Standard Operating Procedure for:

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)

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1 SCOPE AND APPLICATION

1.1 This SOP describes the use of high performance liquid chromatography (HPLC) coupled with Turboionspray mass spectrometry (TIS-MS) detection in positive and negative ion mode analysis of select Pesticides, Herbicides and other Chemicals in water and sediment/waste sample extracts. The preparation of water matrices and sediment/waste samples are described in SOP LC-011.

1.2 This method details the measurement of the acid forms of Chlorophenoxy acid herbicides present in sample extracts. It does not measure the ester forms. Base treatment in the extraction step, proved to be effective in recovering the rest of the 50% ester form of 2, 4-D in Performance Evaluation samples.

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

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

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

The acceptance limits, as provided in EPA Method 8321B and USGS Method O-2060-01, are programmed into the Laboratory's Information Management Systems (LIMS) and applied automatically as part of the data processing, review, and reporting procedures. Table 5.1 of the Quality Manual provides information regarding method limits of detection, accuracy, and precision.

1.4 **Determination of Method Detection Limits and Demonstration of Capability**

Refer to the SOP **LB-007** *Procedure and Policy for Demonstration of Capability for Methods, Instruments and Laboratory Staff* and refer to SOP **LB-031,** *Limit of Detection Verification* for specific procedural details

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The Limit of Detection (LOD) and Demonstration of Capability (DOCs) shall be determined for each main quality system matrix group identified by NELAP (air, drinking water, non-potable water, biological materials and solids/chemical materials). These groups represent the minimum matrix distinctions for LOD determinations and DOCs where there is evidence that the method performance will not be affected by sub-matrix distinctions within these groups, Otherwise LODs and DOCs shall be determined separately for the quality system matrix sub-groups listed below. Where a suitable matrix cannot be found for preparing samples for LOD determinations or DOCs, another similar matrix type may need to be used or samples may need to be prepared in the absence of a matrix (e.g. method blanks). Reasons for matrix substitution may include (but are not limited to): inappropriate background analyte levels in the matrix for the intended study, no analyte-free matrix is available for method blanks, no reference materials are available for a matrix, etc.

1.4.1 Quality System Matrices:

- Aqueous – all aqueous samples excluding drinking water (see below) and salt or estuarine waters;
- Drinking Water – water from a designated potable source;
- Saline/Estuarine – an aqueous sample from an ocean or estuarine. The effects of this matrix type may vary from analysis to analysis and with salt content. Therefore, the decision to what extent if any this matrix has on the LOD and what salt levels may affect the LOD is left to the discretion of experienced supervisory staff;

1.4.2 Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) Determination Procedures

1.4.2.1 The MDLs were established using procedures given in EPA Chapter 40 Part 136, Appendix B of the Federal Register. Also, refer to **Reference 10.4.**

1.4.2.2 Seven replicate samples are analyzed near the MDL. The MDL is the concentration of analyte at which there is analyte present with 99% confidence.

1.4.2.3 The results of the seven replicate data sets are tabulated and the statistical calculations are performed electronically on at least an annual basis to establish or update the MDLs and PQLs for each analyte and matrix. A list of MDLs is given in the Laboratory Comprehensive Quality Control Plan, see Reference 10.6.

1.5 The MDL and PQL values achievable with this method for the following analytes are listed in Table 2.

Note: The detection limits will vary depending on the amount of water, or sediment/waste sample extracted; the final volumes made and type of analysis.

Table 2: Limits Table for Negative Ion Mode and Positive Ion Mode Analysis

Analyte	**Water MDL/PQL µg/L**		**Sediment MDL/PQL µg/kg**	
Negative Ion Mode				
2,4-D-d3	0.002	0.008	0.33	1.3
Dalapon	0.08	0.32		
Picloram	0.08	0.32	3.3	13
Dicamba	0.08	0.32	3.3	13

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Imazapyr	0.004	0.016		
Bentazon	0.0008	0.0032	0.33	1.3
2,4-D	0.002	0.008	0.33	1.3
MCPA	0.002	0.008	0.33	1.3
Triclopyr	0.004	0.016	0.33	1.3
Dichlorprop	0.004	0.016	0.33	1.3
MCPP	0.002	0.008	0.33	1.3
2,4,5-T	0.004	0.016	0.33	1.3
2,4-DB	0.008	0.032	0.33	1.3
Silvex	0.004	0.016	0.33	1.3
Acifluorfen	0.008	0.032	0.33	1.3
Dinoseb	0.004	0.016	0.33	1.3
Benzoic acid	5	20		
Metformin HCl	1	4		
Hydrocodone	0.004	0.016		
Duloxetine HCl	0.008	0.032		
Simvastatin	0.008	0.032		
Endothall-d6	1	4		
Endothall	1	4		
Glyphosate C13 15N	1	4		
Glyphosate	0.1	0.4		
Glufosinate	0.5	2		
AMPA	0.1	0.4		
Sucralose-d6	0.10	0.40	1	4
Sucralose	0.01	0.04	1	4
Positive Ion Mode				
Carbaryl-d7	0.002	0.008	1	4
Aldicarb Sulfoxide	0.002	0.008	1	4
Aldicarb Sulfone	0.002	0.008	1	4
Oxamyl	0.002	0.008	1	4
Methomyl	0.002	0.008	1	4
3-Hydroxycarbofuran	0.002	0.008	1	4
Aldicarb	0.02	0.08	1	4
Propoxur	0.002	0.008	1	4
Carbofuran	0.002	0.008	1	4
Carbaryl	0.002	0.008	1	4
Methiocarb	0.002	0.008	1	4
Bendiocarb	0.002	0.008	1	4
Acetaminophen-d4	0.008	0.032	1	4
Acetaminophen	0.008	0.032	1	4
Carbamazepine-d10	0.0004	0.0016	1	4
Carbamazepine	0.0004	0.0016	1	4
Indaziflam-N15	0.002	0.008	1	4
Indaziflam	0.002	0.008	1	4
Primidone-d5	0.004	0.016	1	4
Primidone	0.004	0.016	1	4
Diuron-d6	0.002	0.008	1	4
Fenuron	0.008	0.016	1	4
Imidacloprid	0.002	0.008	1	4
Diuron	0.002	0.008	1	4
Linuron	0.004	0.016	1	4
Fluridone	0.0004	0.0016	1	4

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Pyraclostrobin	0.002	0.008	1	4
Monuron	0.002	0.008	1	4
Fluometuron	0.004	0.016	1	4
Atrazine-d5	0.002	0.008	1	4
Naproxen	0.02	0.08		
Ibuprofen	0.25	1		
Carbamazepine-d10	0.002	0.008		
Didecyldimethylammonium chloride	1	4		
Diquat-d4	1	4		
Diquat	1	4		
Paraquat	1	4		
Malathion-d6	1	4		
Malathion	1	4		
Carbamazepine-d10	0.002	0.008		
Anatoxin-a	0.25	1		
Cylindrospermopsin	0.25	1		
Microcystin LA	0.25	1		
Microcystin LF	0.25	1		
Microcystin LR	0.25	1		
Microcystin LY	0.25	1		
Microcystin RR	0.25	1		
Microcystin YR	0.25	1		
Nodularin	0.25	1		

1.6 LOD check for all the analytes listed in Table 2 are performed quarterly for each matrix at 1 to 4 times to detection limit.

