Standard Operating Procedure For:

Extraction of Pesticides, Herbicides and other Chemicals from Water or Sediments/Waste

Note: LC-011 (A) is embedded in this SOP pertaining to Sediments/Waste Sample Preparation



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1 SCOPE AND APPLICATION

1.1 The SOP describes the extraction procedures for the isolation of chlorinated acid herbicides, Carbamates, urea herbicides and other chemicals from sediment/waste, ground water, or surface water matrices. If the water samples are not hydrolyzed with sodium hydroxide at basic pH of >12 for 1 to 2 hours, then this procedure isolates ONLY the acid forms of chlorinated acid herbicides, and it's not applicable for the extraction of the salt or ester forms of chlorinated acid herbicides.

1.2 Using this method, the analytes listed in Table 1 are reported in the Laboratory Information Management System (LIMS) for the tests. This method follows the requirements of EPA Method 8321B and USGS Method O-2060-01 for the parameters denoted as such in Table 5.1 of the laboratory's Quality Manual. USGS Method O-2060-01 is an approved test procedure of the National Pollutant Discharge Elimination System (NPDES) under the Clean Water Act (CWA) and Method 8321B is listed in the SW-846 publication under Subtitle C of the Resource Conservation and Recovery Act (RCRA). Furthermore, the NPDES and RCRA compliant tests employed by the laboratory using these methods are listed below.

Table 1: FDEP Laboratory Test IDs and equivalent methods reported by the SOP.

Laboratory Test Name	Test Description
W-CAB-AA-R	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
W-CARB-AA	USGS Method O-2060-01 analysis/EPA Method 3535A preparation, water sample
W-E8321-21	EPA Method 8321B analysis, water sample analysis by direct injection 21 days holding time
W-E8321-DI	EPA Method 8321B analysis, water sample analysis by direct injection 7 days holding time
W-E8321-MS	EPA Method 8321B analysis/EPA Method 3535A preparation, water sample
W-U2060-MS	USGS Method O-2060-01 analysis/EPA Method 3535A preparation, water sample
SPLP-AH-AA	EPA Method 8321B analysis/EPA Method 3010/3535A preparation, SPLP water sample
TPLP-AH-AA	EPA Method 8321B analysis/EPA Method 3011/3535A preparation, TCLP water sample
W-MCYST-AA	EPA Method 8321B analysis, water sample
W-PDQUAT	EPA Method 8321B analysis/Direct Injection preparation, water sample
W-PFAS-MS	EPA Method 8321B analysis, water sample
W-QUAT-NH4	EPA Method 8321B analysis, water sample
S-AHERB-AA	EPA Method 8321B analysis/In House Method preparation, soil sample
S-E8321-MS	EPA Method 8321B analysis/EPA Method 3570 preparation, soil sample
SA-LCMSMS	EPA Method 8321B analysis, surface area wipe sample
O-LCMSMS	EPA Method 8321B analysis, unknown screening sample

1.3 Water samples (filtered if sample is not clear and contains lots of particulate) are extracted by passing through a solid phase column (Graphitized carbon-based solid-phase extraction column or Waters Oasis HLB columns or C18 SPE column) following EPA Method 3535A. Interferences are not removed by extraction of the base phase. Sucralose is sensitive to these acid/base extraction conditions and extraction at neutral pH 7 is used.

1.4 Following USGS method O-2060-01, water samples are extracted by passing through a 0.5 gram graphitized carbon-based solid-phase extraction column (EPA Method 3535A) at neutral pH 7 for Chlorophenoxy acid herbicides, Carbamates, Personal Care Products and Urea herbicides. The extracts are then analyzed using high-performance liquid chromatography/electrospray ionization–mass spectrometry (LC-MSMS) to detect, identify, and quantify polar pesticides and pesticide metabolites in filtered water at concentrations as low as 2 nano grams per liter. This new method was designed to improve sensitivity and selectivity over the prior method, and to reduce known interferences from natural organic matter. These analytes also extract very well from acidified water samples using Waters Oasis HLB columns or C18 SPE columns (Sec. 1.3).

1.5 In sediment/waste extraction, samples are directly extracted with 0.3N sodium hydroxide followed by solid phase extraction. This simple procedure is most effective for the extraction of Chlorophenoxy acid herbicides. The base phase extraction also helps with the hydrolysis of the salts and esters to free acids.

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1.6 EPA Method 3570 is used to prepare soil samples for Carbamates, Urea Herbicides and Personal Care Products. Using this method, soil samples are extracted with 1:1 Acetonitrile – MCAA water and the filtered extract is analyzed by LC-MSMS.

1.7 Water samples are analyzed by direct injection analysis for the Negative and Positive analytes. Higher detection limits are obtained by injecting 50 μL or more and followed by LC-MSMS analysis without pre-concentration factor of 125.

1.8 EPA Method 3535A is used to prepare water samples for Perfluorinated Alkyl Acids. Using this method, water samples are extracted by passing through Waters Oasis HLB Plus LP Extraction Cartridge.

2 SUMMARY OF THE METHOD

2.1 Activate Carbon SPE: Chlorophenoxy acid herbicides, Carbamates, Urea Herbicides and Personal Care Products are extracted from 125 mL of water samples at neutral pH 7 with 125 mg of sodium chloride and 62.5 mg of EDTA using a 0.5 gram graphitized carbon-based solid-phase extraction column (EPA Method 3535A). After extraction, the adsorbed compounds are eluted from the SPE cartridge using two sequential elutions of 2 mL acetonitrile as fraction 1 and followed by 13 mL of an 80:20 ratio of methylene chloride – methanol containing 0.2% trifluoroacetic acid anhydride as fraction 2. The fraction 2 is reduced under nitrogen blowdown to near dryness and then combined with fraction 1. The final volume for the extract is 1 mL in 1:1 acetonitrile-MCAA water and the extract is filtered through 0.45-micron filter and analyzed by LC-MSMS. For Sucralose, the same process is followed except fraction 2 is 80:20 ratio of methylene chloride – methanol without TFAA.

