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Sample Preparation for the Analysis of Organophosphate Flame Retardants and Polybrominated Diphenyl Ethers in Consumer Products Using Sonication

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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 solvent extracted using sonication and prepared for Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis.

2. DEFINITIONS

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

3. PRINCIPLE

Additive organic flame retardants are extracted from foam, batting and/or textile samples by vortexing in DCM, 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.

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4. SAFETY

4.1. Toxicity / Carcinogenicity / SDS

Reagents, organic solvents and analytes can be toxic and/or carcinogenic. Individuals must be familiar with the SDS for chemicals, solvents and analyte standards used in this SOP, as well as policies and procedures detailed in current ECL's Chemical Hygiene Plan prior to performing this procedure.

4.2. Personal Protective Equipment / Fume Hoods

Wear lab coat, eye protection and nitrile gloves while handling toxic substances, and work with these substances in a fume hood. Spills should be cleaned up promptly. Foam and textile subsampling should be completed in designated laboratory area. Care should be exercised when handling and using razor blades.

4.3. Waste Handling

Empty all solvent waste into appropriate waste containers (i.e. chlorinated waste bottle if waste solvent contains DCM and flammable waste bottle if not) and discard any solid waste (i.e. foam and textile samples) into appropriate hazardous waste bags. All waste containers should be labeled with ECL hazardous waste labels filled out during accumulation and storage. When full, liquid waste bottles are taken to the solvent waste cabinet and bags are taken to the hazardous waste bin location. Allow any glassware with residual solvent to dry in hood before washing or disposing of into appropriate glass waste bins. Nitrile gloves should be disposed of in the white hazardous waste bin. Used surgical razor blades should be disposed of in sharps waste container.

5. INTERFERENCES

Foam, batting and textile samples may contain high levels of flame retardants, therefore subsampling should be completed in designated laboratory area to prevent contamination. Transport of foam and fabric samples must be in enclosed vessels or sealed bags. Sample preparation and extraction must be completed 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 subsequent subsampling event.

Trace-level analysis requires careful and clean preparation and analysis technique 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.

6. PRESERVATION AND HOLDING TIMES

6.1. Storage

Keep samples in the packaging they arrived in at room temperature. Samples are stored in sealed glass jars or sealed plastic bags with the sample fully enclosed in aluminum foil.

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Long term storage can be at room temperature in the aforementioned containment vessels.

Sample extracts should be stored in sealed glass vials and in the dark at 4 °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 4 °C storage.

6.2. Holding Times

There is no holding time limit for foam, batting or textile samples.

7. EQUIPMENT AND SETUP

Use the following list of equipment or equivalent when exact model is not available.

- Analytical balance capable of 0.0001 gram accuracy.
- Manual or electronic pipettes and corresponding pipette tips with the following dispensing range.
    - 1-5 mL
    - 50-250 uL
    - 10-100 µL
    - 3-25 µL
    - 1-10 µL
- Vortex
    - IKA MS 3 Basic Minishaker Model Number MS 3 B S1.
- Sonicator
    - Branson Bransonic Ultrasonic Bath Model 3800.
- Centrifuge
    - Scientific Products International Clinical Centrifuge Model CL 30131M-2.
- Vacuum Manifold
    - Supelco Visiprep-DL 24 port Vacuum Manifold Sigma-Aldrich.

8. GLASSWARE/EQUIPMENT CLEANING

8.1. Reusable Glassware

Detailed 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”.

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8.2. Disposable Glassware

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

Clean disposable glassware by wrapping glassware in aluminum foil and baking it in the muffle furnace at 500°C for 3 hours.

8.3. Reusable Plastics

Equipment in this classification includes pipette bulbs.

Wash reusable plastic equipment in the automated dishwasher on the "Plastic Low Heat" program (include soap) then dry equipment in fume hood. Rinse equipment with high purity solvents in the following order: methanol, acetone, toluene, and hexane. Allow equipment to dry in fume hood and then transfer to lab for use.

9. CONSUMABLES

For details on the required consumables, see appendix Table 1.

10. STANDARDS AND REAGENTS USED

10.1. Solvents

The reagents listed below are Residue Analyzed or HPLC Grade. Recommended supplier and supplier part number are listed. When not available an equivalent grade may be substituted.