1.7 This method is essentially the same as EPA methods 8321B and USGS method O-2060-01. The main difference in these methods is the type of instrument or the mass spectrometry technique used. The ABSciex uses polypropylene glycols (PPGs) for calibration of mass spectrometer with a Turboionspray technique to introduce sample. All the new techniques used are well established by the manufacturer and widely used for similar compounds.

2 SUMMARY OF THE METHOD

2.1 An aliquot of the sample extract (prepared in DEP SOP LC-011) is analyzed by LC-MSMS.

2.2 In MS/MS analysis each compound is identified and quantified based on known precursor ions (both in standard and unknown samples) being fragmented into two daughter ions along with retention time.

3 APPARATUS AND EQUIPMENT

3.1 2-mL crimp top glass Auto sampler vials with crimp caps.

3.2 Millipore 13 mm PTFE Syringe Filters 0.45 µ, pack of 1000 (catalog number - SLCR013NK). (and Millex HV PVDF 0.22um Filters only for Doss analysis)

3.3 3 mL disposable Luer tip syringes

3.4 Guard cartridge, reversed phase (Whatman).

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3.5 Phenomenex – Luna, 5 microns, Phenyl-Hexyl, 4.6 x 150 mm; and/or Nova-Pak C8, 60A°, 4 microns, 3.9 x 150 mm; WAT035876 or Nova-Pak C18, 60A°, 4 microns, 3.9 x 150 mm. For the analysis of Didecyldimethylammonium chloride: Phenomenex – Synergy 4-micron Fusion-RP 80A, 50 x 2 mm

3.6 UPLC: Shimadzu UPLC system includes two separate Nexera LC-30AD Liquid Chromatograph, with maximum holding pressure of 19000 psi equipped with Nexera SIL-30AC Autosampler capable of making 0.1 to 500 µL injections, Nexera CTO-30A Column Oven, Prominence DGU-20A5R Degassing Unit and Prominence CBM-20A Communications Bus Module.

3.7 Mass spectrometer system: Applied Biosystems MDS Sciex AB Sciex 6500+ LC-MSMS system (two full systems). A triple quadrupole mass spectrometer capable of scanning from 1 to 1800 amu with Low Mass and High Mass scanning capability. The mass spectrometer must be calibrated with PPG's in both positive mode and negative mode.

3.8 In house liquid Nitrogen gas is used at 120 psi gas pressure before instrument and split with two with additional regulators. Set each gas pressures on the dial to deliver required volumes, curtain gas at 60 psi, source gas at 100 psi and exhaust gas at 60 psi.

3.9 Interface: Turboionspray ionization interface and source that will give acceptable calibration response for the analyte of interest at the concentration required. The source must be capable of generating both positive and negative ions and have a discharge electrode or filament.

3.10 Data system: A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be interfaced to the mass spectrometer. The computer must have software that allows any MS data file to be searched for ions of a specified mass, and such ion abundance's to be plotted versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also allow integration of the abundance's in any EICP between specified time or scan number limits. There must be computer software available to operate the specific modes of the mass spectrometer.

4 REAGENTS AND CHEMICALS

NOTE: Record Lot Numbers of all the reagents and solvents used in the Note Book.

4.1 Solvents, HPLC grade: Acetonitrile and Methanol (Fisher Scientific) and in house deionized water (HPLC water) tested for free of analytes.

4.2 Chemicals: Formic acid, Acros, Reagent grade, ACS, Ammonium Acetate, Fisher HPLC Grade, Ammonium Fluoride, Fisher ACS Grade and Ammonium Formate, Acros, Reagent grade, ACS.

4.3 1 Mole Ammonium formate solution: Dissolve 6.306 grams of ammonium formate in 100 mL of deionized water.

4.4 Mobile phase A: HPLC water containing 5 mmol Ammonium acetate and 0.1% Formic acid. Dissolve 0.77 grams of Ammonium acetate in HPLC water and add 2 mL of Formic acid and dilute to 2-liters. Mobile phase B: Acetonitrile containing 0.1% Formic acid.

4.5 Mobile phase A: HPLC water containing 0.25 mmol Ammonium fluoride (dissolve 0.038 grams of Ammonium fluoride in HPLC water and dilute to 4-liters) and Mobile Phase B: Acetonitrile for Sucralose and direct injection analysis.

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4.6 Mobile phase A: HPLC water containing 5 mmole Heptafluorobutyric acid. Dissolve 1.07 grams of heptafluorobutyric acid in 1000 mL of deionized water. Mobile phase B: Acetonitrile containing 0.1% Formic acid. For PDquat analysis.

4.7 Hydrochloric acid, reagent grade. 1:9 HCl solution - Slowly pour 100 mL of concentrated HCl into 850 mL HPLC grade water in a one-liter volumetric flask. Dilute to volume with HPLC water and shake well to mix.

4.8 See DEP SOP LC-039 for all the required standards and MCAA water is used for the preparation of standards and samples for the Negative and Positive ion (NIPI) analysis.

5 SAMPLE PREPARATION PROCEDURE

5.1 Refer to the extraction SOP LC-011 (Extraction of Pesticides, Herbicides and other Chemicals from Water or Sediments/Waste) for details on sample preparation.

5.2 All sample extracts must be analyzed within 40 days of sample extraction and for Carbamates 21 days, and for direct injection analysis 7 days of collection.

6 SAMPLE ANALYSIS / QUANTITATION

6.1 MASS SPECTROMETER RESOLUTION OPTIMIZATION AND CALIBRATION

6.1.1 Set the liquid-Nitrogen main line pressure gauge at 100 psi and split the line into two lines with two gauges. First Manifold line gauge set at 100 psi for Gas 1 and 2 and Sheath Gas, and second gauge split from main line and set at 60 psi for Exhaust Supply, Curtain Gas Supply and CAD Gas. Turn on the power for all UPLC components. Fill reservoirs with proper mobile phases, and rinse and wash solutions for injector. (Follow the checklist in APPENDIX-A for Shimadzu UPLC). Stop Analyst Services to clear any pending commitments from the Analyst software. Select the required Project. Go to Configure and Hardware Configuration to activate Shimadzu (Mass Spectrometer and UPLC). To completely shut down the LC-MSMS, Deactivate Profile, turn off power on all UPLC components, close Analyst, and Stop Analyst Services to clear any pending commitments from the Analyst software.

6.1.2 Follow the API 6500+ Triple Quad Instrument Manual for PPG calibrations. To do the Manual Tuning, go to Tune, Manual Tuning and open the required Tune File (PPG Q3 Negative/Positive, Q1 Reserpine and Reserpine MS/MS). Do tune only if mass calibration changes, otherwise this tune is required at the time of preventive maintenance and done by the service engineer every six months.

