Volume [µL]	Final Concentration [ng/µL]	Volume Spiked 100 µL Mass Spiked [ng]	Supplier	Part No.
dTPP	1000	62.5	5000	12.5	1250	CIL[12]	DLM-8901-1.2
Component	**Supplier Conc. [ng/µL]**	**Volume [µL]**	**Final Volume [µL]**	**Final Concentration [ng/µL]**	**Volume Spiked 50 µL Mass Spiked [ng]**	**Supplier**	**Part No.**
^{13}C-BDE-77	50	-	-	-	2500	Wellington[13]	MBDE-77

[12]isotope.com, [13]well-labs.com

The above recommended dTPP Surrogate Standard is prepared by transferring 62.5 µL of dTPP to a 5 mL volumetric flask. Bring to 5 mL volumetric mark with toluene and mix thoroughly by swirling or vortexing for 1 minute and inverting for 1 minute. Let the solution rest for 10 minutes at room temperature and then repeat mixing procedure. Record preparation in ECL standards log book.

^{13}C-BDE-77 is spiked at 50 ng/µL with no dilution of supplier stock.

Table 5. Laboratory Control Spike Standard Composition

Component	Supplier Conc. [ng/µL]	Volume [µL]	Final Volume [µL]	Final Concentration [ng/µL]	Volume Spiked 200 µL Mass Spiked [ng]	Supplier	Part No.
TCIPP	100	375	3000	12.5	2500	AccuStandard[14]	PFRS-025S
TDCIPP	100	375	3000	12.5	2500	AccuStandard	PFRS-027S
TPHP	100	375	3000	12.5	2500	AccuStandard	PFRS-020S
BDE-47	50	750	3000	12.5	2500	AccuStandard	BDE-047S
BDE-99	50	750	3000	12.5	2500	AccuStandard	BDE-099S

[14]accustandard.com

The above recommended Matrix Spike Standard is prepared by transferring the appropriate volume of each component listed in Table 5 to a 3 mL volumetric flask. Bring to 3 mL volumetric mark with toluene and mix thoroughly by swirling or vortexing for 1 minute and inverting for 1 minute. Let the solution rest for 10 minutes at room temperature and then repeat mixing procedure. Record preparation in ECL standards log book.

Table 6. Internal Standard Composition

Component	Supplier Conc. [ng/µL]	Volume [µL]	Final Volume [µL]	Final Concentration [pg/µL]	Volume Added 20 µL Final GC Vial Conc. [pg/µL]	Supplier	Part No.
^{13}C-BDE-28	50	50	10000	250	50	Wellington	MBDE-28
^{13}C-BDE-47	50	50	10000	250	50	Wellington	MBDE-47
^{13}C-BDE-79	50	100	10000	500	100	Wellington	MBDE-79
^{13}C-BDE-99	50	50	10000	250	50	Wellington	MBDE-99
^{13}C-BDE-153	50	100	10000	500	100	Wellington	MBDE-153
^{13}C-BDE-154	50	100	10000	500	100	Wellington	MBDE-154
dTNBP	50	125	10000	625	125	Wellington	dTBP
dTCEP	50	250	10000	1250	250	Wellington	dTCEP
dTCIPP	100	125	10000	1250	250	CIL	DLM-9317-1.2
dTDCIPP	50	250	10000	1250	250	Wellington	dTDCPP
dTPHP	50	250	10000	1250	250	Wellington	dTPP
^{13}C-dEH-TBB	50	250	10000	1250	250	Wellington	MEHTBB
^{13}C-dBEH-TEBP	50	250	10000	1250	250	Wellington	MBEHTBP

The above recommended Internal Standard is prepared by transferring the appropriate volume of each component listed in Table 6 to a 10 mL volumetric flask. Bring to 10 mL volumetric mark with toluene and mix thoroughly by swirling or vortexing for 1 minute and inverting for 1 minute. Let the solution rest for 10 minutes at room temperature and then repeat mixing procedure. Record preparation in ECL standards log book.

Table 7. Method Quality Control Criteria

Method Quality Control Requirements					
MB	**LCS/LCSD Recovery**	**LCS/LCSD RPD**	**Reference Material Recovery†**	**Surrogate Recovery**	**Sample Duplicate RPD**
<LOQ‡	50 - 150%	≤30%	50 - 150% 70 - 130%*	50 - 150%	≤50% ≤30%*

