Standard Operating Procedure For:

HPLC Standard Preparation

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1 SCOPE AND APPLICATION

1.1 This SOP details the procedure for preparing reagents and standards used in the HPLC sample extraction and analysis (DEP SOP LC-001 and LC-011).

2 REAGENTS AND CHEMICALS

NOTE: Record Lot Numbers of all the reagents and solvents used in the Notebook. For each new chemical standards, reagents and solvents received, enter the information into the Laboratory Information Management System (LIMS) to obtain the serial number. Record the serial number on the standard as well as the certificate of analysis. Scan the certificate of analysis and save to the directory. File the certificate of analysis in a certification folder. If any expired or expiring stock standard is re-certified, make sure the standard is still good by preparing stock, intermediate and working standard and check against valid standards. Response should agree within +/- 15%. Keep all the documents of re-certification in the certification folder. Re-certification should be limited to stable standards. Do not re-certify ICAL standards and always use un-expired ICAL standards.

2.1 Solvents, HPLC grade: Acetonitrile, methanol (Fisher Scientific) and DI water free of interferences.

2.2 Chemicals: Formic acid, Heptafluorobutyric acid, Monochloroacetic acid, ammonium acetate and Potassium acetate ACS Reagent Grade.

2.3 Commercial Stock Standards and neat standards are available from Ultra Scientific, Fisher Scientific, Accu Standard, CDN Isotopes, Chem Service, Chromadex Inc., Enzo, LGC Standards, O_2Si, Restek, Ria International LLC, Toronto Research Chemicals, Abraxis and other suppliers. Generally, one-milliliter vials of individual and/or mixed standards are purchased for making calibration curve and check standard solutions.

2.4 If a chemical or reagent container and its certificate of analysis have no explicit expiration date, then,

(a) dry inorganic and organic solids are assigned an expiration date ten years from the day they were received in the lab;

(b) all concentrated inorganic acids and organic solvents are assigned an expiration date five years from the day they are received in the lab;

(c) all other liquid reagents are assigned an expiration date five years from the day they are received in the lab;

(d) shorter expiration dates than the ones listed above may be assigned based on historical experience and the discretion of the supervisor. They are documented in the applicable SOPs.

3 EQUIPMENT

3.1 Volumetric Flasks - 1, 2, 5, 10, 25, 50, 100, and 500 mL

3.2 Volumetric pipettes - 0.5, 1, 2, 5, and 10 mL

3.3 Gas-tight micro-syringes - 5, 10, 25, 50, 100, 250, 500, 1000 µL and 2 mL (A certificate is required from the manufacturer stating that the volume is accurate to < 1%. Otherwise calibrate the syringes or volumetric pipets quarterly and record.)

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

3.5 Pipette tips - 200 µL, 30 to 300 µL and 1000 µL.

3.6 2 mL screw cap amber glass auto sampler vials (or 1.8 mL crimp top vials) with colored cap for easy identification.

3.7 Amber glass bottle with Teflon lined screw cap - 25, 50 and 100 mL

3.8 Centrifuge tubes with Teflon lined screw cap - 15 mL. (Calibrate tubes to the required volume and record in the Extraction Logbook)

3.9 Polypropylene tube with screw cap – 15 mL. (Calibrate tubes to the required volume and record in the Extraction Logbook).

3.10 1- and 0.3-mL polypropylene auto sampler vials (crimp top) for samples and standards.

4 PREPARED HPLC MOBILE PHASES AND REAGENTS

4.1 Prepare HPLC mobile phases and reagent solutions as required by each individual method. Generate a new LIMS Tracking Number whenever a new reagent is prepared, including for LC mobile phases. Be sure to enter the LIMS serial number and/or lot number of each solvent and reagent used.

4.2 Unless otherwise stated in the prep or analysis method, expiration for prepared reagents is 1 year from creation, or equal to the nearest expiration of any component used if a component's expiration is less than 1 year.