6.1.3 ACQUISITION INFORMATION IN MS MODE: PPGs:

Peak List for 10MCA scans from PPGs Low Mass Target Ion, Intensity and Width (0.6 to 0.8)

	Target Mass (Da)	Intensity (cps)	Width (Da)
+Q1	59.050	4.98e7	0.626
	175.133	2.66e7	0.738
	500.38	8.39e7	0.742
	616.464	6.08e7	0.755
	906.673	1.80e8	0.713
+Q3	59.050	4.93e7	0.741
	175.133	2.28e7	0.723
	500.38	9.50e7	0.648

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	616.464	5.84e7	0.778
	906.673	2.06e8	0.750
-Q1	44.998	1.05e9	0.643
	411.259	9.02e5	0.771
	585.385	3.96e6	0.688
	933.636	2.01e7	0.716
-Q3	44.998	7.43e8	0.707
	411.259	3.17e5	0.714
	585.385	3.18e6	0.709
	933.636	1.97e7	0.729

6.2 OPTIMIZATION FOR COMPOUNDS BY INFUSION

6.2.1 Tuning parameters are instrument dependent and the values can vary with the type of experiment. Optimize the response for the specific analyte by manual, semiautomatic and/or automatic tuning procedures.

6.2.2 Automatic optimization for MS/MS analysis optimizes certain compound-dependent parameters for MRM (Multiple Reaction Monitoring) transitions. In an automatic optimization experiment, parameters can be optimized for two transitions from the specified precursor ion for each compound. Software determines the optimal conditions for the compound precursor ion and precursor ion to fragment ions to use in MRM analysis and stored under the acquisition method for use.

6.2.3 Tune – Manual Tuning – Infuse compounds individual or as a mix at 0.50 PPM for MS/MS in 1:1 Methanol – Water at 10 μL/minute

6.2.4 MS Method – Q1 MS (Q1) – Polarity – Positive or Negative; Start - 100 amu; Stop – 400 amu; Time -1 second to cover the mass range of compounds of interest

6.2.5 Edit Parameters – Same as PPG Calibration – Curtain Gas – 25; Ion Spray Voltage – 4800; Temperature – 0.0; Ion Source Gas 1 – 25; Ion Source Gas 2 – 5

6.2.6 Start – check and find the mass of compounds of interest. Start - MCA - ON, 10 cycles to check proper intensity of the peaks. If not enough sensitivity, increase flow rate or concentration of the infusing compounds. Stop and close Manual Tuning and do not save.

6.2.7 Quantitative Optimization – Inlet – Infusion; Mass Spectrometer – MS/MS Analysis; Next; Target Ion – MW Ion - Search Window + or – 0.3 amu; Resolution – Unit; Polarity Positive. Product Ion – Auto Search – Criteria – 3 peaks; 2 most intense peaks; exclude 5; amu 40; cps 100; Compound name and target ion and start the auto tune.

6.2.8 User specified optimization is used for the known MRMs (Q1 to Q3).

6.2.9 In Manual optimization, the compound dependent parameters (DP, EP, CE and CXP) for the known MRMs are standardized very efficiently.

6.2.10 Merge Methods – Script – Merge MRM; if more than one method is created and save the final method for use.

6.2.11 Optimize each MRM transition for Curtain Gas, CAD Gas, Ion Spry Voltage, Temperature, Gas 1 and Gas2 by FIA Compound Optimization. Set mobile phase A & B (1:1 ratio) at 0.5 mL/min and make 10 μL injection of standard mix and a run time of 2 minutes.

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6.2.12 Setup for each parameter in increments and the software automatically finds best optimal value and replaces with old value.

6.2.13 Set duration and LC Sync ON and save method.

6.3 ACQUISITION METHOD AND BATCH

6.3.1 LC-MSMS Analyst Software fully controls and communicates with Shimadzu UPLC System.

6.3.2 Setup all the UPLC conditions in the method file which includes divert valve settings, gradient program, flow rate, injection volume and equilibration time.

6.3.3 Select the Acquisition Method to run and equilibrate by going through Acquire, View Queue and Equilibrate and click OK.

6.3.4 Use the suggested gradient program and column for each method.

6.3.5 Build Acquisition Batch – Give new Set name, Select Quantitation – Quick Quant – Method Editor – Use Multiple Methods – Add Samples – Sample Name Prefix – Type Sample Name – Number of digits – 1; Data file Prefix – Data and Set Name checked; New Samples Number – OK to add samples. For each row, type sample name (if same sample is used for different injection volumes than the method specified, then enter DF=? and volume injection) and vial position to run and Injection volume used. Quantitation – Edit Quant Type and Analyte Concentration; Save run sequence and Submit to run.

6.3.6 View Queue – Start Sample - when system is in pre-run mode.

6.3.7 Filter samples prior to analysis with 0.45 μm filters using disposable syringes. Cap and store at 5 °C or proceed directly to UPLC/MSMS analysis.

6.3.8 Each extraction batch must contain one extraction method blank, lab fortified blank (LFB), and duplicate matrix spikes for every sample set, not to exceed 20 samples.

6.3.9 First evaluate Instrument blank by injecting mobile phase followed by an initial calibration curve. Quantitative analysis is accomplished by the external calibration method. The order of the calibration fit used is linear through zero, linear, linear weighted (1/x or 1/x^2) or quadratic through origin. For building an initial calibration curve a minimum of 5 calibration levels must be used. The linearity through origin of the calibration curve is defined by an RSD of ≤20% of the Response Factors, or by a Correlation Coefficient bigger than 0.995. After identification, all positives are quantified. If the quantity found is not within the calibration range the samples are diluted or re-run with a multilevel calibration curve. Check the target and surrogate compounds recovery within ≤20% by analyzing mid-level ICAL standard. If the curve is not satisfactory, a new curve must be regenerated until the requirements are met.

6.3.10 Each run has the following run sequence: Mobile phase, calibration standards (minimum 5 calibration levels are required) and second source check standard to start the run. If the system checks good, then proceed with the rest of the run sequence by injecting five to eight samples followed by a second source check standard as a Continuing Calibration Verification (CCV) standard. This standard must be analyzed to ensure that the system has remained in linear calibration. End the analysis sequence with a CCV and a low-level standard, which is close to MDL. This continuing calibration verification standard's recovery must be within + or - 30%. If at any time the continuing calibration check standard fails to meet this + or - 30% criterion, any samples analyzed after it must be re-

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analyzed. First re-analyze the calibration check standard again and showing it passes the criterion, otherwise repeat the above process by re-analyzing a new five-point calibration curve.

6.3.11 Go to Explore to review the current injection or run to see if the data is collecting properly. Open Data File and check all the MRM transitions by opening their Extracted Ions. Review all the injections for proper collection of data and proceed to Quantitation.