- Methylene Chloride [dichloromethane, CH2Cl2]
  J.T. Baker, Organic Residue Analysis Grade, Part No. 9264-03

- Isooctane [2,2,4-Trimethylpentane, (CH3)2CHCH2C(CH3)3)]
  J.T. Baker, Organic Residue Analysis Grade, Part No. 9335-03

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

For details on the composition and preparation of the Surrogate Standard, Laboratory Control Spike Standard and Internal Standard see Tables 2-4.

10.3. 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 analysis techniques, x-ray fluorescence (XRF), Inductively Coupled Plasma-Mass Spectrometry (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 500 mg of Foam A in DCM for 5 minutes. Remove DCM and repeat sonication process two additional times. Dry cleaned Foam A under ventilation.

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11. PROCEDURE

11.1. 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 15 samples, including QC samples prepared together within a 24 hour period. 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. If QC samples do not meet defined criteria, the cause must be determined and corrective action must be taken. See Table 5 for method QC requirements.

11.1.1. Method blank (MB)

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

A MB is prepared as follows; 50 mg (+/- 5 mg) of analyte free foam is weighed and spiked with Surrogate Standard (see Table 2).

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

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

A LCS and a LCSD are prepared as follows; 50 mg (+/- 5 mg) of analyte free foam is weighed and spiked with Surrogate Standard and Laboratory Control Spike Standard (Table 2 and 3). The percent recovery of the target analytes in the LCS must be within 50-150%. If the LCS does not meet the criteria, the entire batch must be reprocessed.

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

11.1.3. 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.

Duplicate samples must have a relative percent difference (RPD) of ≤ 30% for each target analyte. 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.

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11.1.4. Reference Material

In-house reference material (RM) 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.

A RM is prepared as follows; 50 mg (+/- 5 mg) of RM foam is weighed and spiked with Surrogate Standard (see Table 2). RM results must be within 70-130% of defined value (see Table 5).

11.2. Instrument/Equipment QC requirements

Analytical balance and manual and/or electronic pipettes must not be past calibration due date. Verify calibration sticker on balance and or pipette before use.

Refer to SOP "Determination of Organophosphate Flame Retardants and Polybrominated Diphenyl Ethers in House Dust and Consumer Products by Gas Chromatography – Tandem Mass Spectrometry", DCN 05.0029.00 for data analysis.

11.3. Sample Preparation Procedure

11.3.1. Preliminary Steps and Subsampling

(1) Check out samples from sample storage area and fill out Chain of Custody Log.

(2) Prepare sample labels with ECL sample ID number, date and initials of analyst. Place on sample tubes receiving samples.

For QC samples prepare the following labels, MB, LCS, LCSD and RM with date and initials of analyst.

(3) Verify calibration of the analytical balance using calibration weights. Fill out the Analytical Balance Log Book kept with analytical balance.

(4) For weighing QC foam and samples use the following procedure.

Using a clean surgical razor blade, cut 50 mg (+/- 5 mg) of foam and or fabric subsample from the primary sample material. Tare a plastic weighing boat on the analytical balance and weigh sample material. Record weight on Sample Preparation Worksheet (see appendix Figure 2) and transfer sample to corresponding sample test tube. Seal test tube with test tube screw top.

(5) For the selected sample duplicate weigh an additional 50 mg (+/- 5 mg) of sample and add to sample duplicate test tube.

Record duplicate sample ID on Sample Preparation Worksheet.

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It is important to line the laboratory hood work surface with clean aluminum foil. Clean foil must be used for each sample. This will prevent work surface contamination and sample cross contamination when high levels of flame retardants are present within a sample. Aluminum foil, surgical razor blades and nitrile gloves are to be replaced after each sample.

11.3.2. Extract Collection Tubes

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

11.3.3. Quality Control Standard Spikes

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

(2) Add surrogate standard (see Table 2) 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 3) to LCS and LCSD. Addition of standard should be directly to the surface of the sample material.

11.3.4. Foam and Textile Sonication Extraction

(1) Add 5 mL DCM to each QC and sample tube and ensure tube caps are fastened.

(2) Vortex each QC and sample tube for 1 minute at 3000/min.

