HPLC Standard Preparation

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1 SCOPE AND APPLICATION

1.1 This SOP details the procedure for preparing 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 Note Book. For each new chemical standard received, enter all the information of standard into the Laboratory Information Management System (LIMS) and get the serial number, and file the certificate of analysis in a certification folder. If no expiration date for the chemical received, call the company and if they cannot give any date then enter one (1) year from date of receipt. 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 and 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 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., 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.

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 (Required from the manufacturer, the certificate stating that the volume is accurate to < 1%, otherwise calibrate and keep the record of these gas-tight micro-syringes or volumetric pipets quarterly).

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 tube needs to record the volume in the Extraction Log Book)

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

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

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4 STANDARDS QUALITY ASSURANCE

4.1 New standards must be checked for correctness and condition upon receipt. Information for each new standard is entered into 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 of the preparation information is entered into the Electronic Standard Preparation Log. The Preparation Log will create a serial number and barcode specific for the standard. Place the serial number or barcode onto the vial along with the standard name, concentration, solvent, date and your initials.

4.2 All prepared ICAL (Initial Calibration Levels), spike, surrogate and internal standard intermediate and working standard solutions are good for one year. Replace standards every one year or sooner, if degradation is evident. Before making any standards from commercial sources, check to insure that the expiration date has not passed. Before putting any standard into use, verify that it is acceptable by comparing 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!**

4.3 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 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!!

5 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 insures 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!

5.1 Stock Standards

Individual stock standard solutions (1000 µg/mL) are prepared from neat standards purchased from any certified source.

5.1.1 Accurately weigh 10 mg neat standard and transfer to a 10 mL volumetric flask. If standard purity is certified at 96% or greater concentration may be used as is, without correcting for purity. Dissolve and dilute to volume with acetonitrile or methanol.

5.1.2 Transfer to a clean amber bottle with a Teflon-lined cap. Store in freezer at - 10 °C or less. All standards and spike solutions must be replaced after one year or sooner if degradation of analytes is evident.

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5.2 Negative Ion Mode and Positive Ion Mode Standards

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.

5.2.1 **Monochloroacetic acid buffer**

5.2.1.1 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.

5.2.1.2 Combine 100 mL of the 3M potassium acetate and 156 mL of the 3M monochloroacetic acid.

5.2.2 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).

5.2.3 **Buffered reagent water:** Dilute 10 mL of 1M monochloroacetic acid buffer solution (sec. 5.2.1.2) 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.

5.2.4 Purchase following certified custom mix standards at 100 µg/mL for each analyte from two different sources (AccuStandard, Ultra Scientific and O_2Si or any other vendors) or prepare stock standard from neat (1000 µg/mL and 100 µg/mL in acetonitrile). Negative Ion Mode custom mix standard contains 100 µg/mL of 15 analyte Acid Herbicides (Acifluorfen, Bentazon, 2, 4-D, 2,4-DB, Dalapon, Dicamba, Dichlorprop, Dinoseb, Imazapyr, MCPA, MCPP, Picloram, Silvex, Triclopyr and 2, 4, 5-T). Positive Ion Mode 10 analyte Carbamates custom mix standard contains 100 µg/mL of (Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Methomyl, Oxamyl, Propoxur, Methiocarb, Carbaryl, Carbofuran and 3-Hydroxycarbofuran and Bendiocarb individual standard if needed). Positive Ion Mode custom mix standard contains 100 µg/mL of 12 analyte Urea Herbicides and Personal Care products (Acetaminophen, Carbamazepine, Diuron, Fenuron, Fluometuron, Fluridone, Imidacloprid, Indaziflam, Linuron, Primidone, Pyraclostrobin and Sucralose). Prepare each of the above positive and negative ion mixes at 1 µg/mL in acetonitrile of both 1st and 2nd sources separately. Prepare 10 µg/mL of Negative Ion Mode spike standard in acetonitrile from 100 µg/mL of 1st source for soil samples preparation. Due to different sensitivity, the concentration of Sucralose is adjusted to 4 times higher than other Positive Ion Mode mixed standards.