2.2 Waters Oasis HLB SPE: Chlorophenoxy acid herbicides, Carbamates, Urea Herbicides and Personal Care Products, Dalapon, Imazapyr and Pyraclostrobin are extracted from 125 mL of water samples acidified to pH <2 containing 125 mg of sodium chloride and 62.5 mg of EDTA using Waters Oasis HLB columns (EPA Method 3535A). Adsorbed compounds are eluted from the SPE cartridge using acetonitrile. and the fraction is reduced under nitrogen to near dryness. The final volume for the extract is 1 mL in 10% acetonitrile – MCAA water and the extract is filtered through 0.45-micron filter and analyzed by LC-MSMS. Some Chlorophenoxy acid herbicides may exhibit poor recoveries.

2.3 C18 SPE: Chlorophenoxy acid herbicides, Carbamates, some Urea Herbicides and Personal Care Products are extracted from 125 mL of water samples acidified to pH <2 containing 125 mg of sodium chloride and 62.5 mg of EDTA using C18 columns (EPA Method 3535A). Adsorbed compounds are eluted from the SPE cartridge using acetonitrile. and the fraction is reduced under nitrogen to near dryness. The final volume for the extract is 1 mL in 10% acetonitrile – MCAA water and the extract is filtered through 0.45-micron filter and analyzed by LC-MSMS. Acetaminophen, Dalapon, Imazapyr and Sucralose are not recovered in this procedure.

2.4 Sucralose by Waters Oasis HLB SPE: Following EPA Method 3535A for Sucralose extraction, 125 mL of water sample containing 125 mg of sodium chloride is passed through Waters Oasis HLB columns. Adsorbed compounds are eluted from the SPE cartridge using acetonitrile. and the fraction is reduced under nitrogen to near dryness. The final volume for the extract is 1 mL in 10% acetonitrile – water and the extract is filtered through 0.45-micron filter and analyzed by LC-MSMS.

2.5 Thirty grams of sediment samples are extracted for acid herbicides by sonication into an aqueous NaOH solution. The extract is then acidified and passed through C18 solid phase extraction column. Compounds of interest are eluted with 5 mL of acetonitrile. The 5 mL of acetonitrile eluate from above is then diluted to 10 mL with MCAA water and filtered through 0.45-micron filter and analyzed by LC-MSMS.

2.6 Ten grams of sediment sample is extracted for Carbamates, Urea Herbicides and Personal Care Products by shaking in 10 mL of 1:1 acetonitrile-MCAA water for one hour following EPA Method 3570 Micro scale Solvent Extraction. Part of the extract is filtered through 0.45-micron filter and analyzed by LC-MSMS.

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2.7 For Direct Injection Analysis: Water samples are filtered through 0.45-micron filter and analyzed by LC-MSMS for Negative and Positive analytes.

2.8 For Microcystins analysis, the whole water sample is autoclaved prior to analysis to release (lyse) the Microcystins from the algal and bacterial cells present. Therefore, this method determines the "total" amount of Microcystins present in the sample. After autoclave, water samples are filtered through a 0.45-micron filter and an aliquot is analyzed by LC-MSMS.

2.9 For Perfluorinated Alkyl Acids (PFAA) by Waters Oasis HLB Plus LP SPE: Following EPA Method 3535A for PFAA extraction, 250 mL of water sample is passed through Waters Oasis HLB Plus LP cartridge. Adsorbed compounds are eluted from the SPE cartridge using acetonitrile. and the fraction is reduced under nitrogen to 1 mL. The final volume for the extract is 2 mL in 1:1 acetonitrile – water and the extract is filtered through 0.45-micron filter and analyzed by LC-MSMS.

3 APPARATUS AND EQUIPMENT

3.1 Soil extraction: Metal spatulas, Aluminum weighing boats, Oven for dry weight determinations at 105 °C, Ultrasonic Bath (Fisher Scientific, Model FS220, 320 Watts power), Centrifuge bottles (250 mL and 500 mL), Centrifuge to fit bottles, Glass beakers (400 and 1000 mL), pH paper - 0.0 to 3.0 and 0.0 to 12.0, Top-Loading balance accurate to + or - 0.1 g

3.2 Soil Micro-extraction: Vials – 40 mL with PTFE septa, Wrist Action Shaker, Vortex

3.3 Filtration: Vacuum filtration flasks (500 and 1000 mL), Porcelain Büchner funnels 9 cm, Rubber funnel supports, Whatman binder free glass microfiber filter (type: GF/F, 9 cm, 0.7 μm) or TCLP Glass Fiber filter, Glass beakers (250 mL and 400 mL)

3.4 Water extraction: Baker vacuum manifold or equivalent (capable of holding SPE cartridges with 75 mL polypropylene sample reservoirs), screw cap culture tubes 15 mL, and Glass beakers (400 mL)

3.5 Centrifuge tubes, graduated, with screw cap or ground glass stoppers – 15 mL (Calibrate tubes to the required volume and record in the Extraction Log Book)

3.6 Gas-tight micro-syringes or volumetric pipets - 0.25 mL, 0.5 mL and 2 mL (Manufacturer's Certificate of Accuracy stating accuracy within 1% must be on file, otherwise quarterly calibration is required.)