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

(4) Using a clean pasteur pipette remove liquid extract from sample tube and filter particulate matter using a 3 mL bond elute cartridge fitted with two 20 um pore size frits. Avoid removal of solid sample particulate material. Liquid extract is collected in pre-weighed extract collection tubes (see 11.3.2).

(5) Repeat extraction with an additional 5 mL of fresh DCM. Vortex for 1 minute and sonicate for 10 minutes as described above. Remove liquid extract with clean pasteur pipette and filter particulate matter as described above. Pool both primary and secondary extracts in collection tube.

(6) Centrifuge QC and sample tubes containing the foam or textile matter for 2 minutes at maximum speed to collect additional liquid adsorbed to the solid material. Transfer remaining liquid to filter cartridge. Apply vacuum to cartridge manifold to draw out remaining extract.

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(7) Cap extract collection tubes and weigh using analytical balance. Record weights in laboratory notebook and calculate extract volume for each tube using the density of DCM (1.325 g/mL at 25°C). Add DCM to each collection tube to make up volume (lost to evaporation) to 10 mL. Weigh final extract collection tubes and record final extract weight in laboratory notebook.

(8) Mark extract level, seal with parafilm and refrigerate at 4 °C to prevent solvent evaporation.

11.3.5. 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 content.

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

(3) Using a 200 uL pipette transfer 160 uL of each extract to corresponding autosampler vial.

(4) Using a 50 uL pipette add 40 uL of Internal Standard solution (see Table 4) to each autosampler vial. Be sure to place the pipette tip into the solvent and wash with several cycles of intake and release. Ensure that no liquid is removed.

(5) Vortex each GC autosampler vial for 30 seconds to mix contents. The samples are now ready to be analyzed by GC-MS/MS.

11.3.6. 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. Dilutions must be noted on the sample preparation work sheet.

- To make a 1:10 dilution; transfer 1.25 mL of sample extract to clean tube and add 8.75 mL of DCM. This will yield a 1:8 dilution of the sample extract. Following steps 1-5 in section 10.3.4 will yield a final 10 fold dilution in the GC vial.
- To make a 1:25 dilution; transfer 500 uL of sample extract to clean tube and add 9.50 mL of DCM. This will yield a 1:20 dilution of the sample extract. Following steps 1-5 in section 10.3.4 will yield a final 25 fold dilution in the GC vial.
- To make a 1:50 dilution; transfer 250 uL of sample extract to clean tube and add 9.75 mL of DCM. This will yield a 1:40 dilution of the sample extract. Following steps 1-5 in section 10.3.4 will yield a final 50 fold dilution in the GC vial.
- To make a 1:100 dilution; transfer 125 uL of sample extract to clean tube and add 9.875 mL of DCM. This will yield a 1:80 dilution of the sample

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extract. Following steps 1-5 in section 10.3.4 will yield a final 100 fold dilution in the GC vial.

- To make a 1:500 dilution; transfer 25 uL of sample extract to clean tube and add 9.975 mL of DCM. This will yield a 1:400 dilution of the sample extract. Following steps 1-5 in section 10.3.4 will yield a final 500 fold dilution in the GC vial.
- To make a 1:1000 dilution; transfer 100 uL of sample extract to clean tube and add 9.9 mL of DCM. 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 with the addition of 8.75 mL of DCM. This will yield a 1:800 dilution of the sample extract. Following steps 1-5 in section 10.3.4 will yield a final 1000 fold dilution in the GC vial.

11.4. Data Analysis

Refer to SOP "Determination of Organophosphate Flame Retardants and Polybrominated Diphenyl Ethers in House Dust and Consumer Products by Gas Chromatography – Tandem Mass Spectrometry", DCN 05.0029.00.

11.5. Data Reporting

Record data relevant to sample preparation on the Foam/Textile Sample Preparation Worksheet (see Figure 2).

11.6. Method Performance

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

12. MAINTENANCE AND TROUBLE SHOOTING

12.1. Sonicator

The water bath in the Branson sonicator should be refilled prior to each use, ensuring complete coverage of sample and DCM liquid extract. The water bath should be drained and refilled periodically.

12.2. Analytical Balance

The performance of the analytical balance should be verified prior to each batch.

