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, Per- and Polyfluoroalkyl Substances (PFAS) 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, USGS Method O-2060-01 and EPA Method 1633 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-SAXTN-MS	EPA Method 8321B analysis, water sample
W-PDQUAT	EPA Method 8321B analysis/Direct Injection preparation, water sample
W-PFAS-MS	EPA Method 1633 analysis/EPA Method 3535A preparation, 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
S-PFAS-MS2	EPA Method 1633 analysis/EPA Method 3570 preparation, soil sample
WS-PFAS-MS	EPA Method 1633 analysis/EPA Method 3580A preparation, waste 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 acidified and extracted by passing through a solid phase column (Waters Oasis HLB SPE columns or C18 SPE column or Graphitized carbon-based SPE column) following EPA Method 3535A.

1.4 Following USGS method O-2060-01 water samples are extracted 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-MS-MS) 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. Waters Oasis HLB columns preparation is used due to its simplicity all the time (Sec. 1.3).

1.5 EPA Method 3535A is used to prepare water samples for Per- and Polyfluoroalkyl Substances (PFAS). Using this method, water samples are extracted by passing through Waters Oasis HLB Plus LP or Strata ™-X-AW 33 um Polymeric weak Anion Extraction Cartridge and undergo cleanup using carbon before analysis if

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needed. Some PFAS analytes are commercially available as ammonium, sodium, and potassium salts. This method measures all forms of the analytes as anions while the identity of the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts.

1.6 In sediment/waste extraction for acid herbicides, samples are directly extracted with 0.3 N 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.

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

1.8 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. For Per- and Polyfluoroalkyl Substances (PFAS), 0.3% NH4OH in methanol is used for soil samples extraction and undergo cleanup using carbon before analysis if needed.

1.9 Contribution from the surrogate spike added to the sample is observed in the Microcystin analysis. If added Microcystin LR ethyl-d5 at 10 ug/L level, then Microcystin LR and Desmethyl microcystin LR concentration is increased at the method detection limits. Addition of surrogate is reduced to 5 times lower than before (2 ug/L). Similarly higher levels of 2,4-D in the samples observed to contribute 2,4-D d3 surrogate concentration. In these samples data reported without surrogate recovery for 2,4-D d3 surrogate with comment.

2 SUMMARY OF THE METHOD

2.1 Waters Oasis HLB SPE method: Chlorophenoxy acid herbicides, Carbamates, Urea Herbicides and Personal Care Products are extracted from 125 mL of water samples acidified to pH <2 containing 7.5g of sodium chloride and 1.5g of EDTA using Waters Oasis HLB SPE columns (EPA Method 3535A). Adsorbed compounds are eluted from the SPE cartridge using acetonitrile containing 0.1% formic acid and the fraction is reduced under nitrogen to 0.5 mL. The final volume for the extract is 1 mL in 1:1 acetonitrile and MCAA water and the extract is filtered through 0.45-micron filter and analyzed by LC-MS-MS. Some Chlorophenoxy acid herbicides may exhibit poor recoveries.

2.2 Per- and Polyfluoroalkyl Substances (PFAS) by Strata ™-X-AW 33 um Polymeric weak Anion Extraction Cartridge: Following EPA Method 3535A for PFAS extraction, 125 mL of water sample is fortified with Extracted Internal Standard (EIS) – (a.k.a. isotopically labeled compounds) of the method analytes that function as isotope dilution standards. The entire sample is passed through an SPE cartridge to extract the method analytes and isotope dilution compounds. Adsorbed compounds are eluted from the SPE cartridge with 0.3% ammonium hydroxide in Methanol. The fraction is reduced under nitrogen to 5-mL and Non-extracted Internal Standard is added. The extracts are treated with 10 mg of carbon and filtered through 0.45-micron polypropylene membrane filter and analyzed by LC-MS-MS in the MRM detection mode. The concentration of each analyte is calculated using the isotope dilution technique. For QC purposes, the percent recoveries of the isotope dilution compounds are calculated using the integrated peak areas of isotope performance standards, which are added to the final extract and function as traditional internal standards, exclusively applied to the isotope dilution compounds. Waters Oasis HLB Plus LP Solid Phase Extraction columns also works well.