5.2.5 Surrogate Standards: Prepare or purchase individual surrogates (Acetaminophen-d4, Carbamazepine-d10, Carbaryl-d7, 2, 4-D-d3, Diuron-d6, Indaziflam-N15, Primidone-d5 and Sucralose-d6) from neat standards at 1000, 100 and 10 µg/mL each separately. Prepare at least 50 to 100 mL of 2/8 µg/mL surrogate mix (Sucralose-d6 at 4 times higher due to different sensitivity than others) from above individual standards. Add 25 µL of 2/8 µg/mL surrogate mix to each samples (to a final volume of 1 mL). For soil Acid Herbicides samples add 100 µL of 10 µg/mL of 2, 4-D-d3 surrogate to 30 grams to 10 mL extraction. For soil Carbamates and Urea Herbicides samples add 250 µL of 2/8 µg/mL surrogate mix or 25 µL of 10 µg/mL individual surrogate standard to a final volume of 10 mL extraction. Prepare and replace all the working calibration and CCV standards freshly whenever new surrogate mix is prepared.

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5.2.6 Spike Mix Standards: Spike 25 µL each Spike Standard (1 µg/mL of 1st source prepared in section 5.2.4) to a final volume of 1 mL for the QC samples in the set. For soil Carbamates and Positive Ion Mode samples add 250 µL of spike mix to a final volume of 10 mL extraction. For soil Acid Herbicides samples add 100 µL of 10 µg/mL of Acid Herbicides Mix Spike Standard to a final volume of 10 mL extraction.

5.2.7 Negative Ion Mode Acid Herbicides Mix and Positive Ion Mode Carbamates Mix with surrogate Mix ICAL Standard (500 µg/L): Prepare both ICAL Standards individually at this concentration by taking 0.5 mL aliquot of 1.0 µg/mL 1st source ICAL/Spike Standard from section 5.2.6 and 0.5 mL aliquot of surrogate mix standard (2/8 µg/mL from section 5.2.5), placing in a 2 mL GC vial and cap and mix well.

5.2.8 Calibration Standards for LC-MSMS: Prepare eight different calibration levels in 1:1 Acetonitrile/ MCAA water by taking appropriate aliquots of Negative Ion Mode Mix ICAL Standard, see details in table. Store standard at 5 °C or less...

Final ICAL Concentration (µg/L)	Aliquot of ICAL Standard Used
250 (for high level quantitation only)	0.5 mL of 500 µg/L to 1 mL
100	0.2 mL of 500 µg/L to 1 mL
50	0.1 mL of 500 µg/L to 1 mL
25	0.05 mL of 500 µg/L to 1 mL
10	0.1 mL of 100 µg/L to 1 mL
5	0.1 mL of 50 µg/L to 1 mL
2.5	0.1 mL of 25 µg/L to 1 mL
0.5	0.1 mL of 5 µg/L to 1 mL

5.2.9 Positive Ion Mode Urea Herbicides and Personal Care Products with surrogate Mix ICAL Standard (500/2000 µg/L): Prepare ICAL Standard at this concentration by taking 0.5 mL aliquot of 1/4 µg/mL 1st source ICAL/Spike Standard from section 5.2.6 and 0.5 mL aliquot of surrogate Mix standard (2/8 µg/mL, section 5.2.5), placing in a 2 mL GC vial and cap and mix well.

5.2.10 Calibration Standards for LC-MSMS: Prepare eight different calibration levels in 10% Acetonitrile/water by taking appropriate aliquots of Positive Ion Mode Urea Herbicides and Personal Care Products Mix ICAL Standard with surrogates (sec. 5.2.9), see details in table. Store standard at 5 °C or less...

Final ICAL Concentration (µg/L)	Aliquot of ICAL Standard Used
100/400	0.2 mL of 500/2000 µg/L to 1 mL
50/200	0.1 mL of 500/2000 µg/L to 1 mL
25/100	0.05 mL of 500/2000 µg/L to 1 mL
10/40	0.1 mL of 100/400 µg/L to 1 mL
5/20	0.1 mL of 50/200 µg/L to 1 mL
2.5/10	0.1 mL of 25/100 µg/L to 1 mL
0.5/2	0.1 mL of 5/20 µg/L to 1 mL
0.1/0.4	0.04 mL of 2.5/10 µg/L to 1 mL

5.2.11 Second Source Calibration Check Standard (CCV): Prepare each CCV mix separately at the required concentration as described below.