3.7 Eppendorf 10 μL to 100 μL and 100 μL to 1000 μL adjustable pipette (calibrated quarterly)

3.8 Pippet tips – 200 μL, 30 to 300 μL and 1000 μL or to fit

3.9 Volumetric flasks, 500 and 1000 mL and as needed

3.10 N-Evap®: a heated water bath apparatus used to concentrate the extract with nitrogen gas

3.11 Graduated Polypropylene tube - 15 mL

3.12 Autoclave – Consolidated Stills and Sterilizers, Model # SSR-3A and Autoclave Temperature Indicator Tape.

3.13 0.45-micron filtration disks, 2-mL crimp top vial and Polypropylene Autosampler vials – 0.3 mL

4 REAGENTS AND CHEMICALS

4.1 C18 SPE Cartridge 500 mg (Restek, Resprep or equivalent), Waters Oasis HLB columns 200 mg/6cc, Waters Oasis HLB Plus LP Columns, Supelco ENVIRO-Carb Graphitized Carbon Black 500 mg, 120/400 mesh, 6-mL (catalog number 57094), UCT Enviro-Clean Graphitized Carbon Cartridge 500 mg (catalog number

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EC535156-P), and Millipore 13 mm PTFE Syringe Filters 0.45 micron, pack of 1000 (catalog number - SLCR013NK).

4.2 Hydrochloric acid (HCl), reagent grade; Sulfuric acid (H_2SO_4), reagent grade; Anhydrous NaOH, reagent grade; Formic acid, Acros, Reagent ACS (96%); Trifluoroacetic acid anhydride (TFAA), Pierce Chemical, Inc., reagent grade or equivalent; and L– (+)–Ascorbic acid, J.T. Baker, reagent grade or equivalent.

4.3 Solvents, HPLC grade or equivalent: Acetonitrile, acetone, methylene chloride, methanol and in house deionized water (HPLC water) tested to be free of target analytes.

4.4 1:9 HCl: Slowly add 100 mL of reagent grade HCl to 900 mL of HPLC water in a 1-liter volumetric flask. Mix well.

4.5 1:1 Sulfuric acid aqueous solution – slowly combine equal amounts of sulfuric acid and HPLC water. **REMEMBER: Add acid to water, NOT water to acid, otherwise a violent reaction may occur!**

4.6 Sulfuric acid, 0.1M: Slowly add 2.8 mL of concentrated sulfuric acid (18M) to approximately 450 mL of HPLC water in a 500-mL volumetric flask. Bring to a final volume of 500 mL and mix well. Transfer to re-pipet dispenser bottle.

4.7 0.3N NaOH - Dissolve 6 g NaOH into 500 mL of HPLC grade water.

4.8 Sodium Chloride, reagent grade: Wash with methylene chloride and bake at 700 °C prior to use. Prepare freshly by dissolving 7.5 grams of sodium chloride in 30 mL of water and add 0.5 mL to 125 mL of water sample before extraction.

4.9 EDTA, reagent grade. Prepare freshly by dissolving 3.74 grams of EDTA in 30 mL of water and add 0.5 mL to 125 mL of water sample before extraction and allow the samples stand for one hour before extraction.

4.10 Ottawa Sand for method blanks and lab-fortified blanks: bake at 700 °C prior to use.

4.11 DEP well water: Ground water tested free of interferences and used for method blanks and lab fortified blanks (LFBs).

4.12 Estimate and prepare just enough conditioning and elution solutions for Graphitized Carbon Cartridges.

4.12.1 SPE cartridge conditioning solutions, Solution 1 - 80:20 methylene chloride - methanol. Mix 80 mL of methylene chloride with 20 mL of methanol. Store in a 250-mL stoppered Erlenmeyer flask. Replace solution at least weekly.

4.12.2 SPE cartridge conditioning solutions, Solution 2 - Ascorbic acid solution. Dissolve 2 g of ascorbic acid (4.2) in 200 mL DI water and mix. Refrigerate at 4 °C and replace solution at least weekly.

4.12.3 SPE cartridge elution solution - 80:20 methylene chloride - methanol /0.2-percent trifluoroacetic acid anhydride. Mix 80 mL of methylene chloride, 20 mL methanol, and 0.2 mL of TFAA (4.2). Prepare solution daily and store in a 250-mL stoppered Erlenmeyer flask.

4.13 Negative and Positive Ion (NIPI) spike mix at 0.40 µg/mL and 7-surrogate spike mix at 0.80 µg/mL: add 25 µL of each as required for a final volume of 1 mL extraction and 250 µL to a final volume of 10 mL extraction.

4.14 See SOP LC-039 for Monochloroacetic acid buffer solution (MCAA water) and all other spike standards preparation.

5 SAMPLE COLLECTION, PRESERVATION AND HANDLING

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5.1 Water samples are collected in 500-mL glass amber bottle for acid herbicides, Urea Herbicides and Personal Care Products. For Carbamates, samples are collected in pre-preserved 500-mL glass amber bottles with Monochloroacetic acid to less than pH 5. For Direct Injection Analysis (Endothall, Glyphosate and Sucralose) samples are collected in 40 mL vials and for Diquat and Paraquat samples are collected in pre-preserved 250 mL brown plastic bottles with Heptafluorobutyric acid to less than pH 5. For Malathion, samples are collected in pre-preserved 40 mL vials with Monochloroacetic acid to less than pH 5. All the samples must be iced or refrigerated at 6 °C or less from the time of collection until extraction and must be extracted within 7 days of collection except for Didecyldimethylammonium chloride and Sucralose which must be extracted within 21 days of collection.