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

2.4 For Microcystin Analysis: The whole water sample is autoclaved prior to analysis to release (lyse) the algal toxic materials from the blue green algal and bacterial cells present. Therefore, this method determines the “total” amount of algae causing toxins present in the sample. After autoclave, water samples are filtered through a 0.45-micron filter and an aliquot is analyzed by LC-MS-MS.

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2.5 For samples requiring Microcystins, Anatoxin-a, Neosaxitoxin and Saxitoxin Analysis: Samples are prepared by a Freeze/Thaw (cell lysing) technique prior to sample analysis. The whole water sample bottle is first sonicated for a few minutes and shaken well before taking about 30 mL of sample aliquat for the Freeze/Thaw preparation. Using a –30 °C freezer, the Freeze/Thaw sample aliquots are frozen and thawed three times to release the algal toxins contained within any blue green algal and bacterial cells present within the sample. After completion of the Freeze/Thaw preparation, the water samples are filtered through a 0.45-micron filter and an aliquot is analyzed by LC-MS-MS.

2.6 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 Waters Oasis HLB SPE column. Compounds of interest are eluted with 5 mL of acetonitrile containing 0.1 % of formic acid and then diluted to 10 mL with MCAA water and filtered through 0.45-micron filter and analyzed by LC-MS-MS.

2.7 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-MS-MS.

2.8 Following EPA method 3570, five grams of sediment sample is fortified with Extracted Internal Standard (EIS) of the method analytes that function as isotope dilution standards. The sample is extracted with 10 mL of 0.3% ammonium hydroxide in Methanol to extract the method analytes and isotope dilution compounds. After 24 hours of shaking all the samples in the batch are centrifuged and filtered through 0.45-micron PP membrane filter. Non-extracted Internal Standard and 10 mg of carbon is added and filtered. Analyzed by LC-MS/MS in the MRM detection mode. The concentration of each analyte is calculated using the isotope dilution technique. For QC purposes, the percent recoveries of the isotope dilution compounds are calculated using the integrated peak areas of isotope performance standards, which are added to the final extract and function as traditional internal standards, exclusively applied to the isotope dilution compounds sample.

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 ± 0.1 g

3.2 Soil Micro-extraction: Vials – 40 mL with PTFE septa, Digital Pulse Mixer, 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 Logbook)

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

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

3.11 For PFAS samples - 125-mL polypropylene bottle fitted with a polypropylene screwcap bottle and Graduated Polypropylene tube - 15 mL

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

3.13 Freezer capable of freezing samples -30°C and 50 mL polypropylene bottles.

3.14 0.45-micron filtration disks, 2-mL crimp top vial and Polypropylene Autosampler vials – 1 and 0.3 mL

4 REAGENTS AND CHEMICALS

4.1 Waters Oasis HLB SPE columns 200 mg/6cc, Waters Oasis HLB Plus LP Columns.

4.2 Strata ^{TM}-X-AW 33 um Polymeric weak Anion Extraction Cartridge (500 mg/6 mL).

4.3 Millipore 13 mm PTFE Syringe Filters 0.45-micron, pack of 1000 (catalog number - SLCR013NK), Omicron 13 mm Syringe Filters PTFE (Hydrophilic) 0.45-micron, pack of 100 (catalog number – ICTF13XB) and for PFAS samples, Thermo Scientific 17 mm Titan3 filters 0.45-micron PP Membrane, pack of 200 (catalog number – 44513-PP).

4.4 Hydrochloric acid (HCl), reagent grade; Sulfuric acid (H_2SO_4), reagent grade; Anhydrous NaOH, reagent grade; Formic acid, Acros, Reagent ACS (96%).

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

4.6 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.

4.7 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!**

4.8 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.

4.9 0.3N NaOH - Dissolve 6 g NaOH into 500 mL of HPLC grade water.

4.10 0.3% NH_4OH (ammonium hydroxide) - Mix 3mL of NH_4OH into 1 L of HPLC grade methanol.

4.11 Sodium Chloride, reagent grade: Wash with methylene chloride and bake at 550 °C prior to use. Prepare freshly by dissolving 7.5 grams of sodium chloride in 30 mL of water and add 0.9 mL to 125 mL of water sample before extraction.