4.3 Serial numbers of all the reagents and equipment used during extraction and analysis must be documented by the analyst and reviewed during test authorization.

5 STANDARDS QUALITY ASSURANCE

5.1 New standards must be checked for correctness and condition upon receipt. Information for each new standard is entered in the LIMS Electronic Standard Preparation Log which includes all pertinent information such as date of receipt, analyte list in the mix, concentrations, lot number, solvent, expiration date for the standard preparation. When preparing, a standard make sure that all the preparation information is entered in the Electronic Standard Preparation Log. The Preparation Log will create a serial number specific for the standard. Place the serial number onto the vial along with the standard name, concentration, solvent, date and your initials.

5.2 All prepared ICAL (Initial Calibration Levels), spike, surrogate and internal standard intermediate and working standard solutions are good for six months. Replace working standards every six months or sooner if degradation is evident. Before making any standards from commercial sources, check to ensure that the expiration date has not passed. Before putting any standard into use, verify that it is acceptable by comparing it against the previously used standard or an external check standard. Responses should agree within +/- 15%. If not, check for instrument problems or re-make the new standard. **DISCARD ANY OLD STANDARDS AFTER NEW STANDARDS HAVE BEEN MADE AND FOUND TO BE ACCEPTABLE!**

5.3 Any standard remaining in an open ampoule should be transferred to a 2-mL vial and capped using a Teflon-lined rubber septum. (Make sure Teflon side of septum faces the standard solution!) Replace any pierced septa before returning the standard to storage. Draw a line on the vial at the

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meniscus and date the vial. Always check the meniscus when you use concentrate from a vial to make sure no evaporation has taken place. If it is below the line you drew, open a fresh ampoule. Store the vial in an upright position either in the box the concentrate came in or in the rack with the standards shelf of the refrigerator. Record all standard preparations in the electronic logbook!

6 STANDARDS PREPARATION

NOTICE!

Review relevant SOPs and refer to the Material Safety Data Sheets (MSDS) for hazard and handling instructions before preparing standards.

The HPLC standard in ampoules may precipitate during storage. Visually inspect all standard ampoules before use. Ultra-Sonication of the sealed ampoules prior to preparation usually ensures complete dissolution of the compounds into the solvent and is a good standard practice. Do not use any vial with an un-dissolved precipitate.

Always print labels from LIMS each time you make standard. Do not hand write!

For some calibration curves preparation of all the standard levels may not be necessary. Variable injection volumes may be used on the auto sampler for different levels.

Pre-rinse all glassware used in making standards with 1:9 HCl followed by water and methanol. Let it air dry completely. Store all the standards at - 10 °C or less. Replace all the Positive ion mode standards (especially for Carbamates) once every 30 days.

Reagents used to prepare standards should have their lot and/or LIMS serial numbers recorded in the standard data sheet of each standard entered into the LIMS standard preparation manager.

6.1 Monochloroacetic acid buffer

6.1.1 Dissolve 56.7 g of Monochloroacetic acid in 200 mL of reagent water; this yields a 3 M solution. Dissolve 29.4 g of potassium acetate in 100 mL of reagent water, yielding a 3M solution.

6.1.2 Combine 100 mL of the 3M potassium acetate and 156 mL of the 3M Monochloroacetic acid.

6.1.3 Use 10 mL of buffer per 1-L of sample collected. (This solution is provided for the preservation in Sample Receiving/Bottle Prep; it is placed in the 1-L amber bottles prior to sample collection).

6.1.4 **Buffered reagent water:** Dilute 10 mL of 3M Monochloroacetic acid buffer solution (sec. 5.1.2) with reagent water to 1 liter. Use this buffered water to make up all injection standards, spikes and lab blanks, and in making any sample dilutions. Generate Lims ID and print label.