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5.2.12 Negative Ion Mode Acid Herbicides Mix CCV at 50 µg/L: Mix 50 µL of 1 µg/mL of 2nd source standard (section 5.2.4) and 25 µL of 2/8 µg/mL of surrogate mix (section 5.2.5) and make up to 1.0 mL with 1:1 Acetonitrile/MCAA water.

5.2.13 Positive Ion Mode Carbamates Mix CCV at 25 µg/L: Mix 50 µL of 1 µg/mL of 2nd source standard (section 5.2.4) and 25 µL of 2/8 µg/mL of surrogate mix (section 5.2.5) and make up to 1.0 mL of acetonitrile to prepare 0.5 µg/mL CCV intermediate mix. Dilute 50 µL of this mix to 1 mL in 1:1 Acetonitrile/MCAA water, Store standard at 5 °C or less.

5.2.14 Positive Ion Mode Urea Herbicides and Personal Care Products Mix CCV at 25/100 µg/L: Add 0.25 mL of 1/4 µg/mL of 2nd source standard mix (section 5.2.4) and 0.25 mL of 2/8 µg/mL of surrogate mix (section 5.2.5) and dilute to 10 mL with 10% Acetonitrile/water, store standard at 5 °C or less.

5.2.15 Sucralose Mix CCV at 100/200 µg/L: Add 1.0 mL of 1/4 µg/mL of 2nd source standard mix (section 5.2.4) and 0.25 mL of 2/8 µg/mL of surrogate mix (section 5.2.5) and dilute to 10 mL with 10% Acetonitrile/water, store standard at 5 °C or less.

5.2.16 Second source mixed standard is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

5.2.17 Benzoic acid stock standards are diluted to prepare the required ICAL/Spike from 1st source and CCV from 2nd source with 2, 4-D-d3 surrogate. ICAL mix at 10 and 1 µg/mL (Benzoic acid and 2, 4-D-d3), Benzoic acid Spike solution at 25 µg/mL and 2, 4-D-d3 surrogate at 2 µg/mL (for 250 mL of water sample to a final volume of 1 mL add 40 and 25 µL respectively). Prepare 6 levels of ICAL standards at 10 to 0.25 µg/mL with 10 times lower 2, 4-D-d3 surrogate. Also prepare CCV at 1 and 0.1 µg/mL of Benzoic acid and 2, 4-D-d3 from 2nd source. All the working standards are in 1:1 acetonitrile – MCAA water.

5.3 Endothall

5.3.1 Purchase Endothall stock standards from two different sources (2000 µg/mL from Spex Certiprep and 1000 µg/mL from Accu Standard). From stock standards prepare 10 mL each of intermediate standards at 10 µg/mL in water. Use one source for the preparation of ICAL and spiking standards and another source for CCV preparation.

5.3.2 Endothall-d6 Surrogate: Prepare Endothall-d6 surrogate stock standard at 1000 µg/mL in water (Purchase Endothall-d6 50 mg from CDN Isotopes). Prepare 10 mL of 10 µg/mL intermediate standard in water from 1000 µg/mL stock standard. Prepare 10 mL of 1 µg/mL in water from 10 µg/mL intermediate for routine surrogate spike. Add 125 µL of 1 µg/mL Endothall-d6 surrogate to all the samples in the set for 5 mL of water sample.

5.3.3 Prepare Endothall and Endothall-d6 at 1 µg/mL for ICAL and Spike in 10 mL of water from individual 10 µg/mL of Endothall and Endothall-d6. Add 125 µL of 1 µg/mL of ICAL and Spike mix to 5 mL of QC samples. Prepare the following Calibration Standards for LC-MSMS analysis by transferring appropriate aliquot volume of 1 µg/mL of ICAL and Spike mix and diluting to required volume with water.