4.12 EDTA, reagent grade. Prepare freshly by dissolving 1.5 grams of EDTA in 30 mL of water and add 1.0 mL to 125 mL of water sample before extraction and allow the samples stand for one hour before extraction.

4.13 Ottawa Sand for method blanks and lab-fortified blanks: bake at 550 °C prior to use.

4.14 VOC distilled water or Clean Lab DI water: Ground water tested free of interferences and used for method blanks and lab fortified blanks (LFBs).

4.15 Ammonium acetate preservation: Prepare ammonium acetate preservation dichlorination solution prepared

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by adding 100 g of reagent-grade ammonium acetate into a 200 mL volumetric flask and bringing the flask to volume with reagent-grade water. The solution is dispensed into 4-ounce polypropylene dropper bottles, filling each bottle half full and labeling each bottle. The field operators are instructed to add 5 drops of the ammonium acetate solution to the 125 mL PFAS sample bottles when residual chlorine is detected.

4.16 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. Per- and Polyfluoroalkyl Substances (PFAS) spike mix at 100 µg/L and 20PFAS Extracted Internal Standard surrogate spike mix at 100 µg/L: add 25 µL of each as required for a final volume of 5 mL extraction. For soil, 50 µL of each PFAS spike mix and 20PFAS Extracted Internal Standard surrogate spike mix added for extraction for a 10 mL final volume.

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

4.18 Replacement Charcoal, Supelco (cat. # 2-2451, lot # 4359).

4.19 Heptafluorobutyric acid, Thermo Fisher, for PDquat.

5 SAMPLE COLLECTION, PRESERVATION AND HANDLING

5.1 Water samples are collected in 500-mL glass amber bottles 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 (0.268 g) to less than pH 5. For Microcystins, Anatoxin-a, and Saxitoxins, samples are collected in 250 mL glass brown bottle with ¾ filled. 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 Sucralose which must be analyzed within 21 days of collection.

5.2 For PFAS, samples are collected in a 125-mL polypropylene bottle fitted with a polypropylene screwcap bottle and must be iced or refrigerated at 6 °C or less from the time of collection until extraction. Extract samples within 28 days and analyze within 30 days. If residual chlorine is detected the field operators are instructed to add 5 drops of the ammonium acetate solution (sec. 4.15) to the 125 mL PFAS sample bottles in the field.

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 For PFAS sediment samples are collected in polypropylene or HDPE jars and must be iced or refrigerated at 6 °C or less from the time of collection until extraction. Extract samples within 28 days and analyze within 30 days.

5.5 All sample extracts must be analyzed within 40 days of sample extraction and for Carbamates within 21 days of extraction.

6 SAMPLE PREPARATION PROCEDURE

NOTE: a second person must witness the addition of spike standards to the appropriate 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).

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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 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

6.5.1 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 0.9 mL of sodium chloride solution (sec. 4.11) and 1 mL of EDTA solution (sec. 4.12) and mix well with a glass rod.

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

6.5.3 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.

6.5.4 Use baker manifold and attach 200 mg Waters Oasis HLB Solid Phase Extraction (SPE) columns to plastic reservoirs with plastic adapters onto the manifold. Connect manifold to a 2000 mL vacuum filtration flask with tubing and connect the flask to a vacuum source. Condition SPE columns by passing 15 mL of acetonitrile through the column and let it dry by opening valve and letting air pass through the SPE columns for 5-10 minutes. Follow up with 15 mL of 0.1 M H_2SO_4 acidic water (sec 4.8) and do not let the columns dry by closing the valve and keeping the acidic water within the SPE columns. Pass entire 125 mL water samples through SPE columns at a steady drip rate of about 1.5 drips per second. Rinse empty sample beakers with 15 mL of DI water and add to column. Finally rinse column with 1 mL of DI water once entire sample has passed through and fully open valve to let the SPE column dry for 5-10 minutes. Elute column with 6 mL of acetonitrile containing 0.1% formic acid and 6mL of Methanol either by hand or with gravity method using 10 mL plastic syringes. Use N-Evap® to nitrogen blow down samples at 34 °C down to a volume of 0.5-mL. 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.