5.2 For PFAAs, samples are collected in a 250-mL high density polyethylene or polypropylene bottle fitted with a polypropylene screw-caps. Water samples that are known to be chlorinated must be preserved with Trizma. Trizima at 5.0 ug/L is added to each each sample bottle as a solid prior to shipment to the field (or prior to sample collection)). Samples must be extracted within 14 days of collection and analyzed within 28 days after extraction.

5.3 Sediment/waste samples are collected in glass jars and must be iced or refrigerated at 6 °C or less from the time of collection until extraction. They must be extracted within 14 days of collection.

5.4 Carbamates must be analyzed within 21 days of extraction; all other sample extracts must be analyzed within 40 days of sample extraction.

6 SAMPLE PREPARATION PROCEDURE

NOTE: a second person must witness the addition of spike standards to the proper samples after verifying the correct volume of spike to add, the spike's LIMS number and its concentration. The witness' initials are recorded in the lab notebook or in LIMS along with the bottle number (if more than one bottle is submitted), lot numbers of reagents and solvents used, bath temperature for extract concentration, and balance ID. Calibrate tubes to volume (if necessary).

6.1 Thoroughly clean all glassware by rinsing it with hot tap water, deionized water and acetone, then baking it in an oven at 550 °C for 8 hours. Alternatively, glassware may be thoroughly washed with Alconox and hot water, and then rinsed with hot tap water, deionized water, and acetone.

6.2 All glassware used in these procedures must be pre-rinsed with 1:9 HCl and then thoroughly rinsed with deionized water followed by methanol. This ensures removal of all traces of possible alkaline residue on the glassware, as the analytes are strong organic acids that react readily with alkaline substances and may be lost during extraction otherwise.

6.3 Check out the water and sediment samples from the receiving room walk-in cooler.

6.4 Before proceeding, ensure that the spike and surrogate standards are at room temperature.

6.5 Acid Herbicides Sediment Sample Extraction (Method SOP-LC-011(A)) for the LIMS test S-AHERB-AA

6.5.1 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. There should be one method blank (Ottawa sand), one laboratory fortified blank (Ottawa sand spikes) and duplicate matrix spikes for each set of up to 20 samples.

6.5.2 Note the appearance of each sample in the logbook (e.g., presence of foreign materials, variable particle size, etc.).

6.5.3 Do not decant standing water, large rocks, and other foreign material from the samples at any point. The analyst must consult his/her supervisor for direction if obvious non-representative material is

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found in the sample. For most samples, you will need to remove the sample from the jar and place it on a sheet of aluminum foil (dull side up, shiny side down) to homogenize it fully. Do not use aluminum foil if the sample is to be analyzed for metals. Homogenize each sample by mixing it thoroughly using a metal spatula.

6.5.4 Perform a dry weight determination on all samples, unless this has already been done:

6.5.4.1 Weigh 5 g of each sample into a labeled, pre-weighed aluminum-weighing dish. Record the weight of the dish and the exact weight of the wet sediment in the Dry Weights Notebook. Dry it overnight at 105 °C. The next day, allow it to cool in a desiccator before weighing it a second time. Record the exact weight of the dry sediment in the dry weight notebook.

6.5.4.2 A sample's percent solid is equal to the dry sediment weight (not including the dish weight) divided by the wet sediment weight (not including the dish weight) and multiplied by 100%. Enter the wet and dry weights into the computer-generated extraction log sheet, which will calculate and display the percent solids.

6.5.4.3 Remember to upload the percent solids of all samples into LIMS.

6.5.5 Weigh approximately 30 g of each sample into a properly labeled 500-mL centrifuge bottle and record the weight to the nearest 0.1-g. Prepare one method blank and one laboratory-fortified blank from Ottawa Sand. Select one sample for duplicate matrix spikes. For waste analysis, weigh one gram instead of 30 grams.

6.5.6 Add 250 µL of 0.80 µg/mL 7-Surrogate spike mix to all the samples in the set and 250 µL of NIPI Spike Mix 0.40 µg/mL to the laboratory- fortified blank and matrix spikes to a final volume of 10 mL.

6.5.7 Add 200 mL of 0.3N NaOH solution to each sediment sample and mix with spatula. Sonicate all samples in an ultrasonic water bath for 5 minutes. Then, allow the samples to stand for 25 minutes, mix and sonicate again for 5 minutes, and allow it to stand for another 25 minutes period. Finally, mix and sonicate for a third time for 5 minutes. Centrifuge each for approximately 5 minutes at 50 rpm to settle suspended particulate matter. Decant the NaOH supernatant from each sample into a tarred, labeled centrifuge bottle and record the weight of the decanted extract.

6.5.8 Acidify each aqueous extract with approximately 6 mL of 1:1 sulfuric acid solution (or 1.5 mL concentrated sulfuric acid) and swirl to mix well. Check pH with narrow range pH paper (pH 0.0 to 3.0) to ensure it is less than or equal to 2.

6.5.9 After the extracts have been acidified, they may be stored overnight at 4 °C, if necessary. The sample integrity will be maintained only after pH has been adjusted to 2 or less with acid.

6.5.10 It is likely that precipitate will form during sample acidification. Centrifuge the cloudy extract before adding to SPE cartridge. After transferring the extract, rinse the bottle with 10 mL of 0.1M sulfuric acid, centrifuge again, and then decant the rinse into the reservoir.

6.5.11 Label the C18 cartridge with the appropriate sample number. Firmly push an adapter into the top of each cartridge, and then attach a 75-mL reservoir to each adapter.