API 6500+ Triple Quad/UPLC Negative Ion and Positive Ion Acquisition Method

Table: API 6500+ Acquisition Parameters

Q1 Mass	Q3 Mass	Duration	Analyte	DP	EP	CE	CXP
Negative Ion							
140.819	96.9	10	Dalapon	-5	-7	-12	-13
140.819	104.9	10	Dalapon 2	-5	-7	-10	-13
240.8	196.81	10	Picloram	-30	-10	-14	-11
238.9	194.91	10	Picloram 2	-30	-10	-14	-9
259.978	216	10	Imazapyr	-25	-10	-18	-13
259.978	173	10	Imazapyr 2	-40	-10	-28	-19
218.85	174.911	10	Dicamba	-20	-10	-10	-11
220.9	176.9	10	Dicamba 2	-20	-10	-11	-10
239	132	10	Bentazon	-70	-10	-35	-10
239	175	10	Bentazon 2	-70	-10	-26	-10
221.9	163.9	10	2,4-D-d3	-25	-10	-20.17	-17
222.127	164.25	10	2,4-D-d3 2	-25	-10	-20	-3
218.9	160.9	10	2,4-D	-25	-10	-18	-10
220.9	162.9	10	2,4-D 2	-25	-10	-18	-10
198.9	140.9	10	MCPA	-35	-10	-20	-10
200.9	143	10	MCPA 2	-35	-10	-20	-10
228.9	169	10	Naproxen	-20	-10	-38	-21
228.9	170	10	Naproxen 2	-20	-10	-20	-9
228.9	185	10	Naproxen 3	-20	-10	-10	-11
253.1	194.9	10	Triclopyr	-25	-10	-18	-11
254.9	196.902	10	Triclopyr 2	-25	-10	-16	-11
232.9	160.9	10	Dichlorprop	-20	-10	-18	-10
232.8	124.902	10	Dichlorprop 2	-20	-10	-40	-15
213	141	10	MCPP	-20	-10	-20	-10
215	143	10	MCPP 2	-20	-10	-20	-10
252.9	194.9	10	2,4,5-T	-35	-10	-20	-10
254.9	196.9	10	2,4,5-T 2	-35	-10	-20	-10
246.9	160.9	10	2,4-DB	-20	-10	-16	-10
248.9	162.9	10	2,4-DB 2	-20	-10	-16	-10
266.9	194.9	10	Silvex	-20	-10	-18	-10
268.9	196.9	10	Silvex 2	-20	-10	-18	-10
360	316	10	Acifluorfen	-25	-10	-13	-10
360	195	10	Acifluorfen 2	-25	-10	-35	-10
205	161	10	Ibuprofen	-30	-10	-10	-10
206	162	10	Ibuprofen 2	-35	-10	-10	-5

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205.001	161.001	10	Ibuprofen 3	-13.92	-7.64	-9.34	-13
206.001	162.001	10	Ibuprofen 4	-22.76	-8.07	-9.73	-7.08
239	193	10	Dinoseb	-50	-10	-34	-10
239	134	10	Dinoseb 2	-50	-10	-58	-10
401.058	35.1	10	Sucralose-d6	-45	-10	-66	-15
403.023	35.1	10	Sucralose-d6 2	-45	-10	-66	-15
395.063	35	10	Sucralose	-45	-10	-60	-15
395	359	10	Sucralose 2	-45	-10	-16	-11
190.955	147	10	Endothall-d6	-25	-10	-22	-17
190.955	46	10	Endothall-d6 2	-20	-10	-54	-21
184.915	141	10	Endothall	-20	-10	-20	-17
184.915	43.2	10	Endothall 2	-30	-10	-48	-19
165.929	77.9	10	Acesulfame K-d4	-30	-10	-38	-9
165.929	85.9	10	Acesulfame K-d4 2	-27	-10	-20	-10
161.881	78	10	Acesulfame K	-30	-10	-48	-9
161.881	82	10	Acesulfame K 2	-30	-10	-20	-9
161.881	64.1	10	Acesulfame K 3	-15	-10	-68	-3
121	77	10	Benzoic Acid	-35	-10	-16	-10
121	77.1	10	Benzoic Acid 2	-35	-10	-16	-36
Positive Ion							
130	71	10	Metform	4	10	41	36
130	43	10	Metformin.2	4	10	43	4
130.001	60.001	10	Metformin 3	32.03	9.93	19.15	8.06
130.001	71.001	10	Metformin 4	32.03	9.93	32	6.61
130.001	43.001	10	Metformin 5	161	10	65	20
130	60	10	Metformin 6	161	10	17	8
130.01	71.01	10	Metformin 7	161	10	29	8
156.11	114	10	Acetaminophen-d4	50	10	20	9
152.1	110.1	10	Acetaminophen	50	10	22	10
152.1	65	10	Acetaminophen 2	50	10	39	10
300	199	10	Hydrocodone	4	10	41	12
300	171	10	Hydrocodone 2	1	10	51	20
300.001	128	10	Hydrocodone 3	4	10	73	34
224.077	167	10	Primidone-d5	40	10	17	10
219.1	162	10	Primidone	40	10	17	10
218.981	91	10	Primidone 2	40	10	31	11
165.1	72.1	10	Fenuron	40	10	21	10
165.1	46.2	10	Fenuron 2	40	10	19	10
256.1	209	10	Imidacloprid	35	10	23	10
256.1	175	10	Imidacloprid 2	35	10	26	10
248.129	205.2	10	Carbamazepine-d10	45	10	29	16
237.1	194.1	10	Carbamazepine	45	10	28	10
237.1	193.1	10	Carbamazepine 2	45	10	46	10
298	154	10	Duloxetine	81	10	9	16
298	44	10	Duloxetine 2	81	10	35	20
240.158	78.2	10	Diuron-d6	40	10	20	9
233	72.2	10	Diuron	40	10	36	10

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233	46.2	10	Diuron 2	40	10	31	10
306.95	162	10	Indaziflam-N15	45	10	23	5.3
302.2	158	10	Indaziflam	45	10	23	10
302.2	138.1	10	Indaziflam 2	45	10	35	10
249.1	160.1	10	Linuron	35	10	24	10
249.1	182	10	Linuron 2	35	10	21	10
330.037	310.1	10	Fluridone	150	10	47	10
330.037	259	10	Fluridone 2	150	10	47	10
436.33	199	10	Simvastatin	21	3.5	23	4
419.1	243	10	Simvastatin 2	1	10	21	22
436.2	199	10	Simvastatin 3	1	10	27	22
436.2	419	10	Simvastatin 4	1	10	11	22
388.1	194	10	Pyraclostrobin	35	10	17	10
388.1	163.1	10	Pyraclostrobin 2	35	10	31	10
207.1	132	10	Aldicarb Sulfoxide	45	10	9	10
207.1	89.2	10	Aldicarb Sulfoxide 2	45	10	19	10
237.1	72.1	10	Oxamyl	40	10	27	10
237.1	90.1	10	Oxamyl 2	40	10	12	10
240.1	86.1	10	Aldicarb Sulfone	40	10	25.5	10
240.1	76.1	10	Aldicarb Sulfone 2	40	10	16.5	10
163.1	106	10	Methomyl	40	10	15	10
163.1	88	10	Methomyl 2	40	10	15	10
238.2	163	10	3-Hydroxycarbofuran	45	10	20	10
238.2	107.1	10	3-Hydroxycarbofuran 2	45	10	40	10
208.2	116.1	10	Aldicarb	31	10	10.5	10
208.1	89.1	10	Aldicarb 2	31	10	23	10
210.1	111	10	Propoxur	40	10	20.5	10
210.1	93.1	10	Propoxur 2	40	10	35	10
222.1	165.1	10	Carbofuran	45	10	17	10
222.1	123	10	Carbofuran 2	45	10	30	10
208.967	152.1	10	Carbaryl-d7	45	10	17	16
202.1	144.9	10	Carbaryl	45	10	15	10
202.1	127.1	10	Carbaryl 2	45	10	38	10
226.1	169	10	Methiocarb	40	10	13	10
226.1	121.1	10	Methiocarb 2	40	10	25	10
221.1	179	10	Atrazine-d5	60	10	23	10
222.97	181	10	Atrazine-d5 2	60	10	25	14
198.885	72	10	Monuron	30	10	21	10
198.885	126	10	Monuron 2	30	10	35	14
223.939	167	50	Bendiocarb	30	10	13	20
223.939	81	50	Bendiocarb 2	30	10	43	10
Microcystins							
166.1	131.1	20	Anatoxin-a	50	10	23	12
166.1	91	20	Anatoxin-a 2	50	10	30	12
247.2	204.2	20	Carbamazepine-d10	50	10	27	12
416.1	194.1	20	Cylindrospermopsin	100	10	47	12
416.2	176.1	20	Cylindrospermopsin 2	100	10	49	12