6.2 Stock Standards

6.2.1 Purchase the following required certified standards as neat, individual (at 10, 100 and/or 1000 µg/mL) and/or custom mix standards at available concentration for each analyte from two different sources (AccuStandard, Ultra Scientific, O_2Si and Toronto Research Chemicals or any other vendors) or prepare stock standard from neat at 100 µg/mL and/or 1000 µg/mL in acetonitrile by accurately weighing 1 or 10 mg neat standard in 10

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mL volumetric flask. If standard purity is certified at 96% or greater, the concentration may be used as is, without correcting for purity. Dissolve and dilute to volume with acetonitrile or methanol. Transfer to a clean amber bottle with a Teflon-lined cap. Store in freezer at - 10 °C or less. All standards and spike solutions prepared must be replaced after six months or sooner if degradation of analytes is evident, or use manufacturer suggested expiration.

6.3 Surrogate Standards

6.3.1 Purchase individual surrogates (Acetaminophen-d4, Carbamazepine-d10, Carbaryl-d7, 2, 4-D-d3, Diuron-d6, Diquat-d4, Endothall-d6, Glyphosate 13C 15N, Ibuprofen-d3, Microcystin LR ethyl-d5, Anatoxin-a 13C4, Primidone-d5 and Sucralose-d6) from neat standards at 1000, 100, 10 and 5 µg/mL each separately. Prepare at least 50 to 100 mL of these surrogate mixes from above individual standards depending on the analysis need. Stock surrogate standards expiration may be extended if no degradation is evident.

6.3.2 Prepare for routine extraction, a NIPI surrogate mix at 0.80 µg/mL containing Acetaminophen-d4, Carbamazepine-d10, Carbaryl-d7, 2, 4-D-d3, Diuron-d6, Ibuprofen-d3 and Primidone-d5 and add 25 µL of NIPI surrogate mix to each sample to a final volume of 1 mL.

6.4 Negative and Positive Ion Analysis Mix Standards

6.4.1 Acid Herbicides/Personal Care Products and Urea Herbicides custom mix standard 1st and 2nd sources are at 10 µg/mL each of 2, 4, 5-T, 2, 4-D, 2,4-DB, Acetaminophen, Acetamiprid, Acifluorfen, Afidopyropen, Bentazon, Benzovindiflupyr, Carbamazepine, Clothianidin, Dicamba, Dichlorprop, Dinoseb, Dinotefuran, Diuron, Endothall, Fenuron, Fluridone, Hydrocodone, Ibuprofen, Imazapyr, Imidacloprid, Linuron, Mandestrobin, MCPA, MCPP, Naproxen, Picloram, Primidone, Pyraclostrobin, Silvex, Thiamethoxam, Tolfenpyrad and Triclopyr. Concentration of Bentazon at 1 µg/mL, Carbamazepine and Fluridone at 2.5 µg/mL and Picloram, Dicamba and Ibuprofen at 100 µg/mL due to sensitivity. Hydrocodone individual standard is purchased separately.

6.4.2 Carbamates custom mix standard contains 10 analytes at 100 µg/mL each of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Methomyl, Oxamyl, Propoxur, Methiocarb, Carbaryl, Carbofuran and 3-Hydroxycarbofuran.

6.4.3 Negative Ion and Positive Ion mega mix (NIPI Mix) at 0.40 µg/mL: Prepare NIPI Mix in 10 mL of acetonitrile for both 1st and 2nd sources separately by diluting required volume from above stock solutions (sec 5.4.1 and 5.4.2). Spike 25 µL NIPI Spike Standard (0.40 µg/mL of 1st source to a final volume of 1 mL for the QC samples in the set.

6.4.4 Prepare and replace all the working calibration and ICV standards fresh whenever new surrogate mix is prepared. Store standards at 5 °C or less.

6.4.5 A second source mixed standard is analyzed after each curve to ensure that calibration standards have maintained their integrity and are acceptable for use in analysis.