6.5.12 Condition each cartridge by aspirating 5-mL acetonitrile through the cartridge, followed by 10 mL of 0.1M sulfuric acid. Do not allow the cartridge to go dry during conditioning or sample introduction. When the level of liquid in the cartridge begins to approach the bottom, shut off the valve on the vacuum manifold for that cartridge. Do not let the cartridge go dry during conditioning.

6.5.13 Fill each reservoir with the appropriate sample extract. Open the valves on the vacuum manifold and

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allow the samples to begin passing through the cartridges. The vacuum gauge should not exceed 8-12 inches Hg which corresponds to a sampling rate of about 10mL/min. Do not let any cartridge go dry during sample addition. If a cartridge becomes clogged, and the sample will not pass through it, use a new cartridge.

6.5.14 After the entire volume of a sample has been passed through a cartridge, rinse that sample's beaker with 5 mL of 0.1M sulfuric acid. Pour the rinsate into the sample reservoir and allow it to pass through. After this rinse has completely passed through the cartridge, allow the cartridge to go dry. Remove the reservoir, and let the cartridge vacuum dry for several minutes to remove all traces of water from it.

6.5.15 Elute the analytes from the cartridge by attaching a 5-mL syringe to the adapter on the cartridge. Put 5 mL of acetonitrile into the syringe. Push the acetonitrile through the cartridge using the syringe plunger. Collect eluate in a labeled 15 mL graduated centrifuge tube. If more than one cartridge is used, then when eluting the sample attach the second cartridge to the adapter on the first cartridge and attach the syringe to the adapter on the second cartridge. Use only 5 mL of acetonitrile to elute and push it through both cartridges into a centrifuge tube.

6.5.16 Collect up to 5 mL of acetonitrile eluate from the SPE cartridge and dilute with MCAA water to 10 mL. Vortex to mix and filter through a 0.45-micron filtration disk into a 2-mL crimp top vial. Proceed with LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.6 Acid herbicides Extraction in SPLP and TCLP Extracts for the Lims tests SPLP/TCLP-AH-AA

6.6.1 For SPLP and TCLP extraction, 125 mL of SPLP/TCLP extracts are spiked as in step 6.5.6. The acidified samples extracts are passed through C18 cartridge as in steps 6.5.11 to 6.5.16 to complete the preparation for the tests SPLP/TCLP-AHB-AA. Surrogate and spike amount adjusted to a final volume of 1 mL.

6.7 Water Samples Preparation by Waters Oasis HLB Solid Phase Extraction (EPA Method 3535A) for the LIMS test W-CAB-AA-R, W-CARB-AA, W-E8321-MS and W-U2060-MS

NOTE: Some Acid Herbicides recover low using HLB SPE Columns, therefore C18 SPE Columns are used for these analytes. For Sucralose extraction, samples are not acidified and extracted at neutral pH 7.

6.7.1 Water samples that are not clear and/or contain a lot of particulate need to be pre-filtered before extraction using a 0.7-µm pore size glass-fiber filter. Measure or weigh 125 mL of filtrate or water sample into a beaker and check and record the pH of the sample. Add 0.5 mL of sodium chloride solution (sec. 4.8) and 0.50 mL of EDTA solution (sec. 4.9) and mix well with a glass rod.

6.7.2 For every 20 samples in the set, prepare one extraction blank and one Lab-fortified blank using DI water and one sample duplicate matrix spikes.

6.7.3 For all the samples in the set add 25 µL of 0.80 µg/mL of 7-Surrogate mix. For QC samples add 25 µL of 0.40 µg/mL NIPI Spike mix. Add 1 mL of 1:1 H_2SO_4 to each sample, mix well with a glass rod and ensure the sample pH is <2 with pH paper. Wait for one hour. Record all the sample weights or volumes, volume of spikes and surrogates and LIMS serial numbers in the extraction notebook or in the LIMS prep worksheet.

6.7.4 Pass the water samples through 500 mg Waters Oasis HLB Solid Phase Extraction columns and elute the columns with 5 mL of acetonitrile. Nitrogen blow down at 36 °C to 0.1 mL and re-constitute to 1 mL with MCAA Water. Filter through 0.45-micron PTFE filter for LC-MSMS analysis and store extracts at 4 °C if not analyzed immediately.

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6.8 Water Samples Preparation by C18 Solid Phase Extraction (EPA Method 3535A) for the LIMS test W-E8321-MS and W-U2060-MS

NOTE: HLB Column is used for Acetaminophen and Imazapyr as the C18 SPE Column has low recoveries for these compounds.

6.8.1 Water samples that are not clear and/or contain a lot of particulate need to be pre-filtered before extraction using a 0.7-µm pore size glass-fiber filter. Measure or weigh 125 mL of filtrate or water sample into a beaker and check and record the pH of the sample. Add 0.5 mL of sodium chloride solution (sec. 4.8) and 0.50 mL of EDTA solution (sec. 4.9) and mix well with a glass rod.

6.8.2 For every 20 samples in the set, prepare one extraction blank and one Lab-fortified blank using DI water and sample duplicate matrix spikes.

6.8.3 For all the samples in the set add 25 µL of 0.80 µg/mL of 7-Surrogate mix. For QC samples add 25 µL of 0.40 µg/mL NIPI Spike mix. Add 1 mL of 1:1 H_2SO_4 to each sample, mix well with a glass rod and ensure the sample pH is <2 with pH paper. Wait for one hour. Record all the sample weights or volumes, volume of spikes and surrogates and LIMS serial numbers in the extraction notebook or in the LIMS prep worksheet.

6.8.4 Pass the water samples through 500 mg C18 Solid Phase Extraction columns and elute the columns with 5 mL of acetonitrile. Nitrogen blow down at 36 °C to 0.1 mL and re-constitute to 1 mL with MCAA Water. Filter through 0.45-micron PTFE filter for LC-MSMS analysis and store extracts at 4 °C if not analyzed immediately.