12.3. Manual and Electronic Pipettes

Manual and electronic pipettes should be within calibration date window.

12.4. Contamination

Sample preparation practices must be followed as described in section 5 Interferences.

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13. REFERENCES

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

13.2. IKA MS 3 Basic Minishaker Model Number MS 3 B S1 Manual.

13.3. Branson Ultrasonic Bath Model 3800 Operation's Manual 100-214-294.

13.4. SOP, Determination of Organophosphate Flame Retardants and Polybrominated Diphenyl Ethers in House Dust and Consumer Products by Gas Chromatography – Tandem Mass Spectrometry, DCN 05.0029.00.

13.5. ECL Chemical Hygiene Plan

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APPENDIX

Table 1: Foam and Textile Sample Preparation Consumables List.

Item	Description	Supplier	Part/Catalogue #
Scalpel Blade	Bard-Parker Safety Lock Rib-Back Scalpel Blades, Sterile, Surgical	VWR[1]	89176-354
Weighing Dish	Plastic Micro Boat, Anti-Static EMS, 5 mL Capacity	VWR	100502-714
Threaded Test Tube	Disposable Screw-Cap Finish, Borosilicate Glass, 16 x 100 mm, 12 mL	VWR	53283-802
Test Tube Screw Cap	Black Phenolic Screw Cap with PTFE/Rubber Liner, 15-415 GPI Thread Finish	Kimble[2]	45066C 15
Pasteur Pipette	Durex Borosilicate Glass pipette, 9"	VWR	14673-043
Pipette Bulbs	Small Latex Bulb, 57 mm	VWR	82024-554
GC Vials	12 x 32 amber vial with 300 uL capacity fused insert, Borosilicate Glass	Wheaton[3]	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 Elute Cartridge	3 mL bond elute cartridge Reservoir, 2 - 20 um filter	Agilent[4]	12131014
Flow Control Liners	Disposable Flow Control Valve Liners for VISIPREP-DL	Sigma-Aldrich[5]	57059

[1]us.vwr.com
[2]kimble-chase.com
[3]wheaton.com
[4]agilent.com
[5]sigmaaldrich.com

Table 2. 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	DLM-8901-S
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	MBDE-77

The dTPP Surrogate Standard is prepared by transferring 62.5 uL of dTPP to a 5 mL volumetric flask. Bring to 5 mL volumetric mark with isooctane and mix thoroughly by swirling for 1 minute and inverting for 1 minute. Let the solution rest for 15 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/uL with no dilution of supplier stock.

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Table 3. 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	100.3	374	3000	12.5	2500	AccuStandard	PFRS-025S
BDE-47	49.31	760.5	3000	12.5	2500	AccuStandard	BDE-047S

The Matrix Spike Standard is prepared by transferring the appropriate volume of each component listed in Table 3 to a 3 mL volumetric flask. Bring to3 mL volumetric mark with isooctane and mix thoroughly by swirling for 1 minute and inverting for 1 minute. Let the solution rest for 15 minutes at room temperature and then repeat mixing procedure. Record preparation in ECL standards log book.

Table 4. Internal Standard Composition

Component	Supplier Conc. [ng/µL]	Volume [µL]	Final Volume [uL]	Final Concentration [pg/µL]	Volume Added 40 µL Final GC Vial Conc. [pg/uL]	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-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	92	135.9	10000	1250	250	CIL	DLM-9317-S
dTDCIPP	50	250	10000	1250	250	Wellington	dTDCPP
dTPHP	50	250	10000	1250	250	Wellington	dTPP

The Internal Standard is prepared by transferring the appropriate volume of each component listed in Table 4 to a 10 mL volumetric flask. Bring to 10 mL volumetric mark with isooctane and mix thoroughly by swirling for 1 minute and inverting for 1 minute. Rest solution for 15 minutes and then repeat mixing procedure. Record preparation in ECL standards log book.