Final ICAL Concentration (µg/L)	**Aliquot of ICAL Standard Used**
100	0.10 mL of 1 µg/mL to 1 mL

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75	0.075 mL of 1 µg/mL to 1 mL
50	0.05 mL of 1 µg/mL to 1 mL
25	0.025 mL of 1 µg/mL to 1 mL
10	0.10 mL of 100 µg/L to 1 mL
5	0.10 mL of 50 µg/L to 1 mL

5.3.4 Endothall CCV Standard Mix (25 µg/L): Prepare 1 µg/mL in water from 10 µg/mL of Endothall 2nd source and 10 µg/mL of Endothall-d6. Prepare Endothall CCV Standard Mix (25/25 µg/L) by diluting 0.025 mL of 2nd source 1 µg/mL Endothall and Endothall-d6 and diluting to volume with water. This CCV standard is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

5.4 Diquat and Paraquat

5.4.1 Diquat and Paraquat Standards: Custom mix Diquat and Paraquat (1000 µg/mL) is purchased from Ultra Scientific and Restek.

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.

5.4.2 Weigh out approximately 0.0100 g of each neat standard and dilute to 10 mL final volume with reagent HPLC water (each standard will be at approximately 1000 µg/mL). Use silanized glass or plastic (polypropylene) volumetric glassware. Store at 5 °C or less (DO NOT FREEZE) in amber, polyethylene or polypropylene bottles.

5.4.3 The concentrations for the stock standards must be corrected, as the weight of each is the hydrated salt, not the pure product. Use the following formulas to correct the concentrations:

$$\text{Conc. of Stock Diquat (µg/mL)} = \frac{\text{Amount weighed (µg)} \times 184.3 \text{ g/mol}}{362.1 \text{ g/mol} \times \text{final volume (mL)}}$$

(MW of Diquat Dibromide.H_2O = 362.1 g/mol; MW of free Diquat = 184.3 g/mol)

$$\text{Conc. Stock Paraquat (µg/mL)} = \frac{\text{Amount weighed (µg)} \times 186.3 \text{ g/mol}}{329.2 \text{ g/mol} \times \text{final volume (mL)}}$$

(MW of Paraquat Dichloride.$4H_20$ = 329.2 g/mol; MW of free Paraquat = 186.3 g/mol)

5.4.4 Diquat and Paraquat Mixed Standard (10 µg/mL): Prepare a 10 µg/mL of Diquat and Paraquat by taking 0.1 mL (sec.5.4.1) aliquot, placing into a 10 mL polypropylene tube (silanized or plastic) and diluting to volume with HPLC water. Store at 5 °C or less (DO NOT FREEZE). Use 1st source Diquat and Paraquat 10 µg/mL standard for the preparation of ICAL standards and for spiking. For QC samples spike 25 µL of 10 µg/mL Diquat/Paraquat ICAL/Spike for 10 mL final volume. Use the 2nd source Diquat and Paraquat 10 µg/mL standard for the preparation of CCV standard.

5.4.5 Diquat-d4 Surrogate Standard (10 µg/mL): Prepare 1000 µg/mL stock standard followed by 100 µg/mL intermediate standard 10 mL each in HPLC water. Prepare a 10 µg/mL of Diquat-d4 surrogate standard by taking appropriate aliquot into 15 mL polypropylene tube and diluting to 10 mL with HPLC water. Store at 5 °C or less (DO NOT FREEZE).

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For all the samples in the set and QC samples spike 100 µL of 10 µg/mL of Diquat-d4 surrogate for 10 mL final volume.

5.4.6 Diquat/Paraquat and Diquat-d4 at 1 µg/mL for ICAL: Prepare a 1 µg/mL of Diquat/Paraquat and Diquat-d4 surrogate mix for ICAL by taking 1 mL of sec.5.4.4 and 5.4.5 each to 15 mL of polypropylene tube and diluting to 10 mL with HPLC water. Prepare the following Calibration Standards for LC-MSMS analysis by transferring appropriate aliquot volume of 1 µg/mL of ICAL. Store at 5 °C or less (DO NOT FREEZE).