6.4.6 Negative Ion and Positive Ion mega mix (NIPI Mix) ICAL/NIPI surrogate: Prepare ICAL/Surrogate at 400/800 µg/L by mixing equal volumes of NIPI mix (sec. 5.4.3) and NIPI surrogate mix (sec 5.3.2) and from this mix prepare 100, 50, 25, 10, 5, 2.5, 1, 0.5 and 0.25 µg/L ICAL levels from 1st source 1:1 Acetonitrile with 0.1% Formic acid - MCAA water. Prepare 2.5 µg/L ICV from 2nd source. Note: USGS O-2060-01 requires all working standards must be replaced every six months. When preparing intermediate and ICAL and ICV standards assign six months of expiration.

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6.5 Direct Injection Analysis Standards

6.5.1 W-E8321-DI Analysis: Purchase Glyphosate, Glufosinate, AMPA, Endothall, Acesulfame K and Sucralose individual stock standards from Absolute Standards and O_2Si and prepare custom mix at 10 µg/mL for all and Endothall at 25 µg/mL. Prepare 10 mL of 100 µg/L of all these analytes together both 1st and 2nd sources in DI Water. Also prepare 200 µg/L of three surrogate mix of Endothall d6, Glyphosate 13C 15N and Sucralose d6 (100 µg/L in DI Water. Prepare 10, 5, 2.5, 1, 0.5, 0.25, 0.1 and 0.01 µg/L ICAL levels and 2.5 µg/L of ICV in DI Water (Target analyte to surrogate ratio is 1 to 2). Store all stock and working standards **at 5 °C or less (DO NOT FREEZE).**

6.5.2 Diquat and Paraquat: 50, 25, 10, 5, 2 and 1 µg/L ICAL levels and 10 µg/L of ICV with Diquat-d4 surrogate in water.

Note: Both Diquat and Paraquat strongly adhere to untreated glass. All glassware that contact these compounds must be silanized. Plastic (polyvinyl chloride), polyethylene or polypropylene may be substituted for glassware wherever possible. Use 15 mL polypropylene tubes and dilute with HPLC water. Store at 5 °C or less (DO NOT FREEZE).

6.5.3 Microcystins analysis: Purchase Cylindrospermopsin, Desmethy Microcystin LR, Microcystin HilR, Microcystin HtyR, Microcystins LA, LF, LR, LW, LY, RR, WR and YR and Nodularins-R targets and Microcystin LR ethyl d5 surrogate from Aldrich Chemicals, Enzo and Abraxis). Prepare 500 µg/L in methanol of both 1st and 2nd sources separately. Prepare Microcystin LR ethyl d5 at 100 µg/L in methanol separately. Prepare 50, 25, 10, 5, 2.5, 1, 0.25 and 0.1 µg/L ICAL levels and 10 µg/L of ICV with Microcystin LR ethyl d5 surrogate (Target analyte to surrogate ratio is 10 to 2.5).

6.5.4 Saxitoxin analysis: Prepare Anatoxin-a, Saxitoxin and Anatoxin-a 13C4 at 10 µg/mL from Abraxis and Neosaxitoxin at 20 from Aldrich Chemicals. Prepare target compounds Anatoxin-a, Saxitoxin and Neosaxitoxin at 500/1000/1000 µg/L for the ICAL/Spike and 2nd source in methanol separately. Prepare Anatoxin-a 13C4 surrogate at 250 µg/L in methanol. Use Autoclaved DEP Well Water for working standards and prepare 7 levels from 20 to 0.25 µg/L of ICAL and 10 µg/L of ICV (Target analyte to surrogate ration is 2 to 1).

6.5.5 Store all Microcystin and Saxitoxin standards **at -20 °C or less**

6.6 Per- and Polyfluoroalkyl Substances (PFAS) Analysis Mix Standards

6.6.1 Purchase custom mix standard containing 39 analytes PFAS Standard Mix at 1 µg/mL each from two sources (one source for ICAL and Spike and second source for ICV), 20 analytes Extracted Internal Standard (EIS) surrogate spike mix (Isotope Dilution Compounds Standard) at 1 µg/mL, M3HFPO-DA (EIS) at 50 µg/mL and PFAS Non-extracted Internal Standard mix at 2 µg/mL. Intermediate standards are prepared in methanol and working standards are in 80:20 methanol – water.