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Table 5. Method Quality Control Criteria

Method Quality Control Requirements					
Method Blank [MB]	**Laboratory Control Spike [LCS]**	**Laboratory Control Spike Duplicate [LCSD]**	**LCS/LCSD RPD**	**Reference Material [RM]**	**Surrogate Recovery**
<MDL	50 - 150%	50 - 150%	≤30%	70 - 130%	50 - 150%

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

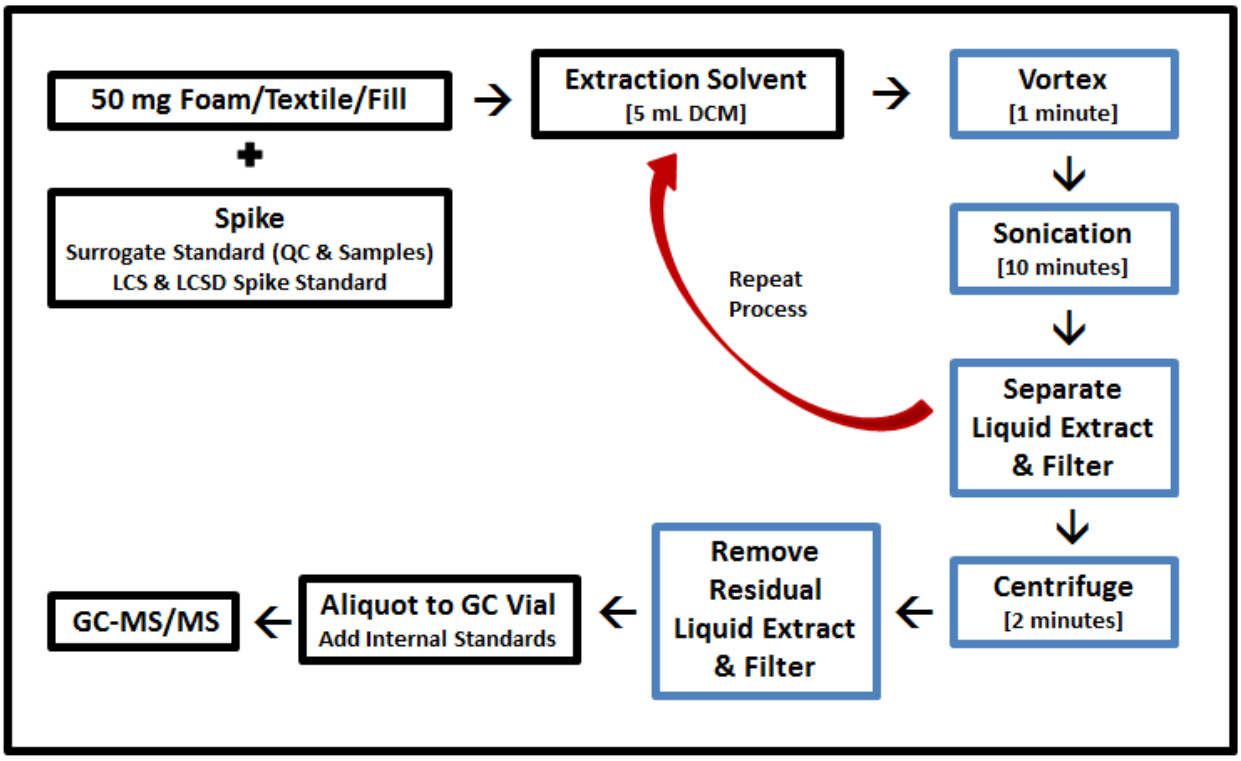

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Figure 2. Foam/Textile Sample Preparation Worksheet

Foam/Textile Sample Preparation Work Sheet

Environmental Chemistry Lab, CA-DTSC

Batch #: _____ Analyst: _____

Lab Notebook #: _____ Date: _____

		ECL #	Sample Weight (mg)	Homogenous / Heterogeneous	Notes/Material Description	Dilution Factor
MB	1					
LCS	2					
LCSD	3					
RM	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	13					
	14					
	15					

Reagent ID & Lot

Reagent: _____
Supplier: _____
Part #: _____
Batch/Lot #: _____

Reagent: _____
Supplier: _____
Part #: _____
Batch/Lot #: _____

Standards

Surrogate ID: _____
LCS Standard ID: _____
Internal Standard ID: _____

_____ _____
Analyst Date

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14. REVISION HISTORY

Revision	Date	Responsible Person	Description of Change
0	November 2015	Ranjit Gill	New Issue

15. ANNUAL REVIEW

Signatures	Date