Final ICAL Concentration (µg/L)	Aliquot of ICAL Standard Used
500	1 mL of 1 µg/mL to 2 mL
250	1 mL of 1 µg/mL to 4 mL
100	1 mL of 1 µg/mL to 10 mL
25	0.1 mL of 1 µg/mL to 4 mL
5	0.5 mL of 100 µg/L to 10 mL
2	0.2 mL of 100 µg/L to 10 mL
1	0.1 mL of 100 µg/L to 10 mL

5.4.7 Diquat/Paraquat CCV at 25 µg/L: Prepare Diquat/Paraquat and Diquat-d4 at 1 µg/mL for CCV in 10 mL of water from individual 10 µg/mL of Diquat/Paraquat 2nd source section 5.4.4 and Diquat-d4 section 5.4.5. Prepare 10 mL of Diquat/Paraquat CCV Standard (25/25 µg/L) by diluting 0.25 mL of 2nd source 1 µg/mL Diquat/Paraquat and Diquat-d4 and diluting to volume with water. This CCV standard is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

5.5 Glyphosate

5.5.1 Prepare Glyphosate Stock Standard Solution at 1000 µg/mL by accurately weighing 0.0100 g in 10 mL of reagent water from two different sources or purchase 100 µg/mL solution in water for each of Glyphosate, Aminomethyl phosphonic Acid (AMPA) and Glyphosate 13C 15N surrogate from Ultra Scientific, AccuStandard, Acros and Cambridge Isotope Laboratory.

Note: It is advantageous to prepare this solution in a clear (not amber) volumetric flask since dissolution of Glyphosate in water at this concentration is not immediate and may have to be facilitated by sonication for approximately 10-15 minutes. Store all the standards at 5 °C or less (DO NOT FREEZE) in amber, screw cap bottle with Teflon liner. It is suggested that CCV and ICAL standards be prepared once every month.

5.5.2 Glyphosate and AMPA Spike Standard (10 µg/mL): Prepare Glyphosate and AMPA mix standard at 10 µg/mL by taking 0.50 mL aliquot of Stock Standard (100 µg/mL, sec. 5.5.1)) and diluting with HPLC water to 5 mL in a volumetric flask.

5.5.3 Glyphosate 13C 15N Surrogate Spike Standard (10 µg/mL): Prepare 10 µg/mL of Glyphosate 13C 15N by taking 0.2 mL aliquot of 100 µg/mL (sec. 5.5.1) and diluting with HPLC water to 2 mL in a volumetric flask or calibrated 15 mL centrifuge tube.

5.5.4 Glyphosate/AMPA and Glyphosate 13C 15N (1/1/5 µg/mL) for ICAL: Prepare this mix for ICAL by taking 0.02 mL of 100 µg/mL of Glyphosate/AMPA mix ICAL and 0.1 mL of 100 µg/mL of Glyphosate 13C 15N surrogate to 2 mL of volumetric flask and diluting with HPLC water. Prepare the following Calibration Standards for LC-MSMS analysis

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by transferring appropriate aliquot volume of 1/1/5 µg/mL of ICAL. Store at 5 °C or less (DO NOT FREEZE), see table below for the details.

Initial Calibration Levels Conc (µg/L)	Final Volume (mL)	Aliquot of Glyphosate/AMPA/Glyphosate 13C 15N (1/1/5 µg/mL) in mL
L1 250/1250	1	0.25
L2 100/500	1	0.1
L3 50/250	1	0.05
L4 25/125	1	0.025
L5 10/50	1	0.1 of L2

5.5.5 Glyphosate/AMPA and Glyphosate 13C 15N CCV at 50/50/250 µg/L: Prepare Glyphosate/AMPA and Glyphosate 13C 15N CCV at 1/1/5 µg/mL mix by taking 0.02 mL of 100 µg/mL of Glyphosate/AMPA mix 2nd source and 0.1 mL of 100 µg/mL of Glyphosate 13C 15N surrogate and diluting to 2 mL of volumetric flask and with HPLC water. Prepare Glyphosate/AMPA and Glyphosate 13C 15N CCV at 50/50/250 µg/L by diluting 0.05 mL aliquot of 1/1/5 µg/mL of Glyphosate/AMPA and Glyphosate 13C 15N mix to 1 mL with HPLC water. This second source standard (CCV) is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