6.6.2 Extracted Internal Standard (EIS) surrogate spike: Prepare 10 mL of 20PFAS and M3HFPO-DA Extracted Internal Standard surrogate spike mix at 100 µg/L and add 5 µL to a final volume of 1 mL to all ICAL, ICV, and add 25 µL to a final volume of 5 mL for all the samples prior to extraction. This results 0.5 µg/L in the final volume.

6.6.3 Non-extracted Internal Standard (NIS): Prepare 10 mL of PFAS Non-extracted Internal Standard mix at 40 µg/L and add 12.5 µL to a final volume of 1 mL to all ICAL, ICV,

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and add 62.5 µL to a final volume of 5 mL of all the samples before the analysis. This results 0.5 µg/L in the final volume.

6.6.4 PFAS 39 ICAL/Spike Standard Mix: Prepare 10 mL of PFAS 39 ICAL/Spike Standard Mix at 100 µg/L and add 25 µL to a final volume of 5 mL to all QC samples prior to extraction. This results 0.5 µg/L in the 5 mL final volume.

6.6.5 Sample Dilution Solution Standard Mix: Prepare 10 mL of Sample Dilution Solution Standard Mix at 0.5 µg/L by mixing PFAS Non-extracted Internal Standard mix and 20PFAS Extracted Internal Standard surrogate spike mix (Isotope Dilution Analogues Standard). This is used for sample dilution.

6.6.6 PFAS ICAL: Prepare ICAL levels at 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 µg/L of 39 PFAS Mix (sec 5.6.4) and each level containing 0.5 µg/L of 20PFAS Extracted Internal Standard surrogate spike mix (sec 5.6.2) and Non-extracted Internal Standard mix (sec 5.6.3).

6.6.7 PFAS ICV: Prepare 0.5 µg/L ICV from 2nd source. A second source mixed standard is analyzed after each curve to ensure that calibration standards have maintained their integrity and are acceptable for use in analysis.

6.6.8 Prepare and replace all the working calibration and ICV standards fresh whenever new surrogate mix is prepared. Store all stock and working standards **at 5 °C or less (DO NOT FREEZE) and protect from light.**

7 REFERENCES

7.1 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)

7.2 DEP SOP LC-011. Extraction of Pesticides, Herbicides and other Chemicals from Water or Sediments/Waste

Appendix of Changes

08/05/2010	Added new analyte Cyanuric acid in water analysis
08/23/2011	Added new analyte Dioctyl sulfosuccinate in water analysis
08/13/2012	Updated standards preparation to current list of analysis
03/06/2013	Updated standards preparation to current list of analysis
08/16/2013	Updated standards preparation to current list of analysis
01/14/2014	Updated standards preparation to current list of analysis
07/14/2014	Updated standards preparation to current list of analysis
09/08/2015	Added new analyte Didecyldimethylammonium chloride in water analysis
01/26/2016	Added new analyte Benzoic acid in water analysis
08/12/2016	Added new analyte Malathion in water analysis
01/16/2017	Updated standards preparation to current list of analysis

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01/25/2018	Updated standards preparation to current list of analysis
10/02/2018	Added Perfluorinated Alkyl Acids
11/15/2019	Updated standards preparation to current list of analysis
12/01/2020	Updated standards preparation to current list of analysis
06/30/2021	Added new Perfluorinated alkyl acids
12/01/2022	Updated standards preparation to current list of analysis
12/05/2023	Updated standards preparation to current list of analysis
04/30/2024	Corrected working standards holding time and added notes to record reagents used for standard preparation.
12/06/2024	Added additional information about records and expiration dates for reagents and mobile phases.

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