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416.1	336.1	20	Cylindrospermopsin 3	100	10	31	12
910.5	402.3	20	Microcystin LA	90	10	35	12
910.5	776.4	20	Microcystin LA 2	90	10	27.5	12
910.5	375.2	20	Microcystin LA 3	90	10	43	12
986.5	478.2	20	Microcystin LF	95	10	37	12
986.5	375.1	20	Microcystin LF 2	95	10	45	12
986.5	852.5	20	Microcystin LF 3	95	10	30	12
991.5	135.1	20	Microcystin LF-d5	95	10	102	12
991.5	213.2	20	Microcystin LF-d5 2	95	10	70	12
995.5	105	20	Microcystin LR	120	10	150	12
995.5	213.2	20	Microcystin LR 2	120	10	75	12
995.5	135.1	20	Microcystin LR 3	120	10	117	12
1002.5	494.2	20	Microcystin LY	90	10	38	12
1002.5	265	20	Microcystin LY 2	90	10	62	12
1002.5	868.4	20	Microcystin LY 3	90	10	30	12
519.9	103.1	20	Microcystin RR	90	10	91	12
519.9	135.1	20	Microcystin RR 2	90	10	36	12
519.9	213.1	20	Microcystin RR 3	90	10	45	12
1045.5	375.2	20	Microcystin YR	160	10	70	12
1045.4	135.1	20	Microcystin YR 2	160	10	130	12
825.4	227	20	Nodularin	160	10	65	12
825.4	135.1	20	Nodularin 2	160	10	99	12
825.4	70.2	20	Nodularin 3	160	10	170	12
Glyphosate							
169.889	151.98	50	Glyphosate 13C 15N 2	-39	-8.83	-13.84	-10.56
169.906	63.052	50	Glyphosate 13C 15N	-5	-10	-28	-9
167.887	62.9	50	Glyphosate	-5	-10	-26	-9
167.887	78.8	50	Glyphosate 2	-5	-10	-52	-11
167.887	149.9	50	Glyphosate 3	-50	-10	-17.16	-7
109.882	63	50	AMPA	-50	-10	-24	-7
109.882	79	50	AMPA 2	-30	-10	-36	-11
109.882	81	50	AMPA 3	-30	-10	-18	-9
179.915	85	50	Glufosinate 2	-35	-10	-26	-11
179.915	95	50	Glufosinate	-35	-10	-24	-11
Benzoic Acid							
221.9	163.9	10	2,4-D-d3	-25	-10	-20.17	-17
121	77	10	Benzoic Acid	-35	-10	-16	-10
121	77.1	10	Benzoic Acid 2	-35	-10	-16	-36
Didecy							
248.129	205.2	25	Carbamazepine-d10	45	10	29	16
326.3	186.2	25	Didecy	80	10	39	12
326.3	41	25	Didecy 2	80	10	92	10
326.3	43	25	Didecy 3	80	10	72	10
Sucralose & Endothall							
401.1	35.1	50	Sucralose-d6	-45	-10	-66	-15
395.1	35	50	Sucralose	-40	-10	-60	-15
395	359	50	Sucralose 2	-30	-10	-16	-11

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190.955	147	50	Endothall-d6	-25	-3	-22	-17
184.915	141	50	Endothall	-20	-3	-20	-17
184.915	43.2	50	Endothall 2	-30	-3	-48	-19
Diquat & Paraquat							
186	158.1	25	Diquat-d4	1	10	31	10
188	156	25	Diquat-d4 2	1	10	31	16
183	157	25	Diquat	136	10	33	11
183	168	25	Diquat 2	136	10	35	18
185	170	25	Paraquat	156	10	29	22
185	169	25	Paraquat 2	156	10	43	8

Negative Ion and Positive Ion (NIPI) Analysis Method:

Synchronization Mode – Lc Sync, Auto-Equilibration – Off, Acquisition Duration – 27 min, Ion Source – Turbo Ion Spray, Polarity – Negative and Positive, Scan Type – MRM(MRM), Resolution Q1 – Unit, Resolution – Q3 – Unit, Intensity Threshold – 0.00 cps, Setting Time – 0 msec, Pause between Mass Range – 5.0 msec, Time – 100 msec, Edit Parameters – Source Gas CUR – 30, Collision Gas CAD – 10, Ion Spray Voltage IS - -3000 and 3000, Temperature TEM – 400, Ion Source Gas 1 GS1 – 55, Ion Source Gas 2 GS2 – 60, Interface Heater Ihe – On, Integrated Valco Valve open to MS: 1 min to 27 min.

Column: Phenomenex – Luna, 5 microns, Phenyl-Hexyl, 4.6 x 150 mm, Part Number 00F-4257-E0.
Mobile phase A: HPLC water containing 5 mmol Ammonium acetate and 0.1 % Formic acid.
Mobile phase B: Acetonitrile containing 0.1 % Formic acid.

Pumps: Pumping Mode – Binary Flow, Total Flow – 0.8 mL/min, Pump B Conc: 5%, Pump B Curve – 0; Configured Pumps: Pump A: LC-30AD, Pump B: LC-30AD; Pressure Limits (Pump A, B) – Minimum: 0 psi, Maximum: 19000 psi.

Autosampler: Model: SIL-30AC, Rack Type: Undefined, Use Autosampler: Checked, Needle Stroke: 52 mm, Control Vial Needle Stroke: 52 mm, Sampling Speed: 5 µL /sec, Cooler Temperature: 15 °C, Measuring Line Purge Volume 100 µL, Rinse Type: External, Rinsing Speed: 35 µL /sec, Rinsing Volume: 500 µL, Rinse Mode: Before and after aspiration and Rinse Dip Time: 0 sec, Rinse Method: Rinse Port only, Rinse Time: 2 sec, Purge Settings: Purge Port: 10 min, Purge Measuring Lines: 10 min. Oven Temperature: 40 °C. Equilibrium Time – 0 min. Injection Volume – 20 µL.