6.9 Acid Herbicides, Carbamates, Urea Herbicides and Personal Care Products Water Samples Preparation by Graphitized Carbon Cartridge Solid Phase Extraction (EPA Method 3535A) for the LIMS tests W-CAB-AA-R, W-CARB-AA, W-E8321-MS and W-U2060-MS

6.9.1 Water samples that are not clear and/or contain a lot of particulate need to be pre-filtered before extraction using a 0.7-µm pore size glass-fiber filter. Measure or weigh 125 mL of filtrate or water sample into a beaker and check and record the pH of the sample. Add 0.5 mL of sodium chloride solution (sec. 4.8) and 0.50 mL of EDTA solution (sec. 4.9) and mix well with a glass rod.

6.9.2 For every 20 samples in the set, prepare one extraction blank and one Lab-fortified blank using DI water and sample duplicate matrix spikes.

6.9.3 For all the samples in the set add 25 µL of 0.80 µg/mL of 7-surrogate mix. For QC samples add 25 µL of 0.40 µg/mL NIPI spike mix. Record all the sample weights or volumes, volume of spikes and surrogates and LIMS serial numbers in the extraction notebook or in the LIMS prep worksheet.

6.9.4 Condition 0.5-gram Supelcoclean or UCT graphitized carbon cartridge by sequentially eluting two 5-mL aliquots of 80:20 methylene chloride - methanol (v/v) without vacuum. This step is followed by elution with 5 mL of methanol through each cartridge. Elute three 5-mL aliquots of the ascorbic acid solution to complete the conditioning. Do all these steps under gravity flow without vacuum. The ascorbic acid conditioning step should take place slowly to ensure activation of binding sites in the cartridge bed. Keep the flow rate of ascorbic acid solution at no more than 3 mL/min. Cover conditioned cartridges with foil and set aside until ready for use. Cartridges can be used up to 8 hours after conditioning. Cartridges that have not been used within 8 hours need to be reconditioned. Collect the conditioning solvents in the vacuum manifold; place these solvents in a chlorinated waste container for proper disposal. NOTE: To avoid deactivation of the SPE sorbent surface, cartridges should never be allowed to dry after conditioning.

6.9.5 Label each conditioned, 6- mL graphitized carbon-based SPE cartridge with appropriate sample

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number. Mount 75 mL reservoir with adapter and load sample. Adjust water sample flow rate through the conditioned cartridges at 20 mL/min.

6.9.6 After sample loading, rinse the cartridge 3 times with 0.5 mL of DI water to remove any salts left on the column and air dry for few minutes and remove the manifold from the vacuum. The cartridges will retain a small amount (around 0.1 mL) of residual water. Chemically dry the cartridge by eluting with 1 mL of acetonitrile twice into a 15 mL glass centrifuge tube labeled as fraction 1 with the appropriate sample identifications. Some compounds are eluted in this fraction. The last drops of acetonitrile in the cartridge bed is removed by applying positive pressure with a bulb on top of the cartridge.

6.9.7 Most of the analytes are collected in the second elution fraction of the SPE cartridge. Place a clean 15 mL centrifuge tube in the elution rack to collect fraction 2. Label these tubes as fraction 2 with the appropriate sample identifications. Make sure the tubes are arranged in the correct order so that they will receive the eluent from appropriate SPE cartridges. Elute each SPE cartridge with three aliquots of 80:20 methylene chloride - methanol (v/v) with 0.2 percent trifluoroacetic acid anhydride (TFAA) solution. Allow each aliquot to reach the cartridge bed but not go dry before adding the next aliquot. The three aliquot volumes are, in sequence, 5, 5, and 3 mL. This improves analyte recoveries compared to continuous elution with a single 13-mL aliquot. When complete, remove the tubes.

6.9.8 Nitrogen blow down the fraction 2 tubes slowly at a bath temperature of 34 °C. When the volume in the tube reaches 5 mL, rinse the top and sides of the tube with a small amount (approximately 0.5 mL) of methanol. Repeat when the volume reaches 0.5 mL. Continue to blow down to 0.2 mL. Increase the bath temperature to 42 °C and transfer the contents of the fraction 1 tube into the tube labeled fraction 2. Rinse the fraction 1 tube with acetonitrile and add to fraction 2. There may be residual methylene chloride left in fraction 2. If this occurs, remove methylene chloride by adding 1 mL of methanol and blowing down to 0.2 mL. Repeat this step until there is no more methylene chloride remaining. Typically, methylene chloride is present as an immiscible layer underneath the aqueous phase, as small bubbles at the bottom of the test tube, or as a cloudy or “milky” appearance. The presence of methylene chloride in a sample may be barely perceptible, so each sample must be carefully examined for the presence of methylene chloride. Continue to concentrate to 0.5 mL and add MCAA water to reach a final volume of 1 mL.

6.9.9 Vortex to mix and filter through a 0.45-micron PTFE filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.10 Sucralose, Urea Herbicides and Personal Care Products Water Samples Preparation by Graphitized Carbon Cartridge Solid Phase Extraction (EPA Method 3535A) for the LIMS tests W-E8321-MS and W-U2060-MS

6.10.1 Follow steps in 6.9 but do not use trifluoroacetic acid anhydride (TFAA) in the elution of Fraction 2. Makeup to 1 mL with 10% acetonitrile – water. Sucralose recovery is very bad when TFAA is used.