6.6 Per- and Polyfluoroalkyl Substances (PFAS) Water Samples Preparation by Oasis HLB Plus LP Solid Phase Extraction (EPA Method 3535A) for the LIMS test W-PFAS-MS

6.6.1 For PFAS, 125 mL of whole water sample bottle is extracted with Oasis HLB Plus LP column.

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

6.6.3 For all the samples in the set add 25 µL of 100 µg/L of 20PFAS Extracted Internal Standard

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surrogate spike mix. For QC samples add 25 µL of 100 µg/L PFAS 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.

6.6.4 Condition SPE column with 6 mL of MeOH and let it dry. Follow up with 6 mL of MeOH, 6mL of distilled VOC DI water and do not let the column dry. Pass the whole 125 mL water samples through Solid Phase Extraction column and rinse bottle with 15 mL of water and add to column. Add 6 mL of methanol to original 125 mL sample bottle shake and add to corresponding column to elute by gravity. Nitrogen blow down at 45 °C to 5 mL and add 50 µL PFAS Isotope Performance Standard mix (40 µg/L). Add carbon 10 mg, mix well and filter through 0.45-micron Polypropylene filter for LC-MSMS analysis and store extracts at 4 °C if not analyzed immediately.

6.7 Per- and Polyfluoroalkyl Substances (PFAS) Water Samples Preparation by Strata ™-X-AW 33 um Polymeric weak Anion Extraction Cartridge (500 mg/6 mL) or Resprep Polymeric SPE Cartridge WAX, 6mL/500mg, 30 um Solid Phase Extraction (EPA Method 3535A) for the LIMS test W-PFAS-MS

6.7.1 For PFAS, 125 mL of whole water sample bottle is extracted with Strata-X-AW 33 um Polymeric weak Anion column or Resprep Polymeric SPE Cartridge. For every 20 samples in the set, prepare one extraction blank, one Lab-fortified blank using DI water, and sample duplicate matrix spikes.

6.7.2 Transfer 125 mL of samples into labeled leak-resistant HDPE NM bottles. If there is visible sediment in the samples, centrifuge the samples for 5 minutes at 40 rpm and then transfer supernatant into labeled bottles. If there is a significant amount of sediment and acquiring 125 mL of sample is not possible after centrifuging and transferring the supernatant, transfer what is possible to labeled bottle and record the amount that was able to be extracted in the extraction sheet for that sample. Add a little more than the difference in amount you need in distilled VOC DI water to the original sediment-filled sample, mix and centrifuge for 5 minutes at 40 rpm, and transfer the entire supernatant to the same labeled bottle for a final volume close to 125 mL. If the final volume is not exactly 125 mL, it is still acceptable to proceed. For example, if 100 mL of supernatant is extracted from the sediment-filled sample, record the amount 100 mL in the extraction sheet, add 25-30 mL of distilled VOC DI water to the original sample bottle, mix and centrifuge, and add entire supernatant into labeled bottle to reconstitute into a final volume of around 125 mL.

6.7.3 For all the samples in the set add 25 µL of 100 µg/L of 20PFAS Extracted Internal Standard surrogate spike mix. For QC samples add 25 µL of 100 µg/L PFAS 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.

6.7.4 Use baker manifold and attach Strata ™-X-AW 33 um Polymeric weak Anion Extraction Cartridge (SPE) columns or Resprep Polymeric SPE Cartridge WAX, 6mL/500mg, 30 um to plastic reservoirs with plastic adapters onto the manifold. Connect manifold to a 2000 mL vacuum filtration flask with tubing and connect the flask to a vacuum source. Condition SPE columns with 6 mL of 0.3% ammonium hydroxide in methanol (sec 4.10) and let it dry by opening valve and letting air pass through the SPE column. Follow up with 6 mL of MeOH, 6 mL of distilled VOC H_2O and do not let the column dry by closing the valve. Pass entire 125 mL water samples through SPE columns at a steady drip rate of about 1.5 drips per second. Rinse empty sample bottles with 15 mL of water and add to column. Add 10 mL of 0.3% ammonium hydroxide in methanol to original 125 mL sample bottle, shake it, and add to corresponding column to elute. Use 10 mL plastic syringes to elute samples by gravity. Use N-Evap® to nitrogen blow down samples at 45 °C down to a volume of 5 mL, add 50 µL PFAS non-extracted internal standard (40 µg/L) and add carbon 10 mg. Mix well for 5 minutes and filter through 0.45-micron Polypropylene filter for LC-MSMS analysis and store extracts at 4 °C if not analyzed immediately.