Gradient Program: A/B (95:5) to A/B (70:30) over 13 min, to A/B (20:80) over 9 min, to A/B (10:90) for 1 min, hold A/B (10:90) for 1 min, to A/B (2:98) for 0.9 min, Total Flow – 0.8 mL/min at 25 min and 1.5 mL/min at 25.5 min, A/B (2:98) at 29.5 min, A/B (95:5) at 30 min Time, Total Flow – 1.5 mL/min at 31 min and 0.8 mL/min at 31.5 min and Stop – 34 min.

Calibration levels (Target to Surrogate Ratio: 1 to 2): 100, 50, 25, 10, 5, 2.5, 1, 0.25, 0.1, 0.05 and 0.01 µg/L; Continuing calibration verification standard (CCV) level: 2.5 µg/L; Surrogate spike level: 20 µg/L and lab fortified blanks and Matrix spike level: 10 µg/L (on column in extract).

Calibration levels for Benzoic acid/2,4-D-d3: 100, 75, 50, 25, 10 and 5 µg/L; Continuing calibration verification standard (CCV) level for Benzoic acid/2,4-D-d3: 50 µg/L; Surrogate spike level: 50 µg/L and lab fortified blanks and Matrix spike level: 50 µg/L.

Sucralose:

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Acquisition Duration – 15 min, Polarity - Negative, Time – 15 msec, Edit Parameters – Source Gas CUR – 40, Collision Gas CAD – 9, Ion Spray Voltage IS - -3000, Temperature TEM – 550, Ion Source Gas 1 GS1 – 60, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve open to MS: 2.5 min to 14 min.

Calibration levels (Target to Surrogate Ratio: 1 to 2): 25, 10, 5, 2.5, 1, 0.25, 0.1, 0.05 and 0.01 µg/L; Continuing calibration verification standard (CCV) level: 2.5 µg/L; Surrogate spike level: 4 µg/L and lab fortified blanks and Matrix spike level: 2 µg/L. Mobile phase A: HPLC 0.25 mmol Ammonium Fluoride in water and Mobile Phase B: Acetonitrile.

Gradient Program: A/B (95:5) to A/B (70:30) for 4 min, to A/B (10:90) for 5 min, A/B (1:99) at 9.2 min, A/B (1:99) at 12.5 min, A/B (95:5) at 12.9 min, A/B (95:5) at 15 min, STOP - 15.1 min. Total Flow – 0.8 mL/min. Injection Volume – 50 µL.

Endothall:

Acquisition and Gradient Program same as Sucralose with following changes. Calibration levels (Endothall/Endothall-d6) - 100, 75, 50, 25, 10 and 5 µg/L; Continuing calibration verification standard (CCV) level: 25 µg/L; Surrogate spike level: 25 µg/L and lab fortified blanks and Matrix spike level: 25 µg/L. Mobile phase A: HPLC water containing 5 mmol Ammonium acetate and 0.1 % Formic acid and Mobile Phase B: Acetonitrile containing 0.1 % Formic acid.

Glyphosate:

Acquisition Duration – 4 min, Polarity - Negative, Edit Parameters – Source Gas CUR – 40, Collision Gas CAD – 5, Ion Spray Voltage IS - -3000, Temperature TEM – 550, Ion Source Gas 1 GS1 – 60, Ion Source Gas 2 GS2 – 70, Interface Heater Ihe – On, Integrated Valco Valve open to MS: 1 min to 3 min.

Calibration levels (Target to Surrogate Ratio: 1 to 5): 25, 10, 2.5, 1, 0.25 and 0.1 µg/L; Continuing calibration verification standard (CCV) level: 1 µg/L; LFBs and Matrix spike level: 1 µg/L. Mobil phase A: HPLC water containing 5 mmol Ammonium acetate and 0.1 % Formic acid and Mobile Phase B: Acetonitrile containing 0.1 % Formic acid.

Gradient Program: A/B (100:0) at 3 min, A/B (2:98) at 4.9 min, Total Flow 0.8 mL/min at 5 min, Total Flow 1.5 mL/min at 5.5 min, A/B (2:98) at 7 min, A/B (100:0) at 7.5 min, Total Flow 1.5 mL/min at 8 min and Total Flow 0.8 mL/min at 8.5 min. Stop – 10 min. Injection Volume – 25µL. Column used - Waters – SymmetryShield RP8, 150 x 3.9 mm, Part Number WAT200655.

Microcystins:

Acquisition Duration – 16 min, Polarity - Positive, Edit Parameters – Source Gas CUR – 30, Collision Gas CAD – 10, Ion Spray Voltage IS - 5250, Temperature TEM – 500, Ion Source Gas 1 GS1 – 55, Ion Source GS2 – 60, Interface Heater Ihe – On, Integrated Valco Valve open to MS: 2 min to 13 min.

Calibration levels (Target to Surrogate Ratio: 1 to 100): 50, 25, 10, 2.5, 1 and 0.25 µg/L; Continuing calibration verification standard (CCV) level: 10 µg/L; LFBs and Matrix spike level: 10 µg/L. Mobil phase A: HPLC water containing 5 mmol Ammonium acetate and 0.1 % Formic acid and Mobile Phase B: Acetonitrile containing 0.1 % Formic acid.

Gradient Program: A/B (95:5) for 1.5 min, A/B (75:25) at 3 min, A/B (45:55) at 8 min, A/B (20:80) at 8.9 min, A/B (2:98) at 9 min, A/B (2:98) at 11.6 min and A/B (95:5) at 12 min. Stop – 16 min. Total Flow – 0.8 mL/min. Injection Volume – 50 µL.

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Didecyldimethylammonium chloride:

Acquisition Duration – 11 min, Polarity - Positive, Edit Parameters – Source Gas CUR – 30, Collision Gas CAD – 10, Ion Spray Voltage IS - 3000, Temperature TEM – 400, Ion Source Gas 1 GS1 – 55, Ion Source Gas 2 GS2 – 60, Interface Heater Ihe – On, Integrated Valco Valve open to MS: 1 min to 10 min.

Calibration levels (Target to Surrogate Ratio: 1 to 50): 100, 50, 25, 10, 5, 2.5 and 0.5 µg/L; Continuing calibration verification standard (CCV) level: 25 µg/L; LFBs and Matrix spike level: 10 µg/L. Mobil phase A: HPLC water containing 5 mmol Ammonium acetate and 0.1 % Formic acid and Mobile Phase B: Acetonitrile containing 0.1 % Formic acid. Column used - Phenomenex – Synergy 4-micron Fusion-RP 80A, 50 x 2 mm, Part Number 00B-4424-B0.

Gradient Program: A/B (95:5) to A/B (30:70) for 5 min, A/B (0:100) over 1.3 min, A/B (95:5) at 6.4 min, A/B (95:5) for 4.5 min, STOP – 11 min. Total Flow – 0.8 mL/min. Injection Volume – 10 µL.