6.11 Carbamates, Urea Herbicides and Personal Care Products Sediment Sample Preparation (EPA Method 3570) for the LIMs tests S-E8321-MS

6.11.1 Follow the steps in section 6.5.1 to 6.5.4 for soil extraction of acid herbicides. Weigh 10 grams of well-mixed sediment sample into 40-mL vial for each sample. Also, prepare duplicate matrix spikes and an Ottawa sand laboratory blank and laboratory fortified blank (LFB).

6.11.2 Add 250 µL of 0.80 µg/mL 7-surrogate spike mix to all samples in the set. Add 250 µL of 0.40 µg/mL NIPI Spike mix to the laboratory-fortified blank and matrix spikes. Note the amount added and the concentration of analytes in the extraction log book.

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6.11.3 Add 10 mL of 1:1 acetonitrile – MCAA Water to each sample and cap it tightly and shake for 1 hour using a wrist action shaker.

6.11.4 After 1 hour, centrifuge the samples and filter the extract through 0.45-µm PTFE filtration disk into a 2–mL crimp top vial. Proceed with LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

NOTE: If pH of sample is greater than 3, recoveries of some Carbamates, particularly Oxamyl, Methomyl and Methiocarb may be low. These Carbamates will degrade in neutral or alkaline aqueous solutions. If soil is alkaline, it is unlikely that these Carbamates will be found.

6.12 Water Samples Preparation for Direct Injection Analysis for the LIMs tests W-E8321-21 & W-E8321-DI

6.12.1 Didecyldimethylammonium chloride (LIMS test W-QUAT-NH4)

Water Samples for the analysis of Didecyldimethylammonium chloride are collected in 40 mL VOC vials only 1/3 filled. Add an equivalent volume of methanol into each vial and record the volume added. Shake and let it stand for 30 minutes. Remove a 2 mL aliquot from each 1:1 methanol-water sample, choosing one sample for duplicate matrix spikes. Prepare a method blank and laboratory fortified blank from 1:1 methanol-reagent water. Add 20 µL of 0.020 µg/mL Carbamazepine-d10 surrogate to all the samples in the set and add 20 µL of 1 µg/mL of Didecyldimethylammonium chloride to each of the QC samples. This results in a 10-ppb spike level for a 2 mL final volume. Vortex to mix and filter all the samples through a 0.45 µm filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.12.2 Endothall (LIMS test W-E8321-DI)

Remove a 5 mL aliquot from each well shaken sample into a labeled 15 mL centrifuge tube, choosing one to use for duplicate matrix spikes. Aliquot two portions of 5 mL of reagent water for a method blank and a laboratory fortified blank. Add 125 µL of 1 µg/mL Endothall-d6 surrogate to all the samples in the set and add 125 µL of 1 µg/mL of ICAL and Spike mix containing Endothall and Endothall-d6 to the QC samples. This results in a 25-ppb spike level in a 5 mL sample volume. Vortex to mix and filter all the samples in the set through a 0.45 µm filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.12.3 Diquat/Paraquat (LIMS test W-PDQUAT)

Remove a 5 mL aliquot from each well shaken sample into a labeled 15 mL centrifuge tube, choosing one to use for duplicate matrix spikes. Aliquot two portions of 5 mL of reagent water for a method blank and a laboratory fortified blank. Add 50 µL of 10 µg/mL Diquat-d4 surrogate to all the samples in the set and add 25 µL of 10 µg/mL of Diquat/Paraquat spike mix to the QC samples. Vortex to mix and filter all the samples in the set through a 0.45 µm filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.12.4 Glyphosate/Glufosinate/AMPA (LIMS test W-E8321-DI)

Remove a 1 mL aliquot from each well shaken sample into a labeled tube, choosing one to use for duplicate matrix spikes. Aliquot two 1 mL portions of reagent water for a method blank and a laboratory fortified blank. Add 10 µL of 1 µg/mL Glyphosate 13C 15N surrogate to all the samples in the set and add 10 µL of 0.20 µg/mL of Glyphosate/AMPA spike mix to the QC samples. Vortex to mix and filter all the samples in the set through a 0.45 µm filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.12.5 Malathion (LIMS test W-E8321-DI)

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Malathion samples are received in Monochloroacetic acid buffer preserved vials. Remove a 1 mL aliquot from each well shaken sample into a labeled 2 mL GC vial, choosing one to use for duplicate matrix spikes. Aliquot two 1 mL portions of MCAA water for a method blank and a laboratory fortified blank. Add 25 µL of 1 µg/mL Malathion-d6 surrogate to all the samples in the set and 20 µL of 0.5 µg/mL of Malathion spike to the QC samples. Vortex to mix and store at 4 °C or proceed directly to LC-MSMS analysis

6.12.6 Negative and Positive Ion Analytes (LIMS test W-E8321-DI)

Remove a 4.5 mL aliquot from each well shaken sample into a labeled centrifuge tube, choosing one to use for duplicate matrix spikes. Aliquot two 4.5 mL portions of reagent water for a method blank and a laboratory fortified blank. Add 0.5 mL of acetonitrile into each tube and mix well. This results in a final solvent of 10% acetonitrile in water. Add 25 µL of 0.80 µg/mL surrogate mix to all the samples in the set and add 25 µL of 0.40 µg/mL of NIPI Spike mix to the QC samples. Vortex to mix and filter all the samples in the set through a 0.45 µm filter for LC-MSMS analysis. Store extracts at 4 °C if not using immediately.

6.18 Water Sample Preparation for Direct Injection Analysis of Microcystins by Autoclave (LIMS test W-MCYST-AA)

6.18.1 Autoclave the entire sample bottles as well as a tube or bottle of at least 10 mL of DEP well water to use for method blanks and lab-fortified blanks. Cap the tubes and bottles loosely or cover with aluminum foil. Place them in a metal tray marked with autoclave tape.