5.6 Microcystins by LC-MSMS

5.6.1 The absorbtivity of 1M solution for the Microcystins at 238 nm (in methanol) is as follows:

Microcystin LR	39800
Microcystin LA	36500
Microcystin YR	38100
Microcystin RR	39800
Nodularin	35500
Cylindrospermopsin	8282 at 262 nm (in methanol)

5.6.2 Conversion Table from µmole to µg/mL (PPM)

Microcystins	**µg/mL (PPM)**	**µmole**	**Molecular Weight**
Microcystin LR	100	100.5	995
Microcystin LA	100	110	909
Microcystin YR	100	95.7	1045
Microcystin RR	100	96.3	1038
Nodularin	100	121.4	824
Cylindrospermopsin	100	241	415

5.6.3 Stock Standard Solution: Dissolve the purchased analytes in methanol and transfer into a 5 mL volumetric flask and make up to the volume. Make 10 times dilution in methanol and measure the UV absorbance at 238 or 262 (sec. 5.6.1). For each compound calculate the concentration in µmole using the respective observed UV absorbance. Then convert µmole into µg/mL (PPM) for each compound using sec 5.6.2. Multiply the value, µg/mL (PPM) by 10 to get the correct concentration of Stock Standard Solution. Store in 15 mL screw cap test tube with Teflon liner at – 10 °C or less. This step gives approximately close to 10 µg/mL concentration of each standard.

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5.6.4 Alternatively, purchase certified calibrated stock individual standards (Anatoxin-a, Cylindrospermopsin, and Microcystins LA, LF, LR, LY, RR and YR at 5 and 10 µg/mL from two different sources (Aldrich Chemicals and Abraxis).

5.6.5 Carbamazepine-d10 Surrogate at 0.1 µg/mL: Prepare this surrogate from stock in 10 mL of water. Spike 20 µL of 100 µg/L of Surrogate to 1 mL of water for all the samples in the set. Microcystin LF-d5 Surrogate at 1 µg/mL: Prepare this surrogate from stock in 10 mL of methanol. Spike 20 µL of 1000 µg/L of Surrogate to 1 mL of water for all the samples in the set.

5.6.6 Microcystins Intermediate Mix Standard (500 µg/L): Prepare Microcystins Intermediate Mix Standard including surrogate by taking appropriate aliquot of the above stock standard solution (sec.5.6.3 to sec. 5.6.5) and diluting with reagent water. Store at 5 °C (DO NOT FREEZE). Prepare two separate 500 µg/L Microcystins Intermediate Mix Standards. One source for ICAL and spike, and other second source for Calibration Check Standard CCV. Spike 20 µL of 500 µg/L of ICAL/Spike mix to 1 mL of water for QC samples.

5.6.7 Calibration Standard Solutions: Prepare standards at a minimum of six concentration levels by making dilutions from Microcystins Intermediate Mix Standard (500 µg/L) in reagent HPLC water, see details in table. Suggested levels are: 100, 50, 20, 10, 5, 2 and 1 µg/L. Store in at 5 °C or less (DO NOT FREEZE).

Final ICAL Concentration (µg/L)	Aliquot of ICAL Standard Used
100	0.10 mL of 1000 µg/L to 1 mL
50	0.05 mL of 1000 µg/L to 1 mL
20	0.02 mL of 1000 µg/L to 1 mL
10	0.10 mL of 100 µg/L to 1 mL
5	0.10 mL of 50 µg/L to 1 mL
2	0.10 mL of 20 µg/L to 1 mL
1	0.10 mL of 10 µg/L to 1 mL

5.6.8 Second Source Calibration Check Standard Mix (CCV) at 10 µg/L: Prepare 1 mL of this CCV mix standard at 10 µg/L from second source Microcystins Intermediate Mix Standard (200 µg/L). This second source standard 10 µg/L is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

5.7 Didecyldimethylammonium chloride

5.7.1 Purchase Didecyldimethylammonium chloride stock standard from two different sources and prepare working standards at 1000, 100, 10, 1 and 0.1 µg/mL in water. Use one source for the preparation of ICAL and spiking standards and another source for CCV preparation. Add 20 µL of 1 µg/mL Didecyldimethylammonium chloride to all the QC samples in the set for 2 mL of water sample.