Diquat and Paraquat:

Acquisition Duration – 13 min, Polarity - Positive, Edit Parameters – Source Gas CUR – 20, Collision Gas CAD – 2, Ion Spray Voltage IS - 5500, Temperature TEM – 700, Ion Source Gas 1 GS1 – 70, Ion Source Gas 2 GS2 – 60, Interface Heater Ihe – On, Integrated Valco Valve open to MS: 6.3 min to 7.5 min.

Calibration levels (Diquat/Diquat-d4/Paraquat): 50, 25, 10, 5, 2 and 1 µg/L. Continuing calibration verification standard (CCV) level: 10 µg/L; Surrogate spike level: 10 µg/L and lab fortified blanks and Matrix spike level: 10 µg/L. Mobile phase A: HPLC water containing 5 mmol Heptafluorobutyric acid. Mobile phase B: Acetonitrile containing 0.1 % Formic acid.

Gradient Program: A/B (95:5) to A/B (70:30) over 8 min, A/B (10:90) for 1 min, A/B (95:5) for 4 min, Total Flow – 0.8 mL/min. Run Time: 13 min. Injection Volume – 25 µL.

6.4 MultiQuant Data Processing

6.4.1 **Quantitate** – Create new results table in MultiQuant – Choose Data file to add runs – Create or Choose Quantitation method and Edit Quantitation method – for each analyte check peak retention time, smooth factor of 1, noise percentage of 90 %, Peak Splitting of 6 – Units in ug/L – Calibration Type Linear and 1/x with other Default Settings.

6.4.2 **Review Results Table** – Check the sample type, Analyte concentration, Dilution Factor, Peak retention time, Integration and Curve fit. Review each sample data, Save the Results Table with same file name as analysis batch. Export file with visible columns for printing.

6.4.3 **QCManager Calculations**: Export data from Results Table (order of columns – Sample Name/Analyte Peak Name/Calculated Concentration (ug/L)/ Analyte Units/Acquisition Date) with .txt extension to Notepad and give the same file name as Run Sequence and Results Table (NIPI180219TQ_QCM.txt). Open the file in Notepad to edit the rows and save to perform QCManager calculations and report the results into LIMs.

6.5 REPORTING

6.5.1 In MS/MS analysis, identification and quantitation of each compound is based on the known precursor ion (in standard and unknown samples) fragmenting to two daughter

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ions. Both MRM transitions from the known precursor ion can be used for Quantitation, confirmation and reporting. For positives, ion ratios are used for further confirmation.

6.5.2 The surrogate, Lab Fortified Blank and Matrix spikes recoveries in each sample must be calculated.

6.5.3 If the surrogate recovery is outside of established limits of 40 to 140, then the results must be flagged, and an explanation offered. If any of the above surrogate falls outside control limits, re-extract the effected sample with proper QC even after expiration and follow DEP SOP LB-027 for reporting.

6.5.4 Check the Lab Fortified Blanks and matrix spikes recoveries, and they must be within the acceptance range with a precision % RPD of <30. This range is set by the method initially and is evaluated once a year.

Table: Recovery Limits Table

Compound	% Rec Water	% Rec Soil
Negative Ion Mode		
2,4-D	40 - 150	50 - 140
2,4,5-T	40 - 150	50 - 140
Silvex	40 - 150	50 - 140
Acifluorfen	40 - 150	50 - 140
Dicamba	40 - 150	50 - 140
Picloram	40 - 150	50 - 140
Bentazon	40 - 150	50 - 140
2,4-DB	40 - 150	50 - 140
MCPP	40 - 150	50 - 140
MCPA	40 - 150	50 - 140
Dichlorprop	40 - 150	50 - 140
Dinoseb	40 - 150	50 - 140
Triclopyr	40 - 150	50 - 140
Dalapon	40 - 150	
Imazapyr	40 - 150	
2,4-D-d3	40 - 150	40 - 150
Benzoic acid	40 - 150	
Metformin HCl	40 - 150	
Hydrocodone	40 - 150	
Duloxetine HCl	40 - 150	
Simvastatin	40 - 150	
Sucralose	40 - 150	
Sucralose-d6	40 - 150	
Endothall	40 - 150	
Endothall-d6	40 - 150	
Glyphosate	40 - 150	
AMPA	40 - 150	
Glufocinate	40 - 150	
Glyphosate C13 15N	40-150	
Positive Ion Mode		
Aldicarb	40 - 150	50 - 140
Aldicarb Sulfone	40 - 150	50 - 140
Aldicarb Sulfoxide	40 - 150	50 - 140
Methomyl	40 - 150	50 - 140

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Oxamyl	40 - 150	50 - 140
Propoxur	40 - 150	50 - 140
Methiocarb	40 - 150	50 - 140
Carbaryl	40 - 150	50 - 140
Carbofuran	40 - 150	50 - 140
3-Hydroxycarbofuran	40 - 150	50 - 140
Bendiocarb	40 - 150	50 - 140
Carbaryl-d7	40 - 150	50 - 140
Acetaminophen	40 - 150	50 - 140
Acetaminophen-d4	30 - 140	50 - 140
Carbamazepine	40 - 150	50 - 140
Carbamazepine-d10	40 - 150	50 - 140
Indaziflam	40 - 150	50 - 140
Indaziflam-N15	40 - 150	50 - 140
Primidone	40 - 150	50 - 140
Primidone-d5	40 - 150	50 - 140
Diuron	40 - 150	50 - 140
Fenuron	40 - 150	50 - 140
Fluometuron	40 - 150	50 - 140
Fluridone	40 - 150	50 - 140
Imidacloprid	40 - 160	50 - 140
Monuron	40 - 150	50 - 140
Pyraclostrobin	40-150	50 - 140
Linuron	40 - 150	50 - 140
Diuron-d6	40 - 150	50 - 140
Neproxen	40-150	
Ibuprofen	40 - 150	
Ibuprofen-d3	40 - 150	
Didecyldimethylammonium chloride	40-150	
Carbamazepine-d10	40-150	
Diquat	40-150	
Paraquat	40-150	
Diquat-d4	40-150	
Anatoxin-a	40 - 160	
Cylindrospermopsin	40 - 160	
Microcystin LA	40 - 160	
Microcystin LR	40 - 160	
Microcystin RR	40 - 160	
Microcystin YR	40 - 160	
Microcystin LF	40 - 160	
Microcystin LY	40 - 160	
Nodularin	40 - 160	
Carbamazepine-d10	40 - 160	
Malathion	60 - 140	
Malathion-d6	60 - 150	

6.5.5 Acceptance ranges for Lab Fortified Blanks and matrix spikes are statutory limits, which are tighter than the statistical limits. In real samples both surrogate and matrix spike recoveries are observed to recover low due to matrix interferences.

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6.5.6 A comment must be added to the qualified data in the final report explaining any deviation from the routine limits whenever the spikes or surrogates are out of range (DEP SOP LB-027).