6.18.2 Turn the Generator Control button ON (located at the bottom) and wait for the autoclave to reach 20 psi pressure on the bottom dial. This takes about 30 minutes.

6.18.3 Open the chamber and insert the sample tray. Close the door and hand tighten. Make sure all the doors are closed. If the door open signal is ON, ensure the tightness of both doors, front and back.

6.18.4 The Fluid Cycle button should be pressed down by default. Start the Autoclave by pressing the ON button. The pressure on the dial will go down and then build back to 20 psi. Once the temperature reaches 120 °C /248 °F (which takes about 5-7 minutes) the timer will start.

6.18.5 When the timer is at zero, the autoclave cycle is complete. This will take about 35 minutes. Press the Generator Control button to turn OFF the Autoclave. Wait for the chamber pressure to drop to zero on the top panel.

6.18.6 Open the chamber and carefully remove the sample tray and allow it to come to room temperature.

6.18.7 When the autoclave is complete, make sure that the indicator tape has turned black. Record in LIMS and affix the Indicator tape to the prep worksheet.

CAUTION: DO NOT OPEN THE AUTOCLAVE UNTIL PRESSURE GAUGE READS ZERO!!
Autoclave contains steam under pressure, which will vent and could cause injury if opened too soon.

6.18.8 Allow the samples to reach room temperature, cap them tightly and shake well to mix. Filter sufficient aliquots of each sample and DEP well water through 0.45 µm filters into labeled tubes.

6.18.9 Remove a 1 mL aliquot from each filtrate into a labeled 2 mL GC vial, choosing one sample to use for duplicate matrix spikes. Aliquot two 1 mL portions of DEP well water for a method blank and a laboratory fortified blank.

6.18.10 Add 20 µL of 5 µg/L Carbamazepine-d10 Spike standard solution and 20 µL of 500 µg/L Microcystin Spike mix to the QC samples. Vortex to mix and proceed with LC-MSMS analysis.

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6.19 Water Samples Preparation by Oasis HLB Plus LP Solid Phase Extraction for the LIMS test W-PFAS-MS

6.19.1 For PFAAs, 250 mL of whole water sample bottle is extracted with Oasis HLB Plus LP column.

6.19.2 For every 20 samples in the set, prepare one extraction blank and one Lab-fortified blank using DI water and sample duplicate matrix spikes.

6.19.3 For all the samples in the set add 100 µL of 20 µg/L of 2-PAAAs Surrogate mix. For QC samples add 100 µL of 20 µg/L PFAAs Spike mix. Record all the sample weights or volumes, volume of spikes and surrogates and LIMS serial numbers in the extraction notebook or in the LIMS prep worksheet.

6.19.4 Condition SPE column with 15 mL of methanol and let it dry followed by 15 mL of water and do not dry the column. Pass the whole 250 mL water samples through Solid Phase Extraction column and rinse bottle with 15 mL of water and add to column. Add 12 mL of acetonitrile to original 250 mL sample bottle shake and add to corresponding column to elute. Nitrogen blow down at 36 °C to 1 mL and re-constitute to 2 mL with 1:1 acetonitrile and water with 0.20 mL of 10 µg/L PFAAs Internal Standard mix. Mix well and filter through 0.45-micron PTFE filter for LC-MSMS analysis and store extracts at 4 °C if not analyzed immediately.

7 SAMPLE ANALYSIS PROCEDURE

7.1 See SOP LC-001, Analysis of Pesticides, Herbicides and other Chemicals in Water and Sediment/Waste Sample Extracts by High Performance Liquid Chromatography with Turboionspray Mass Spectrometry (HPLC/MS/MS).

8 QUALITY CONTROL

8.1 See SOP LC-010, “Quality Assurance/Quality Control in the HPLC Pesticides Laboratory” for specific details on quality control, definitions and interferences.

9 SAFETY/HAZARDOUS WASTE MANAGEMENT

9.1 See SOP OG-001, Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories) for specific details on safety and hazardous waste management.

10 REFERENCES

10.1 EPA Method 3535A. Solid-Phase Extraction (SPE) Revision 1, February 2007

10.2 USGS O-2060-01, “Determination of Pesticides in Water by Graphited Carbon-Based Solid-Phase Extraction and High-Performance Liquid Chromatography/Mass Spectrometry”. U.S. Geological Survey, Denver, CO 80225, 2001.

10.3 EPA Method 3570. Microscale Solvent Extraction (MSE) Revision 0, November 2002

10.4 DEP SOP LC-039. HPLC Standard Preparation.

10.5 DEP SOP LC-001. Analysis of Pesticides, Herbicides and other Chemicals in Water and Sediment/Waste Sample Extracts by High Performance Liquid Chromatography with Turboionspray Mass Spectrometry (HPLC/MS MS)

10.6 DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories)

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Appendix of Changes

08/22/2011	Edited preparation for Sucralose in water
08/15/2012	Edited preparation for Sucralose in water
02/19/2013	Edited Graphitized Carbon Black SPE preparation for low level Chlorophenoxy acid herbicides, Carbamates and Urea herbicides in water
01/10/2014	Merged SOPs LC-005-1, LC-006-2, LC-011-2 and LC-021-2 into LC-011-2
01/14/2015	Merged SOPs LC-038-1 and LC-011-2 into LC-011-2
01/15/2016	Added new analytes
01/26/2016	Added Benzoic acid
08/03/2016	Added Malathion
02/19/2018	Added new Test IDs
10/02/2018	Added Perfluorinated Alkyl Acids

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