5.7.2 Carbamazepine-d10 Surrogate: Prepare Carbamazepine-d10 at 1, 0.2 and 0.02 µg/mL in water for the preparation calibration standards and for spiking. Add 50 µL of 0.02 µg/mL Carbamazepine-d10 surrogate to all the samples in the set for 2 mL of water sample.

5.7.3 Prepare Didecyldimethylammonium chloride and Carbamazepine-d10 at 1000 and 50 µg/L for ICAL in 1 mL of water from individual Didecyldimethylammonium chloride

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and Carbamazepine-d10 respectively. Prepare the following Calibration Standards for LC-MSMS analysis by transferring appropriate aliquot volume of this 1000 and 50 µg/L of ICAL mix and diluting to required volume with water.

Final ICAL Concentration (µg/L)	Aliquot of ICAL Standard Used
100	0.10 mL of 1 µg/mL to 1 mL
50	0.050 mL of 1 µg/mL to 1 mL
20	0.020 mL of 1 µg/mL to 1 mL
10	0.10 mL of 100 µg/L to 1 mL
5	0.10 mL of 50 µg/L to 1 mL
2	0.10 mL of 20 µg/L to 1 mL
1	0.10 mL of 10 µg/L to 1 mL

5.7.4 Second Source Calibration Check Standard Mix (CCV): Prepare 1 mL of Didecyldimethylammonium chloride and Carbamazepine-d10 at 1000 and 50 µg/L for CCV in water as in section 5.7.3 from 2nd source. Dilute 0.1 mL of this mix to 10 mL of water to prepare Didecyldimethylammonium chloride and Carbamazepine-d10 at 10 and 0.5 µg/L of CCV. This CCV standard is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

5.8 Malathion

5.8.1 Purchase Malathion stock standard from two different sources and prepare working standards at 10, 2, 1 and 0.5 µg/mL in methanol. Use one source for the preparation of ICAL and spiking standards and another source for CCV preparation. Add 20 µL of 0.5 µg/mL Malathion to all the QC samples in the set for 1 mL of water sample.

5.8.2 Malathion-d6 Surrogate: Prepare Malathion-d6 at 10 and 1 µg/mL in methanol for the preparation calibration standards and for spiking. Add 25 µL of 1 µg/mL Malathion-d6 surrogate to all the samples in the set for 1 mL of water sample.

5.8.3 Prepare Malathion and Malathion-d6 at 2 µg/mL for ICAL in methanol from individual Malathion and Malathion-d6 respectively. Prepare the following Calibration Standards for LC-MSMS analysis by transferring appropriate aliquot volume 2 µg/mL of ICAL mix and diluting to required volume with MCAA Water (sec. 5.2.3).

Final ICAL Concentration (µg/L)	Aliquot of ICAL Standard Used
250	0.125 mL of 2 µg/mL to 1 mL
100	0.050 mL of 2 µg/mL to 1 mL
50	0.025 mL of 2 µg/mL to 1 mL
25	0.1 mL of 250 µg/L to 1 mL
10	0.10 mL of 100 µg/L to 1 mL
5	0.10 mL of 50 µg/L to 1 mL
1	0.10 mL of 10 µg/L to 1 mL

5.8.4 Second Source Calibration Check Standard Mix (CCV) at 25 µg/L: Prepare from 2nd source Malathion and Malathion-d6 at 1 µg/mL each for CCV in methanol as in section 5.8.3. Take 0.025 mL of this mix (1µg/mL) to 1 mL of MCAA Water (sec. 5.2.3) to prepare Malathion and Malathion-d6 at 25 µg/L of CCV. This CCV standard is analyzed with each run as a check to insure that calibration standards have maintained their integrity and are acceptable for use in analysis.

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6 REFERENCES

6.1 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)

6.2 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

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