6.5.7 Check the method blank for any interfering contaminants. The MDLs may have to be elevated if the blank(s) indicate contamination.

6.6 Spike recoveries:

The recovery of acid herbicide analytes in spiked samples is calculated using the following equation

6.6.1 Spike recoveries:

$$\text{Rec. \%} = \frac{(\text{Amount detected, } \mu g/L)\ (\text{Final extract volume, mL})}{(\text{Spike volume, mL})\ (\text{Spike concentration, } \mu g/L)} \times 100\ \%$$

6.6.2 Calculation of positives in water samples:

$$\text{Conc. in water sample } (\mu g/L) = \frac{(\text{Detected conc., } \mu g/L)\ (\text{Final effective extract volume, mL})}{(\text{Volume of sample extracted, mL})}$$

6.6.3 Calculation of positives in sediment samples

$$\text{Conc. in sediment } \mu g/kg = \frac{(\text{Detected conc., } \mu g/L)\ (\text{Final effective extract volume, mL})}{(\text{Dry weight of sample extracted, g})}$$

Example: If 25 µg/L was detected in sample extract, and sample extract effective final volume was 10 mL and 30 g of sediment sample was extracted:

$$\text{Conc. in sediment sample } (\mu g/kg) = \frac{(25 \mu g/L)\ (10\ mL)}{30\ g} = \mathbf{8.3\ \mu g/kg}$$

6.6.4 When entering the dilution factor for soil samples in QC Manager or Target, use the following formula and make sure that any additional dilutions have been considered!

$$\text{Dilution factor} = \frac{1}{\text{Dry weight (as a fraction of 1)}}$$

(E.g. if dry weight is 8.15%, dilution factor is 1/0.0815 = 12.27)

6.7 If performing dilutions, show all calculations and include copies of the dilution/sample prep logbook entry of the dilution.

6.8 UPLC MAINTENANCE

6.8.1 Flush the system with 100% water followed by 100% acetonitrile or methanol to remove residual formic acid and any strongly trapped organics respectively.

6.8.2 It is suggested that the column be stored in 100% acetonitrile or methanol.

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6.8.3 Call for Preventive Maintenance every six months for API 3200 and once a year for UPLC or if the system needs repairs.

7 REPORTING, DOCUMENTATION AND DATA ARCHIVAL

7.1 When analysis is complete, download the final sample results into the Laboratory Information Management System (LIMS) using QC Manager as a text file. If a sample has been diluted or has a dry weight less than 100 %, then make sure that the information was entered properly into the QC Manager.

7.2 Add the appropriate qualifiers and comments to the sample data in the calculator after performing a complete review of the sample data and QC data. See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data and Correcting Quality Control Problems for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option.

7.3 Definitions of the available qualifying codes are listed in DEP SOP LB-027

7.4 All quantitation reports for samples and QC should be placed in a results folder labeled with the job number, the sample numbers, and the type of analysis.

7.5 Retrieve the field folders (containing the field sheets) and check the data against the field sheets to see if it makes sense (i.e., does the equipment blank contain several hits while an effluent sample does not?) If anything looks suspicious, further research is necessary before posting results.

7.6 Enter the results for each sample in the analysis results form. Print out QC results from the QC Manager. Include a copy of dilutions page if any. Include a print out of sample positives from the data review and file it in the data folder.

7.7 Submit the result folder to the section supervisor. Data folder includes Cover Page, Preparation Sheet, Printout of sample positives, Run Sequence and Results Table. The supervisor will review results and then authorize the sample. The supervisor will then file the folders in the lab. The supervisor will notify the Environmental Manager of the organic chemistry section, who authorizes the job.

7.8 Original analysis data collected in LC-MSMS is archived in to TLHLAB3/LC/. All the QA/QC data and analysis results are archived in the proper file kept in HPLC Lab by chemist who performed the analysis.

8 QUALITY CONTROL

8.1 See DEP SOP LB-027, Standard Operating Procedure for Reporting Qualified Data and Correcting Quality Control Problems for guidelines for qualifying data when quality control results are outside established control limits and re-analysis is not a viable or desired option.

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 8321B. Solvent-Extractable Nonvolatile Compounds by High-Performance Liquid Chromatography/Thermospray/Mass Spectrometry (HPLC/TS/MS) or Ultraviolet (UV) Detection, Revision 2, 2/2007.

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

10.4 Florida Department of Environmental Protection, DEP **SOP LB-007,** *Procedure and Policy for Demonstration of Capability for Methods, Instruments and Laboratory Staff.*

10.5 Florida Department of Environmental Protection, **SOP LB-031,** *Limit of Detection Verification.*

10.6 Quality Manual for State of Florida Department of Environmental Protection Laboratory.

10.7 DEP **SOP LC-039,** *HPLC Standard Preparation.*

10.8 DEP **SOP LB-027,** *Standard Operating Procedure for Reporting Qualified Data and Correcting Quality Control Problems.*

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

10.10 Online solid phase extraction liquid chromatography tandem mass spectrometry (SPE-LC-MS/MS) method for the determination of sucralose in reclaimed and drinking waters and its photo degradation in natural waters from South Florida. Batchu et.al. Chemistry Central Journal 2013, 7:141

10.11 Favorable Effects of Weak Acids on Negative-Ion Electrospray Ionization Mass Spectrometry. Zengru Wu et.al. Analytical Chemistry, December 2007, 1-11

APPENDIX-A

SHIMADZU LC SET-UP CHECK LIST

STARTING UP

1. **Startup:** Fill reservoirs with Mobile Phase A – Aqueous in Bottle A and Mobile Phase B – Organic in Bottle B. Purge each pump and check the flow rate. Fill rinse bottles with 1:1 water-acetonitrile. Check pump head wash solution 10% iso-propanol – water and change once a month.

2. **Shutting Off:** UPLC gradient program stops after one hour automatically by Analyst software along with MSMS and power for UPLC system need to be tuned off manually. Store sample vials in the fridge. OPTIONAL: To flush column, set flow rate to 1.0-mL/min. Allow 100% water to flow through column for 30-60 minutes, followed by 100% acetonitrile or methanol for 40-60 minutes or set up UPLC to run 5 blank injections using same method.

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Appendix of Changes

08/05/2010	Added new analyte Cyanuric acid in water analysis by direct injection. Elaborated MDL determination section 1.4 to include different matrices and supporting references in section 10.
08/22/2011	Added new analyte Dioctyl sulfosuccinate and Sucralose in water analysis. Changed criteria for initial calibration curve.
08/15/2012	Added new UPLC instrument and edited method files to reflect current analysis.
03/06/2013	Edited to include low level Negative Ion Mode Analysis
09/24/2013	Added Diquat and Paraquat in water analysis.
01/13/2014	Added Glyphosate and AMPA in water analysis; Merged SOPs LC-001-2.7, LC-006-2.13 and LC-008-2.15 into LC-001
01/14/2015	Merged SOPs LC-038-1 and LC-001-2 into LC-001-2
01/26/2016	Added new analyte Benzoic acid
01/16/2017	Added new analyte Malathion
03/07/2018	Edited to include two API 6500+ instruments

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