6.8 Waste Samples Preparation (EPA Method 3580A) for the LIMS test WS-PFAS-MS

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6.8.1 For waste PFAS, 0.5 grams of sample is diluted to 5 mL with methanol and 100 µL out of 5 mL is further diluted to 1 mL with required QC (Surrogate/Spike/IS).

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 5 µL of 100 µg/L of 20PFAS Extracted Internal Standard surrogate spike mix.. For QC samples add 5 µL of 100 µg/L PFAA Spike mix. Add 12.5 µL of PFAS Isotope Performance Standard mix (40 µg/L) and carbon 2 mg to all samples in set. Record all the sample weights or volumes, volume of surrogates, spikes and IS and LIMS serial numbers in the extraction notebook or in the LIMS prep worksheet.

6.8.4 Filter through 0.45-micron PTFE filter for LC-MSMS analysis and store extracts at 4 °C if not analyzed immediately.

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

6.9.1 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 100 µL of 1 µg/mL Diquat-d4 surrogate to all the samples in the set and add 50 µL of 1 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.

6.9.2 Water Samples Preparation for Direct Injection Analysis (LIMS tests W-E8321-DI and W-E8321-21): Allow the samples to come to room temperature. Shake each sample well and filter an aliquot of each sample using a 0.45 µm filter and place the sample filtrates into labeled sample tubes. Prepare one method blank and one LFB using DI water and duplicate matrix spikes for a set of up to 20 samples in labeled 2 mL GC vials. Add 25 µL of 200 µg/L DI Surrogate spike to all the samples in set to a final volume of 1 mL. Add 25 µL of 100 µg/L DI Spike mix to LFB and duplicate matrix spike samples. Cap samples and vortex to mix. Proceed with the LC-MSMS analysis. In this method all the analytes in the W-E8321-MS test may be analyzed for confirmation.

6. 10 Water Sample Preparation for the Analysis of Microcystins and Saxitoxins (LIMS test W-MCYST-AA and W-SAXTN-MS)

6.10.1 W-SAXTN-MS Analysis by Freeze/Thaw Technique: The whole water sample bottle is sonicated for a few minutes and shaken well before transferring about 30 mL of sample into a 50 mL polypropylene bottle with screw cap. The Freeze/Thaw samples are placed into a -30°C freezer for 1 hour, or until frozen. Once frozen, the Freeze/Thaw samples are allowed to fully thaw at room temperature for a time of about 30 minutes. Samples may be placed in a 40°C water bath to speed up the thawing process. Alternatively, samples may be sonicated for about 15 minutes or until fully thawed. The Freeze/Thaw procedure is then repeated two more times to fully release the algal toxins from the blue green algal and bacterial cells present within the samples. Method Blank and Lab-Fortified Blank (LFB) samples are also prepared using 10 mL of DEP well water. The Freeze/Thaw samples may be stored in the freezer during the final freeze/thaw cycle until it is time to perform the analysis.

6.10.2 Allow the samples to come to room temperature. Verify that the sample caps are snug, shake each sample well, and then sonicate each sample for a few minutes. Filter an aliquot of each sample using a 0.45 µm filter and place the sample filtrates into labeled sample tubes.

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6.10.3 Prepare one method blank and one LFB using DEP Well water and duplicate matrix spikes for a set of up to 20 samples in labeled 2 mL GC vials.

6.10.4 Add 20 µL of 250 µg/L Anatoxin-a 13C4 Surrogate Spike standard solution to all the samples in set to a final volume of 1 mL. Add 20 µL of 500 µg/L Saxitoxin Spike mix to LFB and duplicate matrix spike samples. Cap samples and vortex to mix. Proceed with the LC-MSMS analysis.

6. 11 Water Sample Preparation for Direct Injection Analysis of Microcystins by Autoclave (LIMS test W-MCYST-AA)

6.11.1 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.

6.11.2 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.

6.11.3 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.

6.11.4 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.

6.11.5 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.

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

6.11.7 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.