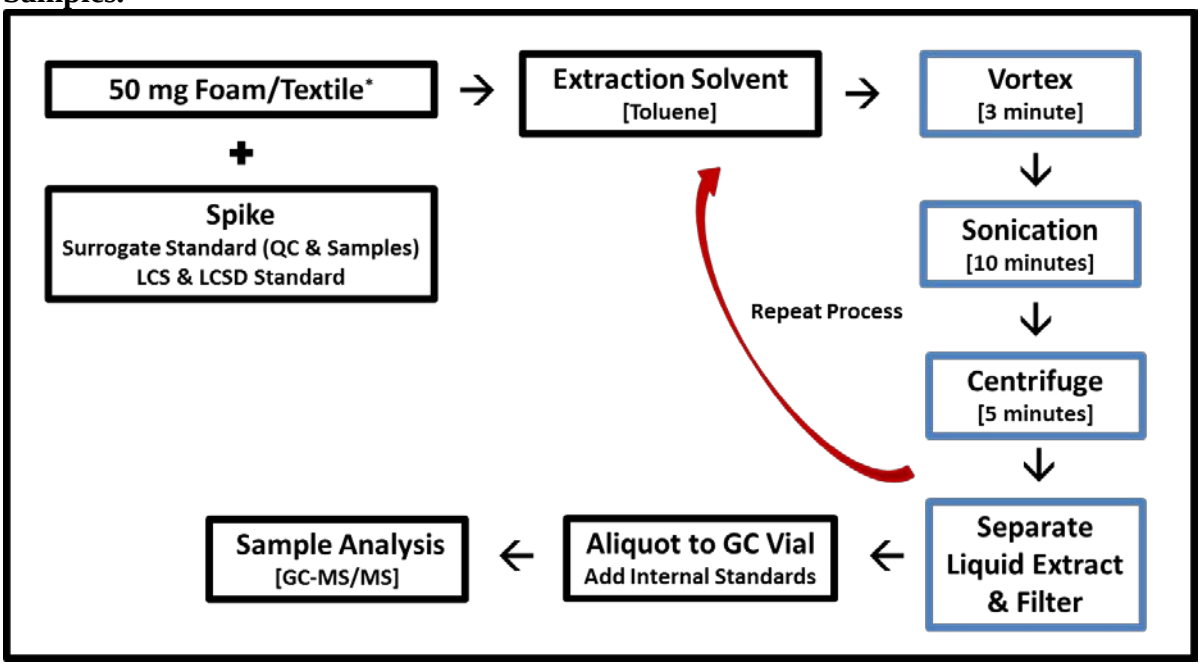
‡LOQ 6.25 mg/kg, †TCEP 75000 mg/kg, *Cryomilled

Table 8. Compound name, abbreviation and CAS No.

Compound Name	Abbreviation	CAS Number
Tri-n-propyl phosphate	TPP	513-08-6
Tri-n-butyl phosphate	TNBP	126-73-8
Tris(2-chloroethyl) phosphate	TCEP	115-96-8
Tris(1-chloro-2-propyl) phosphate	TCIPP	13674-84-5
Tris(1,3-dichloroisopropyl) phosphate	TDCIPP	13674-87-8
Triphenyl phosphate	TPHP	115-86-6
2,2',4-Tribromodiphenyl ether	BDE-17	147217-75-2
2,4,4'-Tribromodiphenyl ether	BDE-28	41318-75-6
2,2',4,4'-Tetrabromodiphenyl ether	BDE-47	5436-43-1
2,2',3,4,4'-Pentabromodiphenyl ether	BDE-85	182346-21-0
2,2',4,4',5-Pentabromodiphenyl ether	BDE-99	60348-60-9
2,2',4,4',6-Pentabromodiphenyl ether	BDE-100	189084-64-8
2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE-153	68631-49-2
2,2',4,4',5,6'-Hexabromodiphenyl ether	BDE-154	207122-15-4
2,2',3,4,4',5',6-Heptabromodiphenyl ether	BDE-183	207122-16-5
2-Ethylhexyl-2,3,4,5-tetrabromobenzoate	EH-TBB	183658-27-7
Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate	BEH-TEBP	26040-51-7
2-Isopropylphenyl diphenyl phosphate**	IPPDPP	64532-94-1

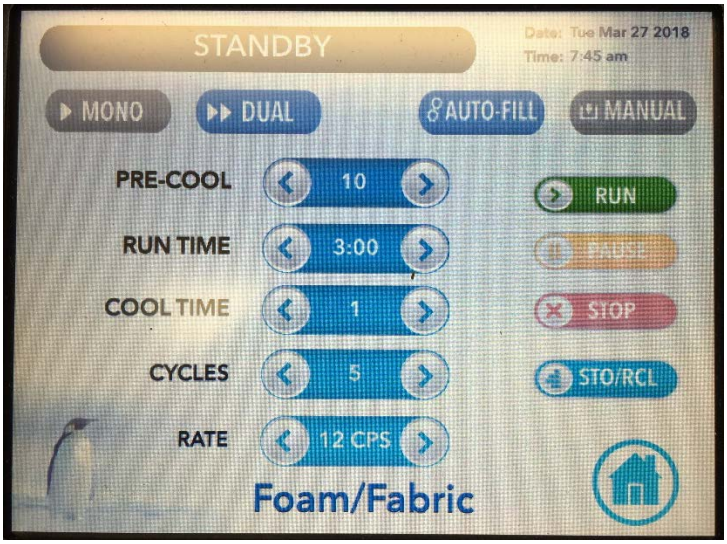
**Tentatively Identified Compound

Figure 1. Process diagram for sonication assisted solvent extraction of Foam and Textile Samples.



*Cryomilled or Unmilled Sample

Figure 2. Freezer Mill Foam/Fabric Program Settings.



13. REVIEW

Signatures	Date