6.11.8 Allow the samples to come to room temperature. Verify that the sample caps are snug, shake each sample well, and then filter an aliquot of each sample using a 0.45 µm filter and place the sample filtrates into labeled sample tubes.

6.11.9 Prepare one method blank and one LFB using DEP Well water and duplicate matrix spikes for a set of up to 20 samples in labeled 2 mL GC vials.

6.11.10 Add 20 µL of 100 µg/L Microcystin LR ethyl-d5 Surrogate Spike standard solution to all the samples in set to a final volume of 1 mL. Add 20 µL of 500 µg/L Microcystin Spike mix to LFB and duplicate matrix spike samples. Cap samples and vortex to mix. Proceed with the LC-MSMS analysis.

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

6.12.1 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.

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

6.12.3 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 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.

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

6.12.4.1 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.

6.12.4.2 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.

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

6.12.5 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.

6.12.6 Add 125 µL of 0.80 µg/mL 11-Surrogate spike mix to all the samples in the set and 125 µL of NIPI Spike Mix 0.40 µg/mL to the laboratory- fortified blank and matrix spikes to a final volume of 10 mL.

6.12.7 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.

6.12.8 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.

6.12.9 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.

6.12.10 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.

6.12.11 Label the Waters Oasis HLB SPE 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.

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6.12.12 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.

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

6.12.14 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.

6.12.15 Elute the analytes from the cartridge by attaching a 5-mL syringe to the adapter on the cartridge. Put 5 mL of acetonitrile with 0.1% formic acid 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 10 mL of acetonitrile to elute and push it through both cartridges into a centrifuge tube.

6.12.16 Collect up to 10 mL of acetonitrile with 0.1% formic acid eluate from the SPE cartridge. Use N-Evap® to nitrogen blow down samples at 34 °C down to a volume of 5-mL. Re-constitute to 10 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.

6.13 Acid herbicides Extraction in SPLP and TCLP Extracts for the LIMS tests SPLP/TCLP-AH-AA

6.13.1 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.

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

6.14.1 Follow the steps in section 6.11.1 to 6.11.4 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).

6.14.2 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 logbook.

6.14.3 Add 10 mL of 1:1 acetonitrile – MCAA Water to each sample and cap it tightly and shake for 24 hours using a Digital Pules Mixer.

6.14.4 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

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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.

6.15 Per- and Polyfluoroalkyl Substances (PFAS) in sediment Sample Preparation (EPA Method 3570) for the LIMs test S-PFAS-MS2

6.15.1 For PFAS, weigh 5 grams of well mixed sediment sample in a 40 mL of VOC vial. For every 20 samples in the set, prepare one extraction blank and one Lab-fortified blank using Ottawa sand and sample duplicate matrix spikes.

6.15.2 For all the samples in the set add 50 µL of 100 µg/L of 20PFAS Extracted Internal Standard surrogate spike mix. For QC samples add 50 µL of 100 µg/L PFAS 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.

6.15.3 Add 10 mL of 0.3% of ammonium hydroxide in methanol to each sample in the set, cap tightly and shake for 24 hours using Digital Pules Mixer. Centrifuge for 10 minutes at 50,000 RPM and take 2 mL of aliquat into centrifuge tube, add required PFAS Non-Extracted Internal Standard mix 40 µg/L and carbon 4 mg and vortex to mix and filter after 5 minutes through 0.45-micron polypropylene filter in to 2 mL vial 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 EPA Method 8321B. Solvent-Extractable Nonvolatile Compounds by High-Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection, Revision 2. (2007, February).

10.5 EPA Method 1633. Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, 4th Draft Method. (2023, July).

10.6 DEP SOP LC-039. HPLC Standard Preparation.

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10.7 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)

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

Appendix of Significant 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 in water
02/12/2020	Updated to current preparations in use
12/15/2020	Updated to current preparations in use
06/30/2021	Added Perfluorinated Alkyl Acids in water
09/15/2021	Added Freeze/Thaw sample preparation technique to Microcystins/Anatoxin-a/Saxitoxins in water
12/08/2023	Added 3 new Perfluorinated Alkyl Acids in water and soil. Updated to current preparations in use.
12/12/2025	Updated the PDquat preparation method

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